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Optimization of sucrose 1'-position modification with 3-(trifluoromethyl)diazirinyl benzylbromide derivatives for photoaffinity labeling

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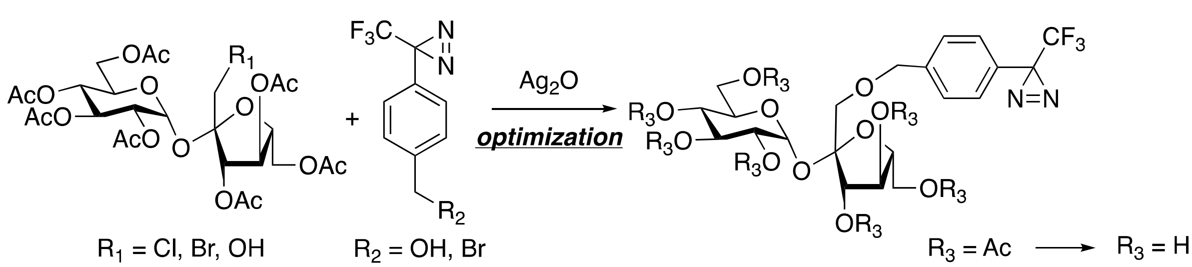
Abstract

Sucrose is well known as naturally occurring sweeteners. Photoreactive sucrose derivative containing 3-(trifluoromethyl)diazirinyl moiety is designed for photoaffinity labeling. As 1'-hydroxyl group of sucrose is well known to be less reactive than other primary alcohols, the optimization of reaction conditions for diazirinyl benzyl bromide derivative at sucrose 1'-postion was examined to elucidate the functional analysis of sweet receptors.



Keywords: Photoaffinity label, diazirine, sucrose, benzylation, functional analysis

Introduction

Humans distinguish gustatory sensations as five basic tastes: bitterness, saltiness, sourness, sweetness, and savouriness. Sweetness is almost universally regarded as a pleasurable experience for human beings. Numerous natural and artificial chemical substances bind to sweet taste receptors, which are G protein-coupled receptors, then recognized as sweetness. Although these sweeteners have various chemical structures, all of the compounds bind to the same sweet taste receptor.[1,2] To study how the receptor can distinguish between sweeteners as well as the structural features of sweeteners that favour the activation of the sweet taste receptor, approaches such as conformational analysis by using X-ray crystallography, NMR spectroscopy, and molecular modelling 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. Functional analysis of sucrose, which is one of the most famous natural sweeteners, is same situation like other sweeteners.

Photoaffinity labeling is a useful biochemical method to explore the structural and functional relationships between low molecular weight bioactive compounds and biomolecules.[3-5] This method is suitable for analyzing biological interactions because it is based on the affinity of bioactive compounds for biomolecules. Various photophores, such as arylazide, benzophenone and phenyldiazirine, are used (Figure 1). 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,[6] 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.[7]

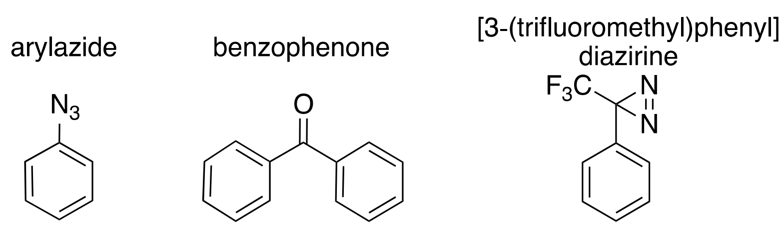


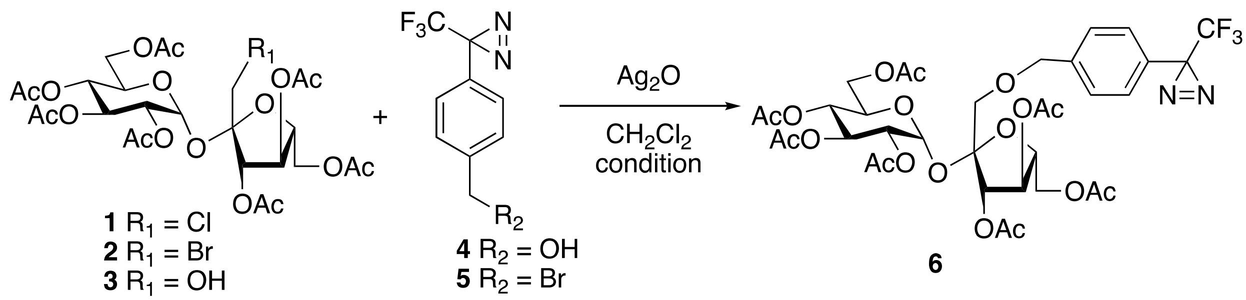
Figure 1. Three major photophores for photoaffinity labelling.

