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# Synthesis of Diazirine-Based Photoreactive Saccharin Derivatives for the Photoaffinity Labeling of Gustatory Receptors

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Saccharin is one of the most common artificial sweeteners that has a bitter taste at high concentrations. Currently, there are no detailed functional analyses of these gustatory receptors. Therefore, we designed and synthesized photoreactive saccharin derivatives that contain a (trifluoromethyl)diazirinyl moiety at the 5- or 6-position for use as functional analysis tools for photoaffinity labeling.

## Introduction

Humans distinguish gustatory sensations as five basic tastes: bitterness, saltiness, sourness, sweetness, and savoriness. Sweetness is almost universally regarded as a pleasurable experience for human beings. Numerous chemical substances, both natural and artificial, have been reported as sweeteners. Saccharin is one of the most common artificial sweeteners in the world and is several hundred times sweeter than sucrose. However, it has a bitter taste at high concentrations. Sweet and bitter taste receptors are both G protein-coupled receptors. For saccharin, the bioactivity underlying its sweetness involves binding with the sweet taste receptor, which has a heterodimeric structure with T1R2 and T1R3 subunits. Each subunit has a large amino-terminal domain linked by a cysteine-rich domain at the extracellular site to a seven transmembrane helical domain.[1] The human heterodimeric sweet taste receptor (hT1R2-hT1R3) responds to a wide variety of chemical substances, both natural and artificial.[2] Although these sweeteners have various chemical structures, all of the compounds bind to the same sweet taste receptor.[3,4] To study how the receptor can distinguish between saccharin and other sweeteners as well as the structural features of saccharin derivatives that favor the activation of the sweet taste receptor, approaches such as conformational analysis by using X-ray crystallography, NMR spectroscopy, and molecular modeling have been used. However, the receptor-bound conformations of the sweeteners remain unclear as a result of limited structural information on the ligands complexes with the receptor.

Photoaffinity labeling is a useful biochemical method to explore the structural and functional relationships between low molecular weight bioactive compounds and biomolecules.[5] This method is suitable for analyzing biological interactions because it is based on the affinity of bioactive compounds for biomolecules. Various photophores, such as phenyldiazirine, arylazide, and benzophenone, are used. To the best of our knowledge, the synthesis of saccharin derivatives for photoaffinity labeling has not been reported yet. Arylazide saccharins, which can be utilized as photoaffinity labeling reagents, have been reported very recently. However, the arylazide moiety was utilized for click reaction substrates and photoaffinity labeling was not employed.[6] Although comparative irradiation studies of these three photophores in living cells indicates that a carbene precursor, [3-(trifluoromethyl)phenyl]diazirine, is the most promising photophore,[7] the relatively complicated synthesis of the [3-(trifluoromethyl)phenyl]diazirinyl ring has resulted in fewer applications in biomolecular studies relative to other photophores. To resolve this problem, we have reported on the post-functional synthesis of a family of [3-(trifluoromethyl)phenyl]diazirines by using many reaction conditions.[8] Suami et al. reported on several structure-activity relationships for saccharin, and found that

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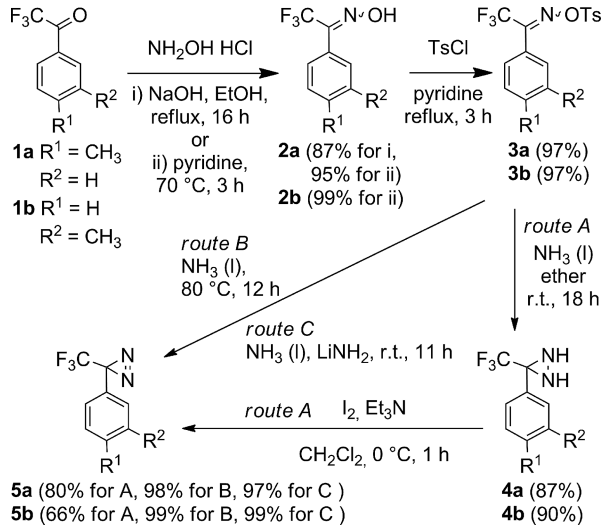
substitutions at the 5- and 6-positions in saccharin were tolerated with regards to its biological activities.[9] In addition, some sweet compounds also interact with other taste modalities. For example, saccharin triggers both the sweet and bitter taste modalities. The mechanisms underlying changes in taste modalities have not yet been elucidated.[10] In this report, we aim to describe the synthesis of photoreactive (trifluoromethyl)diazirinyl saccharin derivatives, which can be used to elucidate the sweet and bitter taste mechanisms. Our approach involves post-functional derivatization[11] for [3-(trifluoromethyl)]phenyldiazirine derivatives.

