Stereoselective Synthesis of (*E*)- and (*Z*)-β-Bromostyrene Containing Trifluoromethyldiazirine for Photoaffinity Labeling

Makoto HASHIMOTO,*[a] Tomoko KOMANO,[a] Kensuke NABETA,[a] and Yasumaru HATANAKA[b]

[a] *Department of Agricultural and Life Science, Obihiro University of Agriculture and Veterinary Medicine; Inada-cho, Obihiro Hokkaido 080–8555, Japan: and* [b] *Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University; 2360 Sugitani, Toyama 930–0194 Japan.*

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Summary **A convenient procedure for the synthesis of (*E*)- and (*Z*)-β-bromostyrene containing trifluoromethyldiazirine is described, which involves the stereoselective reduction of the corresponding trifluoromethyldiazirinyl β,β-dibromostyrene without damaging the photophor. The synthetic route easily introduced deuterium, which can be utilized for the detection of a photolabeled component by MS spectrometry, after construction of a trifluoromethyldiazirinyl skeleton.**

Key words diazirine; photoaffinity label; stable isotope; hydrogenolysis

Photoaffinity labeling is a powerful method in the study of biological structures and functions.[1,2] The method is suitable for the analysis of biological interactions based on the affinity of ligands to receptors. Various photophores, such as phenyldiazirine, arylazide, and benzophenone have been used. Comparative irradiation studies of these three photophors in living cells suggested that a carbene precursor (3-trifluoromethyl)phenyldiazirine is the most promising for *in vivo* applications.[3] However, the complicated synthesis of the diazirinyl ring has resulted in fewer applications for the diazirines in biomolecular studies than other photophors. We have already established the first versatile approach to simplify the time-consuming methods currently used for diazirine synthesis, by starting from only a few simple diazirines, *e.g. m*-methoxy-phenyldiazirine, which can be synthesized on a large scale.[4] Direct substitution on phenyldiazirines (post-functional modification) gave a family of substituted phenyldiazirines without the need to repeat all the steps of diazirine synthesis from the beginning.[5—7] Some tags are needed on photophor to detect photolabeled components. Radiolabeled photophors are used for high sensitive detection. But the synthesis and handling of radiolabeled compounds is quite complicated. We have attempted to resolve these difficulties with a combination of avidin-biotin systems (photoaffinity biotinylation).[8,9] The recent development of mass spectrometry has enabled us to identify important structures in target biomolecules.[10] Elucidation of the different mass numbers, derived from a mixture of unlabeled and stable-isotope labeled photophors, may be useful to identify a photolabeled ligand-biomolecule complex on the MS spectrum. However, to the best of our knowledge, a few protocols for the synthesis of a stable-isotope labeled diazirinyl photophor have been reported.[11] β-Bromostyrenes are extremely useful intermediates in organic synthesis. Their use as precursors to vinyl anions,[12] and as coupling partners in a wide range of transition metal-mediated coupling reactions,[13] has stimulated a great deal of interest. There have been several reports about the stereoselective synthesis of (*E*)- and (*Z*)-β-bromostyrene isomers,[14—18] but the application of these synthetic methods to diazirinyl compounds was not reported. The simple synthesis of diazirinyl (*E*)- and (*Z*)-β-bromostyrene have been required in the stereoselective preparation of conjugated photoreactive polyene and enynes. The conventional synthesis of β-bromostyrene isomers requires reduction steps which may also reduce diazirinyl nitrogen–nitrogen double bond. Here, we would like to describe the first effective, stereoselective synthesis of diazirinyl β-bromostyrenes from diazirinyl aldehyde **1a**, **b**,[5,6] and deuterium introductions to diazirinyl β-bromostyrenes, with commercially available reagents.

