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SYNTHESIS OF METHOXY-SUBSTITUTED DIAZIRINYL

PHENYLALANINE –A NOVEL PHOTOREACTIVE ASPARTAME

DERIVATIVE FOR FUNCTIONAL ANALYSIS OF SWEET RECEPTORS

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Abstract – Photoreactive phenylalanine derivatives are well known as functional analysis reagents for target biomolecules. The photophores are commonly introduced at 4-position on benzene. Aspartame, which consists of dipeptide L-Asp-L-Phe-OMe, is one of the most utilized artificial sweeteners, and substitution effects on its benzene ring have been reported. Substitution at the 4-position, however, does not maintain its sweetness properties. Trifluoromethyl-diazirine, which is one of the most reliable photophores, was introduced to a different site on phenylalanine and the new photoreactive phenylalanine was converted to aspartame derivatives. The new aspartame derivative had slightly higher sweetness potency than sucrose standard solution.

Elucidation of protein functions on the basis of structure–activity relationships can reveal the mechanisms of homeostasis functions in vivo and is of great interests to scientists. In the human body, many proteins are activated and/or inactivated by ligands to maintain homeostasis. Understanding the mechanism of molecular interactions between small bioactive ligands and proteins is an important step in rational drug design and discovery. Photoaffinity labeling is one of the most familiar approaches for chemical biology analysis.¹ Photoaffinity labeling is also applied to functional analysis of α -amino acid, which represent fundamental compounds of life. It seems suitable to introduce photophores at the 4-position of

phenylalanine, because tyrosine, hydroxyl substituted at this position, is one of natural α -amino acids. On this basis many attempts have been made to construct of photophore-substituted phenylalanine rings by targeting the 4-position. Trifluoromethylphenyldiazirine is one of the most promising photophores for photoaffinity labeling, but the need to construct the 3-(trifluoromethyl)diazirinyl moiety each time hampered researchers for further applications of diazirinyl derivatives. Pioneer work for the preparation of diazirinyl phenylalanine derivatives also used substitutions at 4-position of phenylalanine.² Aspartame (L-Asp-L-Phe-OMe) was one of the most famous artificial sweetener and a relationship between the aryl substitution on phenylalanine and sweet taste has been reported, but it has not been reported the details of binding of aspartame to sweet receptors. Methoxy substitution at the 2-position did not reduce the sweetness and 3-substitution also less influence for sweet potency compared with aspartame.³ On the other hand, substitution at 4-position dramatically decreased the sweetness, and 4-diazirinyl aspartame derivative had no sweet taste.^{2a} These results encouraged us to prepare 2-methoxy-5-trifluoromethyldiazirinyl phenylalanine to maintain sweet potency. In this study, synthesis of a novel methoxy-substituted diazirinyl phenylalanine derivative and its introduction to aspartame skeleton is described (Figure 1).

Figure 1. Substitution effects on aromatic in aspartame

Our synthetic methodologies are based on the initial construction of 4-methoxydiazirine,⁴ followed by deriving⁵ to unknown 2-methoxy-5-trifluoromethyldiazirinyl benzyl bromide derivatives and asymmetric synthesis with α -amino acid skeleton. 3-(4-Methoxyphenyl)-3-(trifluoromethyl)-3*H*-diazirine was synthesized according to our previous paper with minor modifications. 4-Trifluoromethyl anisole (**3**) was converted to *N*-hydroxy oxime with hydroxylamine hydrochloride, tosyloxime with tosyl chloride in acetone - TEA, then diaziridine (**4**) with liquid ammonia. These three steps were achieved without purification, and this produced same yield compared with previous report.⁴ Activated manganese oxide was utilized oxidation of diaziridine (**4**) to diazirine (**5**) with an improvement in the yield. The aldehyde was introduced by a Friedel-Crafts reaction with dichloromethyl methyl ether in the presence of titanium chloride at 0 °C, followed by reduction of aldehyde (**6**) with sodium borohydride in ethanol with good

yield. The hydroxyl group (**7**) was converted to benzyl bromide (**8**) with carbon tetrabromide and triphenylphosphine (Scheme 1).