## Results and Discussion

Although several methods for the synthesis of saccharin have been reported,[12] we were required to choose the simplest synthetic methods for the diazirine three-membered ring structure. Each synthetic step for oxidative cyclization of *N*-*t*Bu-*o*-toluenesulfonamide derivatives to construct saccharin skeleton[13] could be applied without decomposition of the (trifluoromethyl)diazirine moiety. Under previously reported conditions,[14] 2,2,2-trifluoro-1-(*p*-tolyl)ethanone (**1a**) was treated with hydroxylamine hydrochloride in the presence of sodium hydroxide in ethanol at reflux temperatures for 16 h, which resulted in 87% yield of diazirine. The isolated yield was increased to 95% within 3 h when pyridine was used as a solvent at 70 °C.[15] Regioisomer **1b** was also converted into corresponding oxime **2b** under identical conditions with **1a** in 99% yield. The oximes were converted into tosyl oximes **3a** and **3b** by using Tosyl(Ts)Cl in pyridine in very good yield. Then, we subjected the tosyl oximes to classical stepwise conversions to give diazirines **5a**[14] and **5b**[16] through diaziridines **4a** and **4b** with up to 70% yield for the two steps (Scheme 1, route A). We recently reported on effective one-pot conversions into diazirine derivatives from the corresponding tosyl oximes.[17] The tosyl oximes were subjected to two conditions. The first was the treatment of tosyl oximes in liquid ammonia at 80 °C for 12 h in a stainless pressure-resistant tube. A detailed product analysis revealed that diaziridine formation occurred within an hour, then slow conversion into diazirine with in situ generated $^{-}NH_2$ species at high temperature (Scheme 1, route B). The reaction rate for the latter step was further improved by adding lithium amide to enhance the concentration of $^{-}NH_2$ at lower temperatures (room temperature; Scheme 1, route C). The chemical yields of both one-step conversions into diazirine from tosyl oxime were almost quantitative.

3-(*p*- or *m*-Tolyl)-3-(trifluoromethyl)-3*H*-diazirines (**5a** and **5b**) were subjected to aromatic chlorosulfonation at adjacent positions of the toluene methyl group with chlorosulfonic acid at –20 °C. Addition of strong acid at higher temperatures promoted decomposition of the diazirinyl ring.[18] The sulfonyl chloride was converted into *N*-alkylated sulfonamide with *t*BuNH$_2$ at room temperature. The reaction in the triethylamine and *t*BuNH$_2$ system (both

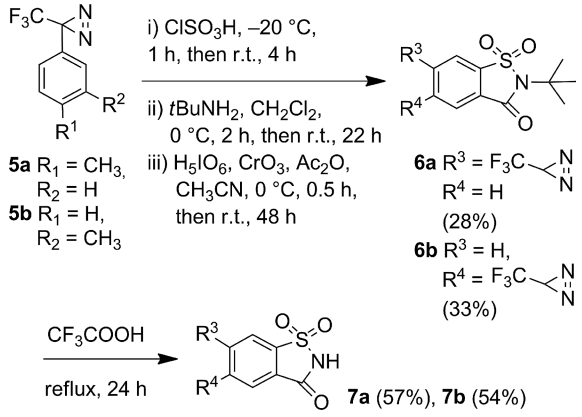



Scheme 1. Improved synthesis of 3-(*m*- or *p*-tolyl)-3-(trifluoromethyl)-3*H*-diazirines.

2 equiv.; experimental conditions are reported in ref.[13]) afforded a more complex mixture than that in *t*BuNH$_2$ only (4 equiv.). Oxidative cyclization with $H_5IO_6$ and $CrO_3$ between the methyl group and *o*-oriented *N*-*tert*-butyl sulfonamide synthesized saccharin skeletons **6a** and **6b** in a moderate yield. Purification for each step generated lower isolated yields for cyclized compounds **6a** and **6b** (< 10%). We performed these three steps as one-pot reactions (≈ 30% yields). Deprotection of the *t*Bu group with trifluoroacetic acid (TFA) under reflux conditions afforded diazirinyl saccharin derivatives **7a** and **7b** (Scheme 2). The synthesized photoreactive saccharin derivatives were insoluble in water. We performed further purification of the synthesized saccharin derivatives by converting them into the corresponding sodium salts with aqueous sodium hydroxide and then subjecting them to reversed phase HPLC. Both samples were eluted at 22.5 and 21.0 min on an ODS column with 30% methanol for sodium salt of **7a** and **7b**, respectively, at 215 nm (Figure 1). These peaks were also detected at 350 nm. $^{19}F$ NMR spectra for the (trifluoromethyl)-3*H*-diazirines (–65 ppm) revealed the three-membered ring, which was identical to those previous reported.[18] These results indicate that the diazirinyl groups are preserved in the final compounds.

Irradiation studies of a methanolic solution (1 mM) of **7a** and **7b** by using 15 W black light indicated that the diazirinyl moiety of both saccharin derivatives decomposed very rapidly (Scheme 3). Reports have suggested that diazo derivatives, which are one of the main by-products of the irradiation of diazirines, cannot generate carbenes under irradiation at 350 nm at a diazirine concentration over 10 mM.[19] However, a diluted (< 1 mM) solution can generate carbene from diazo compounds when irradiated at 350 nm for periods > 10 min. The absorbance at around 350 nm, which is characteristic of a diazirinyl three-membered ring,[20] diminished with increasing irradiation time (Figure 1). The half-life of diazirinyl saccharin derivatives **7a** and **7b** were




Scheme 2. Post-functional synthesis of (trifluoromethyl)diazirinyl saccharin derivatives at the 5- or 6-positions.