Chemical designs are important for applications of photoaffinity labeling to elucidate the functional analysis of sweetness. We have reported that diazirinyl photophore was appropriate for the analysis of sweet [8-11] and bitter [12,13] receptors. It was found that several sucrose-based oligosaccharides have sweet activities and 1-kestose (GF2), which has an additional fructose, was linked at 1′-position of the fructose unit of sucrose and acted as a sweetener.[14] These results indicated that the 1′-position of the sucrose will be acceptable for substitutions. Although selective esterification of 1′- position was achieved by biotransformation,[15] the ester linkage seems easily hydrolyzed under physiological conditions and metabolism during analysis. On the other hand, benzyl ether linkages seem more stable than esters for functional analysis.[16] Ag_2O-mediated O-benzylation has been used as an indispensable strategy due to its mild conditions, easy postprocessing, and low environmental impact. Nonetheless, many reports suffered the excess use of reagents, preparation of fresh Ag_2O, poor solubility of the substrate, low reaction yields, or long reaction times.[17, 18] We here present the optimization for synthesis of 1′-benzyloxy derivative of sucrose, including trifluoromethyldiazirinyl moiety as the photophore for photoaffinity labeling.

Results and Discussion

To synthesis diazirinyl benzylation of 1'-position of sucrose, 1'-halo substituted heptaacetylsucroses (**1** and **2**) [19] was reacted with diazirinyl benzyl alcohol derivative **4** [20] in the presence of silver oxide at 60 °C in CH_2Cl_2, which is common solvent in carbohydrate synthesis. Although excess amounts of benzyl alcohol derivative (up to 10 eq) and Ag_2O (up to 15 eq) were subjected to the reaction, no desired product was observed at any conditions (Table 1 Entries 1-4). To compare these synthetic conditions, the reaction with 1'-OH acetylsucrose **3**[19,21,22] (0.075 mmol) and diazirinylbenzyl bromide derivative **5** [20] (2 eq) in the presence of Ag_2O (3 eq) was conducted in CH_2Cl_2 (5 mL). The 1'-O-diazirinyl benzyl substituted heptaacetyl sucrose **6** was afford less than 8% at 60 °C (Table 1 Entry 5). These results indicated that the combination of 1'-OH acetyl sucrose **3** and