Scheme 1. Synthesis of diazirinyl benzyl bromide derivatives (**8**). i) $\text{NH}_2\text{OH} \cdot \text{HCl}$, pyridine, $80\text{ }^\circ\text{C}$, 3 h, ii) TsCl , TEA, acetone, $0\text{ }^\circ\text{C}$, 1 h, iii) NH_3 (l), ether, rt, 8 h, 85% (3 steps), iv) MnO_2 , ether, rt, 6 h, 93%, v) Cl_2CHCHO , TiCl_4 , $0\text{ }^\circ\text{C}$, 1 h, 98%, vi) NaBH_4 , EtOH, rt, 4 h, 92%, vii) CBr_4 , Ph_3P , rt, 4 h, 95%

The diazirinyl benzyl bromide (**8**) was subjected asymmetric synthesis with glycine derivative (**9**) in the presence of phosphazene base BTPP (**10**) and catalytic amounts of chinchonidine bromide (**11**) at $-78\text{ }^\circ\text{C}$.⁶ The condensed product (**12**) was slightly decomposed during silica gel-column chromatography, so the reaction mixture was directly subjected to deprotection with TFA to afford new photoreactive L-phenylalanine derivative (**13**) (Scheme 2).

Scheme 2. Synthesis of diazirinyl L-phenylalanine derivative (**13**). i) CH_2Cl_2 , $-78\text{ }^\circ\text{C}$, 8 h, ii) TFA, $0\text{ }^\circ\text{C}$, 2h, 65% (2 steps)

Scheme 3. Synthesis of diazirinyl L-phenylalanine derivatives (**13**). i) SOCl_2 , MeOH, rt, 8 h, 85%, ii) $N\text{-Boc-L-Asp}(\text{OtBu})\text{OSu}$ (**15**), $(i\text{-Pr})_2\text{NEt}$, THF, rt, 8 h, 86%, iii) TFA, $0\text{ }^\circ\text{C}$, 3 h, 97%.

The chirality of the synthetic **13** was checked with chiral HPLC to reveal over 96% ee. The racemate **13** was obtained when the reaction temperature proceeded at $0\text{ }^\circ\text{C}$. The diazirinyl phenylalanine was converted to methyl ester (**14**) with thionyl chloride in methanol. The methyl ester was reacted with $N\text{-Boc-L-Asp}(\text{OtBu})\text{-OSu}$ (**15**) in the presence of diisopropylethylamine at room temperature, followed by deprotection of tBu ester and Boc groups with TFA to afford new photoreactive aspartame derivative (**2**) in good yield (Scheme 3). The synthetic compound **2** was subjected to photoirradiation experiment

with black light to ensure the photoreactivity. The decay around 370 nm was measured in a time-dependent manner (Scheme 4). Analysis with semi-logarithm plot calculated half-life of compound **2** as 1.3 min. The result indicated the compound **2** has enough reactivity for photoaffinity label.

Scheme 4. Photolysis of photoreactive aspartame (**2**) in methanol (1 mM) with black light (100W). UV spectra of the photolysis reaction were recorded every 1 min until 15 min, then every 5 min until 30 min.

The synthesized photoreactive aspartame derivative **2** was subjected to preliminary sensory evaluations using a filter-paper disk.⁶ The synthetic compound had less sweet potency than mother compound aspartame, but a slightly higher potency than sucrose, which is most popular standard for sweet test. It is well known high concentration of sweet compound can also produced bitter taste. (Table 1).

Concentration (mg/mL)	Compound 2	Aspartame	Sucrose
40	- ^[a]	No test	9
10	5	No test	0
1	3	9	0
0.1	1	6	0

Table 1. Preliminary sensory evaluations by filter-paper disk assay (n=9). Numbers indicated that the recognition of sweetness at the indicated concentration. [a] all test person recognized as bitter.

We have developed synthesis of substituted diazirinyl compounds to produce a photoreactive aspartame derivative to improve sweetness potency. The phenyl ring of phenylalanine was substituted with a methoxy and diazirinyl group at 2- and 5- position, respectively. The phenylalanine derivative was prepared by asymmetric synthesis in the presence of a catalytic amount of chinchonidinium salt without decomposition of the diazirinyl ring. The photoreactive phenylalanine derivative was condensed with the aspartic acid derivative to construct the aspartame skeleton. The synthetic aspartame derivatives easily generated carbene and had a sweetness potency that was higher than standard sucrose solution. Further functional analysis of sweet receptor by the synthetic photoreactive reagent is underway.

EXPERIMENTALS

General methods. NMR spectra were measured by JEOL EX-270 and ECA-500 spectrometers. ESI-TOF-MS data were obtained with a Waters UPLC ESI-TOF mass spectrometer. Optical rotation data were obtained with a JASCO DIP-370 polarimeter at 23°C.