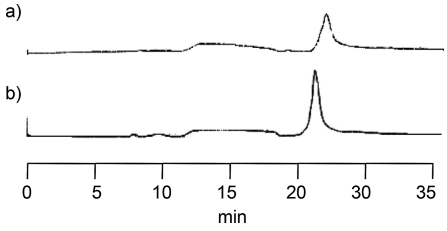
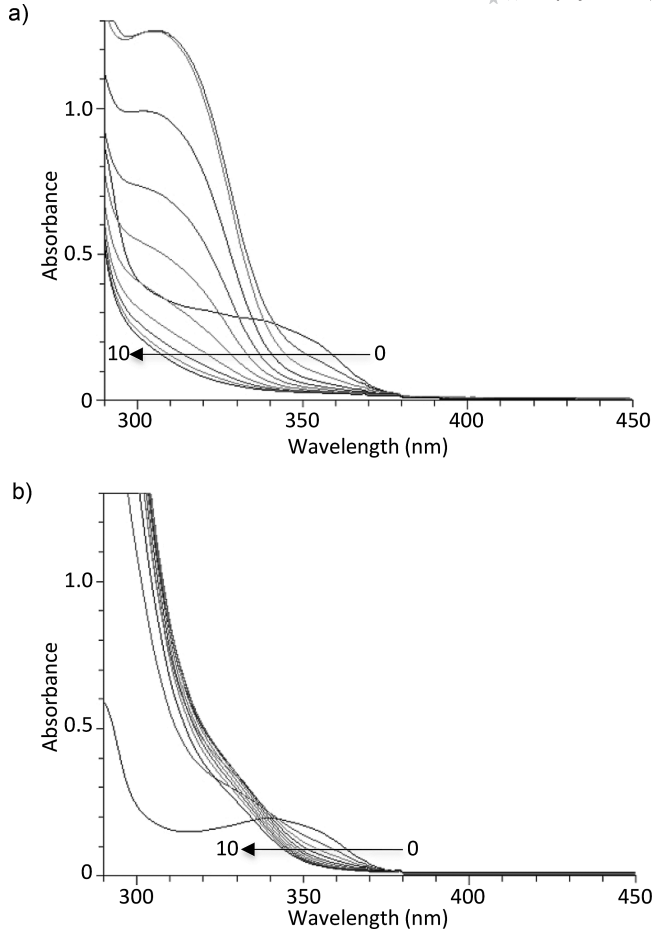



Figure 1. HPLC profiles of 5- or 6-(trifluoromethyl)diazirinyl-sodium saccharin. The chromatograms for sodium salts of **7a** and **7b** are a) and b), respectively. HPLC conditions; Tosoh TSKgel ODS-80 Ts (4.6×250 mm), 30% MeOH/$H_2O$, flow rate 1 mL/min, detection at 215 nm.

calculated at 77 and 107 s, respectively. These half-lives are shorter than those of the diazirinyl α-amino acid derivatives[21] that we reported with the photoreactive D-isomer acting as the sweetener.[22] An electron-donating moiety at the *p*-position (carbonyl and sulfonamide moieties for **7a** and **7b**, respectively) may promote rapid photolysis of diazirinyl saccharins.

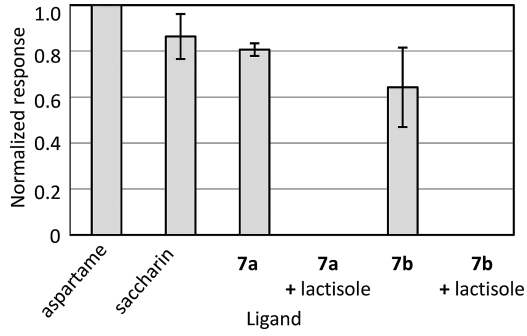
A required characteristic for photoaffinity labeling was sufficiently met because no influence of irradiation effects on other biomolecules occurred. The photoreactivities of the studied compounds were also consistent with previous $^{19}F$ NMR spectroscopic studies in which the $^{19}F$ NMR spectroscopic chemical shifts changed from –65 to –78 ppm after 10 min of irradiation. No diazo isomer signal (–58 ppm) was detected in the photoirradiated mixture.[19] The results indicate that the diazirine moieties generated carbenes, which were then quenched by solvent.

The synthesized photoreactive saccharin derivatives were subjected to preliminary gustatory receptor assays at 10 mM. Saccharin, **7a**, and **7b** have 90, 80, and 65% relative sweetness activity, respectively, against the same concentration of aspartame for the hT1R2-hT1R3 expressed HEK-293 T cell.[23] The response was completely inhibited by addition of lactisole, which is one of the specific inhibitors in the sweet taste assay. (Scheme 4). The bitter receptor hT2R31 response to **7a** and **7b** at 10 mM activity was calculated as 60 and 80%, respectively. Other bitter receptors, such as hT2R43,[10] did not respond to 10 mM **7a** and **7b**.




Scheme 3. Photolysis of diazirine-based saccharin derivatives **7a** (a) and **7b** (b) in methanol (1 mM) with black light (15 W). UV spectra of the photolysis reaction were recorded every 1 min for 10 min.

The photoreactive compounds will be subjected to further detailed biological analyses for their gustatory responses.