Table 1. 1'-O-diazirinyl benzylation of sucrose in CH_2Cl_2

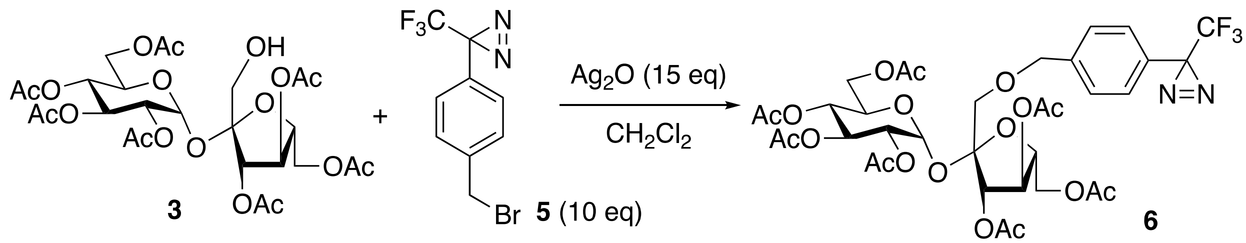


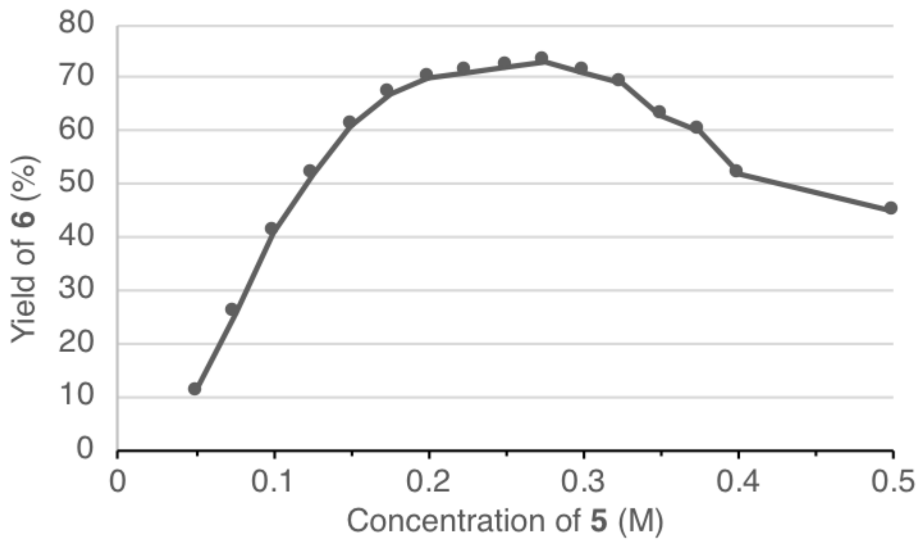
Entry	Sucrose derivative	Diazirine derivative (eq)	Ag_2O (eq)	Temp (°C)	Time (h)	Yield of **6** (%)
1	**1**	**4** (2)	3	60	24	0
2	**1**	**4** (10)	15	60	48	0
3	**2**	**4** (2)	3	60	24	0
4	**2**	**4** (10)	15	60	48	0
5	**3**	**5** (2)	3	60	48	8
6	**3**	**5** (2)	3	70	32	6
7	**3**	**5** (2)	3	50	43	15
8	**3**	**5** (2)	3	40	60	10
9	**3**	**5** (4)	6	50	24	34
10	**3**	**5** (6)	9	50	24	45
11	**3**	**5** (8)	12	50	24	52
12	**3**	**5** (10)	15	50	24	65
13	**3**	**5** (14)	21	50	24	63

Carbohydrate (0.075 mmol) and CH_2Cl_2 (5 ml) were applied each reaction.

diazirinyl benzybromide **5** was suitable to synthesize aimed product. Screening the temperature revealed that the reaction at 50 °C was suitable for the reaction (Table 1 Entry 5-8). The amounts of benzyl bromide derivative and Ag_2O were screened at 50 °C. The excess of both reactants improved the chemical yield drastically (Table 1 Entry 9-13). Chemical yield reached to 65% with 10 equivalent benzyl bromide derivatives **5** and 15 equivalent Ag_2O (Table 1 Entry 12).

Following the equivalents of reactants, we examined the concentration of the reactants in the reaction. The previous reaction was set up to 0.15 M for benzylbromide derivatives. The concentrations of the reactants **5** were applied from 0.05 to 0.5 M of benzylbromide derivative **5**. The chemical yield of **6** was observed 10% at 0.05 M of **5** and increased concentration dependent manner until 0.3 M. The higher concentration of **5** over 0.3 M hampered the effective O-benzylation. (Figure 2)





1’-OH-heptaacetylsucrose **3** (0.075 mmol) was subjected to the benzylation in various concentration of **5** at 50 °C. Ratio of [**3**] : [**5**] : [Ag_2O] was set up to 1 : 10 : 15.

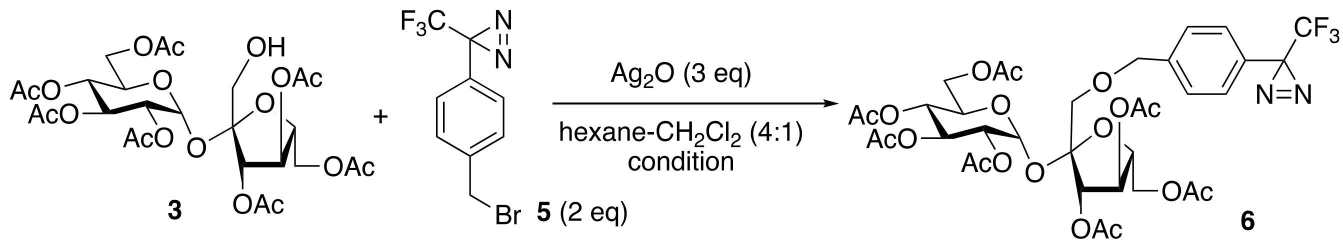
Figure 2. Relationship between reactant **5** concentration and chemical yield of **6** in CH_2Cl_2.