3-(4-Methoxyphenyl)-3-(trifluoromethyl)-diaziridine (4)

2, 2, 2-trifluoro-4'-methoxyacetophenone (**3**) (5.00 g, 24.5 mmol) and hydroxylamine hydrochloride (2.78 g, 40.0 mmol) were dissolved in pyridine (50 mL) at room temperature. The reaction mixture was stirred at 70 °C for 3 h and concentrated. The residue was partitioned between AcOEt and 1N HCl. The organic layer was washed with H₂O and brine, dried over MgSO₄, filtrated, and concentrated. The crude oxime was dissolved in acetone (170 mL) and cooled to 0 °C. Triethylamine (8.5 mL, 26.8 mmol) and p-toluenesulfonyl chloride (5.10 g, 26.8 mmol) were added in succession. The reaction mixture was stirred for 1 h at the same temperature, filtrated, and then concentrated. In a pressure tube, the ether solution of the crude tosyl oxime was added to excess liquid ammonia at -78 °C. The reaction mixture was warmed to room temperature and then stirred for 8 h. After excess ammonium gas was removed in a draft chamber, the reaction mixture was concentrated. The residue was purified by column chromatography (CH₂Cl₂) to afford **4** (4.55 g, 85 %) as colorless amorphous mass. The analytical data is identical to a previous report.⁴

3-(4-Methoxyphenyl)-3-(trifluoromethyl)-3*H*-diazirine (5)

The diaziridine **4** (1.88 g, 8.6 mmol) was dissolved in Et₂O (80 mL). Excess MnO₂ (activated) was added until the starting material was consumed completely. The insoluble material was filtrated with Celite. The filtrate was concentrated, and the residue was purified by column chromatography (hexane/CH₂Cl₂ 1/1) to afford diazirine **5** (1.74 g, 94 %) as yellow oil. The analytical data is identical to a previous report.⁴

2-Methoxy-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzaldehyde (6)

To a mixture of **5** (479 mg, 2.22 mmol) and dichloromethyl methyl ether (2.6 mL, 28.86 mmol), TiCl₄ (1.1 mL, 9.99 mmol) was added at 0 °C dropwise. The reaction mixture was stirred at the same temperature for 1 hour and poured into cold water/AcOEt. The organic layer was washed with sat. NaHCO₃, 1 M HCl, and brine, dried over MgSO₄, filtrated, and concentrated. The residue was purified by column chromatography (hexane/AcOEt 8/1) to afford **6** (531 mg, 98 %) as yellow solid. ¹H-NMR (CDCl₃) δ: 10.43 (1H, s), 7.66 (1H, d, *J* = 2.3 Hz), 7.47 (1H, dd, *J* = 2.6, 8.9 Hz), 7.04 (1H, d, *J* = 8.6 Hz), 3.96 (3H, s); ¹³C-NMR (CDCl₃) δ: 188.47, 162.43, 133.91, 127.13, 124.88, 122.01 (q, ¹*J*_{CF} = 274.4 Hz), 121.46, 112.45, 56.00, 27.97 (q, ²*J*_{CF} = 40.2 Hz); ¹⁹F-NMR (CDCl₃) δ: -65.56; FD-MS: [M]⁺ calculated for C₁₀H₇F₃N₂O₂ 244.0460, found 244.0433.

2-Methoxy-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl alcohol (7)

5-Diaziriny-2-methoxybenzaldehyde **6** (113 mg, 0.46 mmol) was dissolved in EtOH and cooled to 0 °C. NaBH₄ (19 mg, 0.51 mmol) was added to the reaction mixture at same temperature. The reaction mixture

was stirred at room temperature for 4 h. After acidification of the reaction mixture with 1 M HCl at 0 °C, the product was extracted with Et₂O. The organic solution was dried over MgSO₄, filtrated, and concentrated. The residue was purified by column chromatography (CH₂Cl₂) to afford **7** (105 mg, 92 %) as yellow solid. ¹H-NMR (CDCl₃) δ: 7.18-7.15 (2H, m), 6.88 (1H, dd, *J* = 2.0, 7.3 Hz), 4.67 (2H, d, *J* = 6.3 Hz), 3.88 (3H, s), 2.12 (1H, t, *J* = 6.4 Hz); ¹³C-NMR (CDCl₃) δ: 158.33, 129.98, 127.66, 126.79, 122.26 (q, ¹*J*_{CF} = 276.6 Hz), 120.97, 110.47, 61.38, 55.55, 30.80 (q, ²*J*_{CF} = 40.1 Hz); ¹⁹F-NMR (CDCl₃) δ: -65.55; FD-MS: [M]⁺ calculated for C₁₀H₉F₃N₂O₂ 246.0616, found 246.0619.