Scheme 4. Sweetness potential represented as normalized responses for 10 mM of aspartame, sucrose and synthetic photoreactive compounds (**7a** and **7b**). Cell responses, which are triggered with 10 mM aspartame, were set as normalization standard. The degree of cell response to 10 mM of each chemical was reported as a normalized ratio. Each column represents the mean standard error of three independent experiments.

## Conclusions

These results indicate that the preparation of the diazirinyl saccharin derivatives is effective, and that these

photoreactive compounds have enough affinity for the sweet and bitter taste receptors to elucidate the binding sites of their ligands in these receptors. This will ultimately allow an understanding of the underlying molecular mechanisms of gustatory receptors.

## Experimental Section

**General Remarks:** NMR spectra were measured with JEOL EX-270 or Bruker AMX500 spectrometers. All solvents were of reagent grade and distilled by using the appropriate methods. ESI-TOF-MS data were obtained with a Waters UPLC ESI-TOF mass spectrometer.

**2,2,2-Trifluoro-1-(*p*-tolyl)ethanone Oxime (2a). Method (i) in EtOH:** 2,2,2-Trifluoro-1-(*p*-tolyl)ethanone (**1a**; 0.104 g, 0.55 mmol) in EtOH (5 mL) was added to hydroxylamine hydrochloride (0.0459 g, 0.66 mmol) and NaOH (0.064 g, 1.6 mmol) in EtOH (5 mL). The reaction mixture was heated to reflux for 16 h, and then concentrated. The residue was partitioned between ether and water. The organic layer was washed with HCl (0.01 M) and water, dried with $MgSO_4$, filtered, and concentrated to afford a colorless amorphous solid (0.0974 g, 87%). **Method (ii) in pyridine:** 2,2,2-Trifluoro-1-(*p*-tolyl)ethanone (**1a**; 1.13 g, 6.0 mmol) was dissolved in pyridine (30 mL), then hydroxylamine hydrochloride (0.500 g, 7.2 mmol) was added. The mixture was stirred at 70 °C for 1 h and was then subjected to rotary evaporation to remove the pyridine. The residue was dissolved in ethyl acetate and washed with HCl (1 M), the organic layer was washed with $H_2O$ and brine, dried with $MgSO_4$, and concentrated to afford a colorless amorphous solid (1.17 g, 96%). The product was mixture of *syn*- and *anti*-isomers. $^1$H NMR (270 MHz, $CDCl_3$): $\delta$ = 9.01 (br. s, 1 H), 7.37–7.44 (m, 2 H), 7.21–7.29 (m, 2 H), 2.39 (s, 0.8 H), 2.38 (s, 2.2 H) ppm. $^{13}$C NMR (68 MHz, $CDCl_3$): $\delta$ = 148.2 [q, $^2J(C,F)$ = 30.6 Hz], 141.2 and 141.0, 129.42 and 129.36, 128.7 and 128.3, 123.0, 118.5 (q, $^1J_{C,F}$ = 283.0 Hz), 21.3 and 21.2 ppm. $^{19}$F NMR (470 MHz, $CDCl_3$): $\delta$ = –62.4, –66.6 ppm. HRMS (ESI): calcd. for $C_9H_9F_3NO$ 204.0636; found 204.0632.

**2,2,2-Trifluoro-1-(*m*-tolyl)ethanone Oxime (2b):** Treatment of 2,2,2-trifluoro-1-(*m*-tolyl)ethanone (**1b**; 1.13 g, 6.0 mmol) in pyridine was carried out as described above to afford **2b** (1.21 g, 99%) as a colorless amorphous solid. $^1$H NMR (270 MHz, $CDCl_3$): $\delta$ = 8.76 (br. s, 0.6 H), 8.59 (br. s, 0.4 H), 7.29–7.40 (m, 4 H), 2.40 (s, 1.8 H), 2.39 (s, 1.2 H) ppm. $^{13}$C NMR (68 MHz, $CDCl_3$): $\delta$ = 148.4 (q, $^2J_{CF}$ = 30.7 Hz), 148.1 (q, $^2J_{C,F}$ = 32.4 Hz), 138.6, 131.6 and 131.4, 129.8 and 129.1, 129.0 and 128.6, 125.9 and 125.7, 125.5, 120.7 (q, $^1J_{C,F}$ = 274.8 Hz), 118.4 (q, $^1J_{C,F}$ = 282.9 Hz), 21.2 and 21.1 ppm. $^{19}$F NMR (470 MHz, $CDCl_3$): $\delta$ = –62.4, –66.8 ppm. HRMS (ESI): calcd. for $C_9H_9F_3NO$ 204.0636; found 204.0628.