It is not feasible to utilize large excess amounts of diazirinyl compound to synthesis 1’-photophore introduced sucrose. It is essential to develop the reaction condition with less amounts of the reactants. We have recently reported that the benzylation with benzyl halide and Ag_2O in co-solvent system (hexane – CH_2Cl_2 = 4 : 1) acted to prevent hydrolysis of benzyl halide derivatives and to soluble the 1’-OH-heptaacetylsucrose **3**. The typical condition, 2 eq benzyl bromide derivative and 3 eq Ag_2O at 60 °C, was subjected to synthesis **6** at 80% in previous report without optimization.[22] So we examined optimization of the detail condition settings in co-solvent system and summarized the results in Table 2. The benzylation at room temperature afforded best results for chemical yield of **6** (90%), but it took long reaction time (120 h) (Table 2 entry 1). The reaction times were shortened at higher temperature and the chemical yields of **6** reached less than 85% (Table 2 entries 2-4). Like as CH_2Cl_2 solvent, the concentration of the reactant **5** from 0.02 to 0.4 M was also examined at 50 °C for

24 h (Table 2, Entries 3 and 5-9). The concentration of benzyl bromide derivative **5** at around 0.1 M afforded best result to chemical yield of **6**.

Compound **6** was subjected deacetylation with methanolic ammonia at room temperature for 12 h to afford **7** with good yields (Figure 3). Deprotection with sodium methoxide afforded the complex reaction mixture to isolate the product.

Table 2. 1'-*O*-diazirinyl benzylation of sucrose in hexane - CH_2Cl_2



Entry	Temperature (°C)	Concentration of **5** (M)	Time (h)	Yield of **6** (%)
1	rt	0.1	120	90
2	40	0.1	50	87
3	50	0.1	24	85
4	60	0.1	16	80
5	50	0.02	24	61
6	50	0.05	24	72
7	50	0.15	24	82
8	50	0.2	24	78
9	50	0.4	24	74

Carbohydrate (0.05 mmol) and CH_2Cl_2 (5 ml) were applied each reaction.

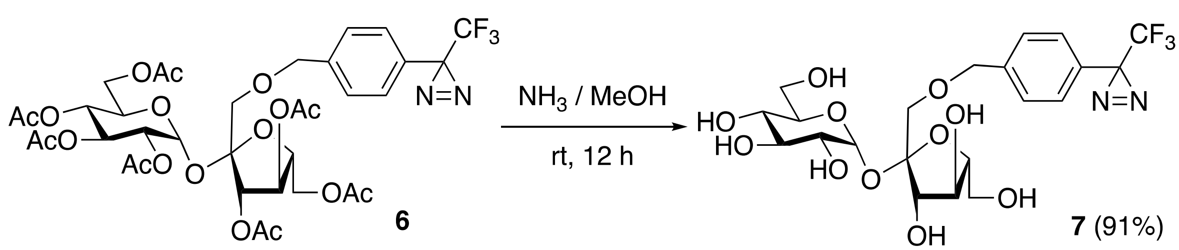


Figure 3. Deacetylation of 1'-modified heptaacetylsucrose.

Conclusions

In summary, optimization for diazirinyl O-benzylation of sucrose 1'-position, which is less reactive hydroxyl group, with silver oxide were examined in CH_2Cl_2 only and co-solvent from hexane and CH_2Cl_2. The chemical yields for the benzylation at 1'-position of sucrose depended on reaction temperature and concentration of reactants. These results will contribute to the reactions with less reactive hydroxyl groups. Further studies for sweetness property for the synthetic compound are in progress.

Experimental Section

General. ^{1}H NMR and ^{13}C NMR were recorded on JEOL EX-270 (^{1}H: 270 MHz, ^{13}C: 67 MHz) and Bruker AMX-500 (^{19}F: 470MHz), using solvent peak as internal reference. The chemical shifts (δ) and coupling constants (J) are expressed in ppm and hertz respectively. FTIR spectra were recorded on a FT-IR 4100 spectrometer (JASCO, Tokyo, Japan). HRMS spectra were obtained with a Waters UPLC ESI-TOF mass spectrometer (Waters, Milford, CT, USA). All reactions were carried out under nitrogen. Other reagents and starting materials were directly used as obtained commercially.

Optimization for equivalent of reactants. Heptaacetylsucrose derivatives (**1**-**3**, 0.075 mmol) in CH_2Cl_2 (5ml) was treated with compound **4**-**5** and Ag_2O, that amounts indicated in the table, in the presence of molecular sieves 4A. The reaction mixture was heated indicated temperature and reaction time, then filtrated with Celite.

The filtrate was concentrated and the residue was subjected silica gel column chromatography to afford colorless oil.

Optimization for concentration of reactants. 1'-OH-Heptaacetylsucrose (**3**, 0.075 mmol), benzyl bromide derivative (**5**, 0.75 mmol), Ag_2O (1.125 mmol) and molecular sieves 4Å was suspended in CH_2Cl_2. The concentration of 5 was set up to indicated value in table. The reaction mixture was heated at 50 °C for 24 h then filtrated with Celite. The filtrate was concentrated and the residue was subjected silica gel column chromatography to afford colorless oil.