2-Methoxy-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl bromide (8)

To a solution of **7** (91 mg, 0.37 mmol) and CBr₄ (246 mg, 0.74 mmol) in dry CH₂Cl₂, Ph₃P (194 mg, 0.74 mmol) was slowly added at 0 °C. The reaction mixture was stirred at room temperature for 4 h and concentrated. The residue was purified by column chromatography (hexane/CH₂Cl₂ 1/1) to afford **8** (108 mg, 95 %) as yellow oil. ¹H-NMR (CDCl₃) δ: 7.19-7.17 (2H, m), 6.89 (1H, d, *J* = 8.6 Hz), 4.50 (2H, s), 3.91 (3H, s); ¹³C-NMR (CDCl₃) δ: 158.46, 129.14, 128.79, 127.05, 122.16 (q, ¹*J*_{CF} = 274.9 Hz), 121.06, 111.29, 55.86, 28.07 (q, ²*J*_{CF} = 39.7 Hz), 27.64; ¹⁹F-NMR (CDCl₃) δ: -65.50; ESI-TOF-MS: [M+H]⁺ calculated for C₁₀H₈BrF₃N₂O 307.9772, found 307.9743.

L-2-Methoxy-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenylalanine (13)

The diazirinyl benzyl bromide derivative **8** (97 mg, 0.32 mmol), tert-butylglycinate benzophenone imine **9** (102 mg, 0.35 mmol), and *O*-allyl-*N*-9-anthracenylmethylcinchonidium bromide **11** (30 mg, 0.050 mmol) was dissolved in dry CH₂Cl₂ and cooled to -78 °C under N₂. To the solution was slowly added phosphazene base BTPP (1-*tert*-Butyl-2,2,2-tri(1-pyrrolidinyl)phosphazene) **10** (125 μL, 0.47 mmol) followed by stirring for 8 h. After removal of the solvent, the residue was dissolved in Et₂O. The organic layer was washed with water twice and brine, dried over MgSO₄, and concentrated to afford crude **12**. The crude **12** was dissolved in TFA (2 mL) at 0 °C. The reaction mixture was stirred at room temperature for 2 h. After concentration, the residue was partitioned between Et₂O and 1 M HCl, and the organic layer was extracted with 1 M HCl three times. The aqueous solutions were combined and concentrated. The residue was purified by column chromatography on washed silica gel (MeCN/MeOH/H₂O 8/1/1) to afford diazirinyl phenylalanine **13** (86 mg, 65% for 2 steps) as white solid. ¹H-NMR (CD₃OD) δ: 7.28 (1H, dd, *J* = 1.5, 8.7 Hz), 7.16-7.03 (2H, m), 4.24 (1H, t, *J* = 6.4 Hz), 3.91 (3H, s), 3.40-3.33 (2H, m), 3.12 (1H, dd, *J* = 7.3, 14.2 Hz); ¹³C-NMR (CD₃OD) δ: 171.08, 160.68, 130.80, 129.52, 125.01, 123.70 (q, ¹*J*_{CF} = 273.2 Hz), 121.86, 112.67, 56.27, 53.86, 32.57, 29.23 (q, ²*J*_{CF} = 40.2 Hz); ¹⁹F-NMR (CD₃OD) δ: -67.29 ppm. ESI-TOF-MS: [M+H]⁺ calculated for C₁₂H₁₃F₃N₃O₃ 304.0909, found 304.0894; [α]_D -9.1 (c 1.0 MeOH)

L-2-Methoxy-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenylalanine methyl ester (14)