**2,2,2-Trifluoro-1-(*p*-tolyl)ethanone *O*-Tosyl Oxime (3a):** To a solution of oxime **2a** (0.910 g, 4.5 mmol) in acetone (30 mL) at 0 °C, triethylamine (1.87 mL, 13.4 mmol) was added. Then, *p*-toluenesulfonyl chloride (0.945 g, 5.0 mmol) was added to the reaction mixture that was stirred at room temperature for 1 h. After evaporation, the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:3) to afford a colorless amorphous solid (1.50 g, 93%, mixture of *syn*- and *anti*-isomers). $^1$H NMR (270 MHz, $CDCl_3$): $\delta$ = 7.90 (d, $J$ = 8.4 Hz, 2 H), 7.28–7.40 (m, 4 H), 7.22 (d, $J$ = 8.4 Hz, 2 H), 2.48 (s, 0.6 H), 2.46 (s, 2.4 H), 2.40 (s, 0.6 H), 2.39 (s, 2.4 H) ppm. $^{13}$C NMR (67 MHz, $CDCl_3$): $\delta$ = 154.1 (q, $^2J_{C,F}$ = 32.1 Hz), 146.2 and 146.1, 142.6 and 142.4, 131.5 and 131.3, 129.9, 129.5, 129.2 and 129.1, 128.8 and 128.4, 124.8, 117.5 (q, $^1J_{C,F}$ = 283.9 Hz), 21.5, 21.3, 21.2 ppm. $^{19}$F NMR (470 MHz, $CDCl_3$): $\delta$ = –61.5, –66.5 ppm. HRMS (ESI): calcd. for $C_{16}H_{15}F_3NO_3S$ 358.0725; found 358.0745.

**2,2,2-Trifluoro-1-(*m*-tolyl)ethanone *O*-Tosyl Oxime (3b):** Treatment of **2b** (1.04 g, 5.1 mmol) was carried out as described above to afford **3b** as a colorless amorphous solid (1.68 g, 92%, mixture of *syn*- and *anti*-isomers). $^1$H NMR (270 MHz, $CDCl_3$): $\delta$ = 7.87–7.92 (m, 2 H), 7.30–7.40 (m, 4 H), 7.16–7.23 (m, 2 H), 2.48 (s, 0.7 H), 2.46 (s, 2.3 H), 2.39 (s, 0.7 H), 2.37 (s, 2.3 H) ppm. $^{13}$C NMR (68 MHz, $CDCl_3$): $\delta$ = 154.3 (q, $^2J_{C,F}$ = 32.3 Hz), 146.3 and 146.1, 138.8 and 138.7, 132.6 and 132.5, 131.6 and 131.3, 129.9 and 129.4, 129.3 and 129.1, 128.7 and 128.6, 127.7, 126.0, 125.5, 117.4 (q, $^1J_{C,F}$ = 283.8 Hz), 21.5, 21.2 and 21.1 ppm. $^{19}$F NMR (470 MHz, $CDCl_3$): $\delta$ = –61.5, –66.9 ppm. HRMS (ESI): calcd. for $C_{16}H_{15}F_3NO_3S$ 358.0725; found 358.0710.

**3-(*p*-Tolyl)-3-(trifluoromethyl)diaziridine (4a):** To liquid $NH_3$ (20 mL) at –78 °C in a sealed tube, tosyloxime **3a** (1.02 g, 2.9 mmol) in $Et_2O$ (5 mL) was added. The reaction mixture was stirred at room temperature for 8 h. After evaporation of $NH_3$ gas, the reaction mixture was partitioned between ether and water. The organic layer was washed with brine, dried with $MgSO_4$ and the solvents evaporated. The residue was subjected to silica-gel column chromatography (EtOAc/hexane, 1:5) to afford 3-(*p*-tolyl)-3-(trifluoromethyl)diaziridine (**4a**) as a colorless amorphous solid (0.502 g, 87%). $^1$H NMR (270 MHz, $CDCl_3$): $\delta$ = 7.50 (d, $J$ = 8.0 Hz, 2 H), 7.22 (d, $J$ = 8.0 Hz, 2 H), 2.76 (d, $J$ = 8.0 Hz, 1 H), 2.38 (s, 3 H), 2.19 (d, $J$ = 8.0 Hz, 1 H) ppm. $^{13}$C NMR (68 MHz, $CDCl_3$): $\delta$ = 140.3, 129.5, 128.9, 128.1, 123.7 (q, $^1J_{C,F}$ = 278.1 Hz), 57.8 (q, $^2J_{C,F}$ = 35.9 Hz), 21.1 ppm. $^{19}$F NMR (470 MHz, $CDCl_3$): $\delta$ = –75.7 ppm. HRMS (ESI): calcd. for $C_9H_{10}F_3N_2$ 203.0796; found 203.0785.

**3-(*m*-Tolyl)-3-(trifluoromethyl)diaziridine (4b):** Treatment of **3b** (1.030 g, 2.9 mmol) was carried out as described above to afford **4b** as a colorless amorphous solid (0.524 g, 90%). $^1$H NMR (270 MHz, $CDCl_3$): $\delta$ = 7.40–7.42 (m, 2 H), 7.31 (t, $J$ = 7.8 Hz, 1 H), 7.25 (d, $J$ = 7.8 Hz, 1 H), 2.77 (d, $J$ = 8.0 Hz, 1 H), 2.38 (s, 3 H), 2.21 (d, $J$ = 8.0 Hz, 1 H) ppm. $^{13}$C NMR (68 MHz, $CDCl_3$): $\delta$ = 138.7, 131.7, 131.0, 128.73, 128.69, 125.3, 123.6 (q, $^1J_{C,F}$ = 278.2 Hz), 58.0 (q, $^2J_{C,F}$ = 35.9 Hz), 21.2 ppm. $^{19}$F NMR (470 MHz, $CDCl_3$): $\delta$ = –76.0 ppm. HRMS (ESI): calcd. for $C_9H_{10}F_3N_2$ 203.0796; found 203.0796.