To achieve the stereoselective synthesis of diazirinyl β-bromostyrene, diazirinyl β,β-dibromostyrenes, **2a**, **b** may be one of the best precursors. The diazirinyl benzaldehydes **1a**, **b**, which were prepared by the Friedel–Crafts reaction of the corresponding diazirine, were converted to **2a**, **b**, with carbon tetrabromide and triphenylphosphine in dichloromethane at room temperature.[19] β,β-Dibromostyrene derivatives are also one of precursors to prepare phenylethyn derivatives.[20] The compounds **2a**, **b**, were smoothly converted to the alkynyl bromides **3a**, **b**, with DBU in DMSO at room temperature, without damage to the diazirinyl ring. Stereoselective synthesis of (*E*)-bromostyrene was reported by Kuang *et al.*[14] They reported microwave irradiation in the presence of sodium ethoxide and diethyl phosphite was very effective. We found that microwave irradiation in the presence of sodium methoxide caused decomposition of the diazirinyl skeleton. But the reactions proceeded at room temperature in the same way without microwave irradiation. During the investigation of optimal conditions, undesired alkynyl bromide **3a**, **b** was synthesized when sodium methoxide was added before the addition of diethyl phosphite. To prevent this side reaction, sodium methoxide and diethyl phosphite should be mixed first, then the β,β-dibromostyrene derivatives should be added. The isomer, (*Z*)-β-bromostyrene, was calculated at less than 3% from NMR analysis. The stereoselective synthesis of (*Z*)-β-bromostyrene was achieved by a slight modification of the method reported by Uenishi *et al.*[17,18] A large excess of $(n\text{-Bu})_3$SnH (3.6 eq) promoted further hydrogenolysis to diazirinyl β-bromostyrene to produce diazirinyl styrene as a byproduct (over 40%). NMR studies of the reaction mixture revealed that the starting materials, **2a** or **2b**, converted to **5a** or **5b** within 50 min, and few amount of diazirinyl styrene (<1%) was produced in the presence of 1.2 eq of $(n\text{-Bu})_3$SnH. (*E*)-iso-

* To whom correspondence should be addressed. e-mail: hasimoto@obihiro.ac.jp

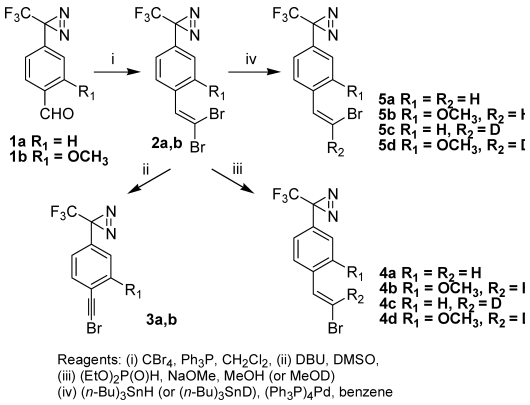

Fig. 1. Stereoselective Synthesis of Diazirinyl (*E*)- and (*Z*)-*β*-Bromostyrene

mer was calculated at less than 3% from NMR analysis. Stereoselective hydrogenolysis was applied to introduce deuterium by commercially available reagents. It has been reported that hydrogen of solvent, has been utilized for the synthesis of (*E*)-*β*-bromostyrene. The reaction, performed with unlabeled diethylphosphonate in labeled CD_3OD solution, produced deuterated compound **5c** at a rate of over 90%. On the other hand, deuterated (*Z*)-compound **5d** was obtained with $(n\text{-Bu})_3SnD$ and unlabeled Pd catalysis in unlabeled solvent at a rate of over 95% deuterium incorporation.

Experimental

IR spectra were recorded with a JASCO IR spectrophotometer. NMR spectra were recorded on a JEOL ECA-500 instrument (^1H 500 MHz, ^{13}C 125 MHz), and chemical shifts are expressed in ppm downfield from tetramethylsilane. Mass spectra were recorded on JEOL JNM-LA400 mass spectrometers. CD_3OD and $(n\text{-Bu})_3SnD$ were purchased from Cambridge Isotope Laboratories and Aldrich, respectively. All other solvents were reagent grade and distilled using the appropriate methods.