The diazirinyl phenylalanine **13** (56 mg, 0.13 mmol) was dissolved in MeOH and cooled to 0 ° C. To the solution SOCl₂ (91 μ L, 1.04 mmol) was slowly added. The reaction mixture was stirred at room temperature for 8 h and concentrated. The residue was dissolved in AcOEt, washed with sat. NaHCO₃, and dried over MgSO₄. After removal of the solvent, the residue was purified by column chromatography (CH₂Cl₂/MeOH 19/1) to afford **14** (34 mg, 85 %) as colorless amorphous mass. ¹H-NMR (CDCl₃) δ: 7.09 (1H, dd, *J* = 1.6, 8.2 Hz), 6.96 (1H, d, *J* = 1.6 Hz), 6.85 (1H, d, *J* = 8.6 Hz), 3.83 (3H, s), 3.77 (1H, t, *J* = 6.6 Hz), 3.69 (3H, s) 3.06 (1H, dd, *J* = 5.9, 13.2 Hz) 2.87 (1H, dd, *J* = 7.9, 13.5 Hz); ¹³C-NMR (CDCl₃) δ: 175.37, 160.59, 130.46, 128.60, 127.34, 123.74 (q, ¹*J*_{CF} = 273.8 Hz), 121.49, 112.26, 56.20, 54.88, 52.47, 36.02, 29.25 (q, ²*J*_{CF} = 40.2 Hz); ¹⁹F-NMR (CDCl₃) δ: -65.49; ESI-TOF-MS: [M+H]⁺ calculated for C₁₃H₁₅F₃N₃O₃ 318.1066, found 318.1086. [α]_D + 22.3 (c 1.0 MeOH).

***L*-N-tert-Butoxycarbonyl-β-tert-butylaspartyl-2-methoxy-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]-*L*-phenylalanine methyl ester (**16**)**

The diazirinyl phenylalanine methyl ester **14** (51 mg, 0.16 mmol) and *L*-N-Boc-β-tert-butyl-aspartic acid succinimide ester **15** (73 mg, 0.19 mmol) was dissolved in THF and cooled to 0 °C. To the mixture, DIPEA (56 μ L, 0.32 mmol) was added, followed by stirring at room temperature for overnight. After the reaction, the solutions were concentrated in vacuo. The residue was purified by column chromatography (hexane/AcOEt 2/1) to afford protected diairiny aspartame **16** (81 mg, 86%) as colorless amorphous mass. ¹H-NMR (CDCl₃) δ: 7.22 (1H, d, *J* = 7.3 Hz), 7.14 (1H, dd, *J* = 2.0, 8.6 Hz), 6.93 (1H, d, *J* = 8.9 Hz), 5.58 (1H, d, *J* = 6.9 Hz), 4.71 (1H, dd, *J* = 6.3, 13.5 Hz), 4.43 (1H, br s), 3.90 (3H, s), 3.67 (3H, s), 3.09 (2H, d, *J* = 6.6 Hz), 2.83 (1H, dd, *J* = 4.3, 17.1 Hz), 2.53 (1H, dd, *J* = 6.9, 17.1 Hz), 1.43 (9H, s), 1.39 (9H, s); ¹³C-NMR (CDCl₃) δ: 171.34, 171.18, 170.74, 158.68, 155.23, 129.46, 129.29, 125.55, 122.19 (q, ¹*J*_{CF} = 274.3 Hz), 120.95, 110.78, 81.58, 80.19, 55.68, 53.08, 52.10, 50.67, 37.67, 32.14, 28.23, 27.90 (q, ²*J*_{CF} = 36.9 Hz); ¹⁹F-NMR (CD₃OD) δ: -65.52 ppm; ESI-TOF-MS: [M+H]⁺ calculated for C₂₆H₃₆F₃N₄O₈ 589.2485, found 589.2470. [α]_D -7.5 (c 1.0 MeOH).

***L*-Aspartyl-2-methoxy-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]-*L*-phenylalanine methyl ester (**2**)**

The Boc- and tBu- protected diazirinyl aspartame **16** (40 mg, 0.068 mmol) was dissolved in TFA (2 mL) at 0 °C. The mixture was stirred at room temperature for 3 h. After concentration, the residue was partitioned between diethyl ether and 1 M HCl, and the organic layer was extracted with 1 M HCl three times. After concentration of aqueous layer, the residue was purified by column chromatography on washed silica gel (MeCN/MeOH/H₂O 8/1/1) to afford diazirinyl aspartame **2** (36 mg, 97%) as a colorless amorphous mass. ¹H-NMR (CD₃OD) δ: 7.15 (1H, dd, *J* = 2.3, 8.6 Hz), 7.01 (1H, d, *J* = 8.9 Hz), 6.94 (1H, d, *J* = 2.0 Hz), 4.69 (1H, dd, *J* = 6.4, 8.7 Hz), 4.07 (1H, dd, *J* = 4.6, 8.6 Hz), 3.84 (3H, s), 3.61 (3H, s), 3.18 (1H, dd, *J* = 6.3, 13.5 Hz), 2.98-2.86 (2H, m), 2.76 (1H, dd, *J* = 8.6, 18.1 Hz); ¹³C-NMR