**3-(*p*-Tolyl)-3-(trifluoromethyl)-3*H*-diazirine (5a). Route A (stepwise conversion through the diaziridine):** Diaziridine **4a** (0.143 g, 0.71 mmol) was dissolved in $CH_2Cl_2$ (5 mL) and triethylamine (0.29 mL), and cooled to 0 °C. Iodine (0.199 g, 0.78 mmol) was added dropwise. The reaction mixture was stirred for 1 h and washed with NaOH (1 M), $H_2O$, and brine. The organic layer was dried with $MgSO_4$, filtered and concentrated. The residue was subjected to silica-gel column chromatography ($CHCl_3$/EtOAc, 19:1) to afford **5a** as a colorless oil (0.114 g, 80%). **Route B (one-pot synthesis):** To liquid $NH_3$ (10 mL) at –78 °C in a sealed tube, tosyloxime **3a** (0.711 g, 2.0 mmol) was added. The reaction was stirred at 80 °C for 11 h. The sealed tube was cooled to –78 °C and the reaction mixture was diluted with $Et_2O$ (50 mL). The sealed tube was warmed to room temperature to remove the ammonia gradually. The organic layer was washed by $H_2O$, brine, dried with $MgSO_4$, and carefully evaporated (0 °C) to afford a colorless oil (0.392 g, 98%). **Route C (one-pot with $LiNH_2$):** To liquid $NH_3$ (5 mL) at –78 °C in a sealed tube, tosyloxime **3a** (0.711 g, 2.0 mmol) and $LiNH_2$ (0.230 g, 10 mmol) was added. The reaction was stirred at room temperature for 12 h. The sealed tube was cooled to –78 °C and the reaction mixture was diluted with $Et_2O$

(50 mL). The sealed tube was warmed to room temperature to remove the ammonia gradually. The organic layer was washed by $H_2O$ (three times), brine, dried with $MgSO_4$, and carefully evaporated (0 °C) to afford a colorless oil (0.388 g, 97%). $^1$H NMR (270 MHz, $CDCl_3$): $\delta$ = 7.20 (d, $J$ = 8.2 Hz, 2 H), 7.08 (d, $J$ = 8.2 Hz, 2 H), 2.36 (s, 3 H) ppm. $^{13}$C NMR (68 MHz, $CDCl_3$): $\delta$ = 140.0, 129.6, 127.8, 126.5, 122.4 (q, $^1J_{C,F}$ = 274.5 Hz), 28.3 (q, $^2J_{C,F}$ = 40.4 Hz), 21.0 ppm. $^{19}$F NMR (470 MHz, $CDCl_3$): $\delta$ = –65.4 ppm. HRMS (ESI): calcd. for $C_9H_8F_3N_2$ 201.0640; found 201.0632.

**3-(*m*-Tolyl)-3-(trifluoromethyl)-3*H*-diazirine (5b). Route A (stepwise conversions through the diaziridine):** Treatment of **4b** (0.143 g, 0.71 mmol) was carried out as described above to afford **5b** (0.094 g, 66%) as a colorless oil. **Route B (one-pot synthesis):** Treatment of **3b** (0.715 g, 2.0 mmol) was carried out as described above to afford **5b** (0.3960 g, 99%) as a colorless oil. **Route C (one-pot with $LiNH_2$):** Treatment of **3b** (0.722 g, 2.0 mmol) was carried out as described to afford **5b** (0.400 g, 99%) as a colorless oil. $^1$H NMR (270 MHz, $CDCl_3$): $\delta$ = 7.28 (t, $J$ = 7.6 Hz, 1 H), 7.22 (d, $J$ = 7.6 Hz, 1 H), 7.01 (d, $J$ = 7.6 Hz, 1 H), 6.97 (s, 1 H), 2.35 (s, 3 H) ppm. $^{13}$C NMR (68 MHz, $CDCl_3$): $\delta$ = 138.9, 130.5, 129.2, 128.8, 127.1, 123.7, 122.4 (q, $^1J_{C,F}$ = 274.5 Hz), 28.3 (q, $^2J_{C,F}$ = 40.4 Hz), 21.2 ppm. $^{19}$F NMR (470 MHz, $CDCl_3$): $\delta$ = –65.2 ppm. HRMS (ESI): calcd. for $C_9H_8F_3N_2$ 201.0640; found 201.0644.