1'-(4-(Trifluorodiazirinyl)benzyl)heptaacetylsucrose (6). To a solution of 1'-OH-heptaacetylsucrose (**3**) (64 mg, 0.1 mmol) in cosolvent (n-hexane/CH_2Cl_2, 0.8 mL/0.2 mL) in a glass sealed tube were added diazirinyl benzyl bromide **5** (2.0 eq.), Ag_2O (3.0 eq.), and molecular sieves 4 Å (200 mg), respectively. The reaction mixture was stirred at room temperature in the dark in the presence of N_2. After the reaction was finished, the mixture was filtered by Celite and concentrated, and the residue was purified through a silica gel column chromatography (EtOAc/n-hexane = 3:2) to afford colorless oil. (75.1mg, 90%): $[\alpha]_D$ +53 (c 1 $CHCl_3$). ^{1}H NMR (270 MHz, $CDCl_3$): δ_H 7.39 (2H, d, $^3J_{HH}$ 8.2 Hz), 7.20 (2H, d, $^3J_{HH}$ 8.2 Hz), 5.70–5.68 (2H, m), 5.47–5.39 (2H, m), 5.08 (1H, t, $^3J_{HH}$ 9.3 Hz), 4.86 (1H, dd, $^3J_{HH}$ 9.9, 4.1 Hz), 4.60 (2H, s), 4.32–4.11 (6H, m), 3.60 (1H, d, $^3J_{HH}$ 10.5 Hz), 3.41 (1H, d, $^3J_{HH}$ 10.5 Hz), 2.13 (3H, s), 2.12 (3H, s), 2.09 (3H, s), 2.04 (6H, s), 2.01 (3H, s), 1.94 (3H, s). ^{13}C NMR (68 MHz, $CDCl_3$): δ_C 170.7, 170.6, 170.2, 170.0 (2C), 169.8, 169.6, 139.4, 128.6, 128.0, 126.6, 122.1 (q, $^1J_{CF}$ 275.3 Hz), 104.3, 89.5, 78.4, 75.5, 74.4, 72.7, 70.1 (2C), 69.7, 68.2, 68.1, 63.3, 61.6, 28.2 (q, $^2J_{CF}$ 39.9 Hz), 20.5, 20.4 and 20.2 (7C). ^{19}F NMR (470 MHz, $CDCl_3$): δ_F −65.28. IR (neat) ?: 2930, 1745, 1250. HRMS-ESI (*m/z*) $[M + Na]^+$ calcd for $C_{35}H_{41}N_2O_{18}F_3Na$ 857.2204, found 857.2191.

1'-(4-(Trifluorodiazirinyl)benzyl)sucrose (7). To a solution of 1'-diazirinyl benzylated sucrose derivative **6** (0.2 mmol) in methanol (4 mL) was bubbled NH_3 gas at 0 °C. The mixture was then stirred at room temperature for 12 h. After removal of the solvent, the residue was purified through silica gel column chromatography (EtOAc/MeOH = 5:1) to afford 1'-diazirinyl benzylated sucrose **7** as colorless solid (98.3 mg, 91%): mp 73–75 °C. $[\alpha]_D$ +50 (c 1 CH_3OH). ^{1}H NMR (270 MHz, CD_3OD): δ 7.50 (2H, d, $^3J_{HH}$ 8.4 Hz), 7.25 (2H, d, $^3J_{HH}$ 8.4 Hz), 5.39 (1H, d, $^3J_{HH}$ 3.9 Hz), 4.70 (1H, d, $^3J_{HH}$ 12.7 Hz), 4.63 (1H, d, $^3J_{HH}$ 12.7 Hz), 4.23 (1H, d, $^3J_{HH}$ 8.5 Hz), 4.09–3.97 (1H, m), 3.84–3.57 (9H, m), 3.39 (1H, dd, $^3J_{HH}$ 5.7, 2.6 Hz), 3.35 (1H, s). ^{13}C NMR (68 MHz, CD_3OD): δ 142.2, 129.4, 129.3, 127.7, 123.8 (q, $^1J_{CF}$ 273.7 Hz), 105.2, 94.1, 83.6, 78.7, 75.4, 74.7, 74.4, 73.7, 73.2, 71.4, 71.3, 63.3, 62.3, 29.4 (q, $^2J_{CF}$ 40.5 Hz). ^{19}F NMR (470 MHz, CD_3OD): δ −67.15. IR (neat) ?: 3300, 2900, 1450, 1070. HRMS-ESI (m/z) $[M + Na]^+$ calcd for $C_{21}H_{27}N_2O_{11}F_3Na$ 563.1465, found 563.1488.

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