(CD₃OD) δ : 172.91, 169.37, 160.52, 130.29, 128.72, 127.22, 123.70 (q, $^1J_{CF}$ = 273.8 Hz), 121.50, 112.32, 64.21, 56.30, 53.85, 52.77, 50.89, 36.22, 33.28, 29.23 (q, $^2J_{CF}$ = 40.8 Hz); ^{19}F -NMR (CD₃OD) δ : -67.26; ESI-TOF-MS: $[\text{M}+\text{H}]^+$ calculated for C₁₇H₂₀F₃N₄O₆ 433.1335, found 433.1342; $[\alpha]_D$ +2.9 (c 1.0 MeOH).

Photolysis of compound 2

A 1 mM methanolic solution of the diazirinyl aspartame **2** was placed in a quartz cuvette. Photolysis was performed with black-light (100 W). Spectra were measured after each minute, and then the half-life was calculated from the decrements of the absorbance around 370 nm.

Preliminary sensory evaluations by filter-paper disk

Nine healthy test persons aged between 20 and 28 years were recruited from authors' laboratory members. Onto filter-paper discs with a width of 5 mm, 10 μL solutions were adsorbed, each at 40, 10, 1 and 0.1 mg/mL concentrations, and the discs were put on the same area of the tongue. The lowest concentration (0.1 mg/mL) was applied first, followed by the next level of concentration, and so on, until the test person recognized a sweet taste. Forty milligrams per milliliter of sucrose is well known as standard for sweet flavor.

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REFERENCES

1. a) J. Brunner, *Annu. Rev. Biochem.*, 1993, **62**, 483; b) Y. Hatanaka, H. Nakayama, Y. Kanaoka, *Rev. Heteroatom Chem.*, 1996, **14**, 213; c) G. Dormán, G. D. Prestwich, *Trends Biotechnol.*, 2000, **18**, 64; d) T. Tomohiro, M. Hashimoto, Y. Hatanaka, *Chem. Records*, 2005, **5**, 385; e) M. Hashimoto, Y. Hatanaka, *Eur. J. Org. Chem.*, 2008, 2513; f) L. Dubinsky, B. P. Krom, M. M. Meijler, *Bioorg. Med. Chem.*, 2012, **20**, 554.
2. a) M. Nassal, *J. Am. Chem. Soc.*, 1984, **106**, 7540; b) L. B. Shih, H. Bayley, *Anal. Biochem.*, 1985, **144**, 132; c) G. Baldini, B. Martoglio, A. Schachenmann, C. Zugliani, J. Brunner, *Biochemistry*, 1988, **27**, 7951; d) C. W. G. Fishwick, J. M. Sanderson, J. B. C. Findlay, *Tetrahedron Lett.*, 1994, **35**, 4611; e) H. Nakashima, M. Hashimoto, Y. Sadakane, T. Tomohiro, Y. Hatanaka, *J. Am. Chem. Soc.*, 2006, **128**, 15092. f) K. Masuda, A. Koizumi, T. Misaka, Y. Hatanaka, K. Abe, T. Tanaka, M. Ishiguro, M. Hashimoto, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 1081.
3. M. Kawai, M. Chorev, J. Marin-Rose, M. Goodman, *J. Med. Chem.*, 1980, **23**, 420.

4. a) Y. Hatanaka, M. Hashimoto, H. Kurihara, H. Nakayama, Y. Kanaoka, Yuichi, *J. Org. Chem.*, 1994, **59**, 383; b) M. Hashimoto, Y. Kato, Y. Hatanaka, *Tetrahedron Lett.*, 2006, **47**, 3391.
5. M. Hashimoto, Y. Kanaoka, Y. Hatanaka, *Heterocycles*, 1997, **46**, 119.
6. E. J. Corey, F. Xu, M. C. Noe, *J. Am. Chem. Soc.*, 1997, **119**, 12414.
7. K. Berling, J. Knutsson, A. Rosenblad, M. Unge, *Acta Oto-Laryngologica*, 2011, **131**, 488.