***N*-*tert*-Butyl-6-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]-1,2-benzisothiazole-3-one 1,1-Dioxide (6a):** Chlorosulfonic acid (0.380 mL, 5.7 mmol) was cooled to –20 °C. Compound **5a** (0.115 g, 0.57 mmol) was added dropwise and the reaction mixture was stirred at the same temperature for 1 h, warmed to room temperature, and stirred for 4 h, then poured into ether and ice water. The organic layer was washed with saturated $NaHCO_3$, dried with $MgSO_4$ and filtered. The filtrate was concentrated to afford crude 2-methyl-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzene-1-sulfonyl chloride as a pale yellow oil. $^1$H NMR (270 MHz, $CDCl_3$): $\delta$ = 7.80 (d, $J$ = 1.7 Hz, 1 H), 7.53 (dd, $J$ = 1.1, 8.0 Hz, 2 H), 7.50 (d, $J$ = 8.0 Hz, 2 H), 2.80 (s, 3 H) ppm. The crude residue in $CH_2Cl_2$ (1 mL) was added to $t$BuNH$_2$ (0.130 mL, 1.2 mmol) in $CH_2Cl_2$ (1 mL) at 0 °C. The reaction mixture was stirred at the same temperature for 2 h, and then warmed to room temperature for 3 h. The reaction mixture was washed with HCl (0.1 M) and saturated $NaHCO_3$, dried with $MgSO_4$ and filtered. The filtrate was concentrated to afford *N*-*tert*-butyl-2-methyl-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzenesulfonamide as a pale yellow oil. $^1$H NMR (270 MHz, $CDCl_3$): $\delta$ = 7.84 (d, $J$ = 2.3 Hz, 1 H), 7.35 (d, $J$ = 8.0 Hz, 1 H), 7.29 (dd, $J$ = 2.3, 8.0 Hz, 1 H), 4.45 (s, 1 H), 2.67 (s, 3 H), 1.23 (s, 9 H) ppm. $CrO_3$ (6.0 mg, 0.06 mmol) and acetic anhydride (0.430 mL, 4.5 mmol) were added to *ortho*-periodic acid (1.06 g, 4.6 mmol) in $CH_3CN$ (10 mL). The crude material in a minimum volume in $CH_3CN$ was added at 0 °C. The reaction mixture was stirred at room temperature for 2 d and concentrated. The residue was dissolved in EtOAc and washed with saturated $NaHCO_3$, saturated $Na_2S_2O_3$, and brine. The organic layer was dried with $MgSO_4$, filtered, and concentrated. The crude oil was subjected to silica-gel column chromatography (hexane/$CH_2Cl_2$, 3:1) to afford **6a** as a pale yellow amorphous solid (0.056 g, 28%). UV/Vis ($CH_3OH$): $\lambda_{max}$ [log($\varepsilon$/m$^{-1}$ cm$^{-1}$)] = 290 (850), 340 (300) nm. $^1$H NMR (270 MHz, $CDCl_3$): $\delta$ = 8.03 (d, $J$ = 8.0 Hz, 1 H), 7.64 (s, 1 H), 7.59 (d, $J$ = 8.0 Hz, 1 H), 1.77 (s, 9 H) ppm. $^{13}$C NMR (68 MHz, $CDCl_3$): $\delta$ = 158.8, 138.7, 136.2, 131.9, 128.2, 125.2, 121.4 (q, $^1J_{C,F}$ = 275.5 Hz), 118.4, 61.8, 28.4 (q, $^2J_{C,F}$ = 41.6 Hz), 27.8 ppm. $^{19}$F NMR (470 MHz, $CDCl_3$): $\delta$ = –64.7 ppm. HRMS (ESI): calcd. for $C_{13}H_{13}F_3N_3O_2S$ 348.0630; found 348.0646.

***N*-*tert*-Butyl-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]-1,2-benzisothiazole-3-one 1,1-Dioxide (6b):** Treatment of **5b** (0.136 g, 0.68 mmol) was carried out as described above to afford 2-methyl-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzene-1-sulfonyl chloride. $^1$H NMR (270 MHz, $CDCl_3$): $\delta$ = 8.10 (d, $J$ = 8.6 Hz, 1 H), 7.24 (d, $J$ = 8.6 Hz, 1 H), 7.16 (s, 1 H), 2.80 (s, 3 H) ppm. Treatment of the residue was carried out as described above to afford *N*-*tert*-butyl-2-methyl-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl] benzenesulfonamide. $^1$H NMR (270 MHz, $CDCl_3$): $\delta$ = 8.05 (d, $J$ = 8.6 Hz, 1 H), 7.12 (d, $J$ = 8.6 Hz, 1 H), 7.04 (s, 1 H), 4.44 (s, 1 H), 2.66 (s, 3 H), 1.22 (s, 9 H) ppm. Treatment of the residue with $H_5IO_6$, $CrO_3$, and acetic anhydride as described for **6b** (0.0781 g, 33%). UV/Vis ($CH_3OH$): $\lambda_{max}$ [log($\varepsilon$/m$^{-1}$ cm$^{-1}$)] = 280 (900), 340 (300) nm. $^1$H NMR (270 MHz, $CDCl_3$): $\delta$ = 7.89 (d, $J$ = 8.0 Hz, 1 H), 7.82 (s, 1 H), 7.63 (d, $J$ = 8.0 Hz, 1 H), 1.77 (s, 9 H) ppm. $^{13}$C NMR (68 MHz, $CDCl_3$): $\delta$ = 158.7, 138.5, 135.8, 132.2, 128.3, 122.7, 121.4 (q, $^1J_{C,F}$ = 281.1 Hz), 120.9, 61.8, 28.3 (q, $^2J_{C,F}$ = 38.8 Hz), 27.7 ppm. $^{19}$F NMR (470 MHz, $CDCl_3$): $\delta$ = –64.7 ppm. HRMS (ESI): calcd. for $C_{13}H_{13}F_3N_3O_3S$ 348.0630; found 348.0626.