General Procedure for the Synthesis of Diazirinyl *β*,*β*-Dibromostyrene (2) To an ice cold stirred solution of **1a** (0.443 g, 2.07 mmol) and CBr_4 (1.5 eq), in dry CH_2Cl_2, was added PPh_3 (3.0 eq) in CH_2Cl_2. The reaction mixture was stirred at room temperature for 2 h and concentrated. The residue was purified with silica gel column chromatography (hexane) to give colorless oil.

β,*β*-Dibromo-4-(3-trifluoromethyl-3*H*-diazirin-3-yl)styrene (**2a**): Yield 97%, ^1H-NMR ($CDCl_3$) δ: 7.57 (d, 2H, J=8.3 Hz), 7.47 (s, 1H), 7.18 (d, 2H, J=8.3 Hz), ^{13}C-NMR ($CDCl_3$) δ: 136.55, 135.56, 129.51, 129.24, 128.70, 126.40, 121.97, (q, J_{CF}=274.6 Hz), 91.61, 28.36 (q, $^2J_{CF}$=40.8 Hz), MS (EI) 368, 370, 372, HR-MS (EI) Calcd for $C_{10}H_5Br_2F_3N_2$ (M^+) 367.8772, Found 367.8765.

β,*β*-Dibromo-2-methoxy-4-(3-trifluoromethyl-3*H*-diazirin-3-yl)styrene (**2b**): Yield 85%, ^1H-NMR ($CDCl_3$) δ: 7.72 (d, 1H, J=8.0 Hz), 7.54 (s, 1H), 6.80 (d, 1H, J=8.0 Hz), 6.58 (s, 1H), 3.84 (s, 3H), ^{13}C-NMR ($CDCl_3$) δ: 132.1, 131.4, 130.7, 129.5, 125.9, 122.0 (q, J_{CF}=274.7 Hz), 118.4, 109.9, 108.3, 91.4, 55.6, 28.4 (q, $^2J_{CF}$=40.8 Hz), MS (EI) 398, 400, 402, HR-MS (EI) Calcd for $C_{11}H_7Br_2F_3N_2O$ (M^+) 397.8877, Found 397.8868.

General Procedure for the Synthesis of Diazirinyl 1-Bromo-2-phenylethyne (3) Compound **2** was dissolved in DMSO. DBU (3 eq) was added to the solution. The reaction mixture was stirred at room temperature for 1 h, then purified with silica gel column chromatography (hexane) to afford a colorless oil.

1-Bromo-2-[4-(3-trifluoromethyl-3*H*-diazirin-3-yl)phenyl]ethyne (**3a**): Yield 84%, ^1H-NMR ($CDCl_3$) δ: 7.46 (d, 2H, J=7.9 Hz), 7.12 (d, 2H, J=7.9 Hz), 1.55 (s, 1H), ^{13}C-NMR ($CDCl_3$) δ: 132.3, 129.4, 126.3, 124.2, 121.9 (q, J_{CF}=274.7 Hz), 78.9, 52.5, 28.4 (q, $^2J_{CF}$=40.8 Hz), MS (EI) 288, 290, HR-MS (EI) Calcd for $C_{10}H_4BrF_3N_2$ (M^+) 287.9510, Found 287.9520 IR (neat) 2206 cm^{-1}.

1-Bromo-2-[2-methoxy-4-(3-trifluoromethyl-3*H*-diazirin-3-yl)-phenyl]ethyne (**3b**): Yield 68%, ^1H-NMR ($CDCl_3$) δ: 7.42 (d, 1H, J=8.0 Hz), 6.74 (d, 1H, J=8.0 Hz), 6.57 (s, 1H), 3.88 (s, 3H), ^{13}C-NMR ($CDCl_3$) δ: 160.6, 134.0, 130.9, 125.2 (q, J_{CF}=274.7 Hz), 120.0, 111.8, 111.4, 110.6, 76.4, 55.8, 28.4 (q, $^2J_{CF}$=40.8 Hz), MS (EI) 318, 320, HR-MS (EI) Calcd for $C_{11}H_6BrF_3N_2O$ (M^+) 317.9616, Found 317.9608, IR (neat) 2199.42 cm^{-1}.