**6-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]-1,2-benzisothiazole-3-one 1,1-Dioxide (7a):** Compound **6a** (13.5 mg, 39 μmol) was dissolved in TFA (2 mL). The reaction mixture was heated to reflux for 24 h and then concentrated. The residue was dissolved in EtOAc and washed with saturated $NaHCO_3$, HCl (1 M), and brine. The organic layer was dried with $MgSO_4$ and filtered. The filtrate was concentrated, then the residue was recrystallized from EtOAc and hexane at –20 °C to afford **7a** (6.5 mg, 57%) as a colorless amorphous solid. UV/Vis ($CH_3OH$): $\lambda_{max}$ [log($\varepsilon$/m$^{-1}$ cm$^{-1}$)] = 290 (920), 337 (303) nm. $^1$H NMR (270 MHz, $CD_3OD$): $\delta$ = 8.11 (d, $J$ = 8.0 Hz, 1 H), 7.90 (s, 1 H), 7.84 (d, $J$ = 8.0 Hz, 1 H) ppm. $^{13}$C NMR (68 MHz, $CD_3OD$): $\delta$ = 161.3, 142.3, 137.3, 133.8, 130.6, 126.8, 123.1 (q, $^1J_{C,F}$ = 275.1 Hz), 120.4 29.7 (q, $^2J_{C,F}$ = 41.2 Hz) ppm. $^{19}$F NMR (470 MHz, $CD_3OD$): $\delta$ = –66.7 ppm. HRMS (ESI): calcd. for $C_9H_5F_3N_3O_3S$ 292.0004; found 292.0020.

**5-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]-1,2-benzisothiazole-3-one 1,1-Dioxide (7b):** Compound **6b** (13.2 mg, 38 μmol) in TFA (2 mL) was treated in the same manner as described for **6a**. The residue was recrystallized from EtOAc and hexane at –20 °C to afford **7b** as a colorless amorphous solid (6.0 mg, 54%). UV/Vis ($CH_3OH$): $\lambda_{max}$ [log($\varepsilon$/m$^{-1}$ cm$^{-1}$)] = 280 (1005), 340 (280) nm. $^1$H NMR (270 MHz, $CD_3OD$): $\delta$ = 8.11 (d, $J$ = 8.0 Hz, 1 H), 7.86 (s, 1 H), 7.83 (d, $J$ = 8.0 Hz, 1 H) ppm. $^{13}$C NMR (68 MHz, $CD_3OD$): $\delta$ = 161.2, 142.3, 136.5, 134.3, 130.5, 123.9, 123.1, 123.0 (q, $^1J_{C,F}$ = 275.1 Hz), 29.5 (q, $^2J_{C,F}$ = 41.2 Hz) ppm. $^{19}$F NMR (470 MHz, $CD_3OD$): $\delta$ = –62.7 ppm. HRMS (ESI): calcd. for $C_9H_5F_3N_3O_3S$ 292.0004; found 292.0014.

**HPLC Purification for 7a and 7b:** The suspension of **7a** and **7b** in aqueous solution were made alkaline with NaOH (1 M). The sodium salts were subjected to HPLC [Tosoh TSKgel ODS-80 Ts (4.6 × 250 mm), 30% MeOH, 1 mL/min, detection at 215 nm or 350 nm].

**Photolysis of a Methanolic Solution of 7a and 7b:** Methanolic solutions of **7a** and **7b** (1 mM) were irradiated with black light (15 W) at a distance 3 cm. Spectra were recorded at minute-long intervals. The decrease in absorbance at around 350 nm was plotted and used to calculate half-life. $^{19}$F NMR (470 MHz, $CD_3OD$): $\delta$ = –78.0 ppm (from **7a** and **7b**). HRMS (ESI): calcd. for $C_{10}H_9F_3NO_4S$ 296.0199; found 296.0191 (from **7a**), 296.0194 (from **7b**).

**Gustatory-Tasting Effect Assay:** Synthetic compounds **7a** and **7b** were tested in a gustatory-tasting effect assay. This test used $Ca^{2+}$

imaging analysis on cultured cells. Briefly, HEK293 T cells were transfected with $G_{16}$/gust25 and hT1R2-hT1R3 (sweet), hT2R31 and hT2R43 (bitter) constructs. $Ca^{2+}$ imaging analysis was performed essentially as described by Ueda et al.[23] Cells were defined as responding positively when the fluorescence ratio F340/F380 of the calcium sensor increased above 0.15 after the addition of a reagent.

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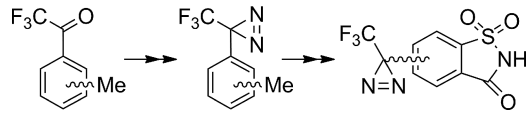


Saccharin is one of the most common artificial sweeteners. Synthesis of photoreactive saccharin derivatives that contain a (trifluoromethyl)diazirinyl moiety at the 5- or 6-position is reported. These saccharin derivatives could be applied for functional analysis of gustatory receptors.



**Keywords:** Synthetic methods / Photoaffinity labeling / Receptors / Nitrogen heterocycles / Saccharin

  