General Procedure for the Synthesis of Diazirinyl (*E*)-*β*-Bromostyrene (4) Compound **2** (30.0 mg, 0.081 mmol), in EtOH (0.25 ml), was added to a solution of diethyl phosphite (1.1 eq) and sodium methoxide solution (28%, 1.1 eq) in EtOH. The reaction mixture was stirred at room temperature for 1 h. After concentration, the residue was purified with silica gel column chromatography (hexane) to afford colorless oil.

(*E*)-*β*-Bromo-4-(3-trifluoromethyl-3*H*-diazirin-3-yl)styrene (**4a**): Yield 96%, ^1H-NMR ($CDCl_3$) δ: 7.32 (d, 2H, J=8.3 Hz), 7.14 (d, 2H, J=8.3 Hz), 7.09 (d, 1H, J=14.2 Hz), 6.84 (d, 1H, J=14.2 Hz). ^{13}C-NMR ($CDCl_3$) δ: 137.1, 136.1, 135.4, 128.9, 126.9, 126.3, 122.0 (q, J_{CF}=274.8 Hz), 110.4, 108.4, 28.4 (q, $^2J_{CF}$=40.8 Hz), MS (EI) 290, 292, HR-MS (EI) Calcd for $C_{10}H_6BrF_3N_2$ (M^+) 289.9666, Found 289.9657.

(*E*)-*β*-Bromo-2-methoxy-4-(3-trifluoromethyl-3*H*-diazirin-3-yl)styrene (**4b**): Yield 72%, ^1H-NMR ($CDCl_3$) δ: 7.90 (d, 1H, J=8.0 Hz), 7.22 (d, 1H, J=14.3 Hz), 6.97 (d, 1H, J=14.3 Hz), 6.75 (d, 1H, J=8.0 Hz), 6.59 (s, 1H), 3.86 (s, 3H). ^{13}C-NMR ($CDCl_3$) δ: 156.6, 132.0, 130.0, 128.2, 126.3, 122.0 (q, J_{CF}=274.7 Hz), 118.9, 109.9, 108.7, 55.6, 28.4 (q, $^2J_{CF}$=40.8 Hz). MS (EI) 320, 322, HR-MS (EI) Calcd for $C_{11}H_8BrF_3N_2O$ (M^+) 319.9772, Found 319.9777.

(*E*)-*β*-Bromo-*β*-deutrium-4-(3-trifluoromethyl-3*H*-diazirin-3-yl)styrene (**4c**): Yield 89%, ^1H-NMR ($CDCl_3$) δ: 7.32 (d, 2H, J=8.3 Hz), 7.14 (d, 2H, J=8.3 Hz), 7.09 (d, 1H, J=14.2 Hz), 6.84 (d, 1H, J=14.2 Hz). ^{13}C-NMR ($CDCl_3$) δ: 137.1, 136.1, 135.4, 128.9, 126.9, 126.3, 122.0 (q, J_{CF}=274.8 Hz), 110.4, 108.4 (t, J_{CD}=28.8 Hz), 28.4 (q, $^2J_{CF}$=40.8 Hz), MS (EI) 291, 293, HR-MS (EI) Calcd for $C_{10}H_5DBrF_3N_2$ (M^+) 290.9728, Found 290.9734.

(*E*)-*β*-Bromo-*β*-deutrium-2-methoxy-4-(3-trifluoromethyl-3*H*-diazirin-3-yl)styrene (**4d**): Yield 56%, ^1H-NMR ($CDCl_3$) δ: 7.90 (d, 1H, J=8.0 Hz), 7.25 (1H, s), 6.75 (d, 1H, J=8.0 Hz), 6.59 (1H, s), 3.86 (3H, s). ^{13}C-NMR ($CDCl_3$) δ: 156.6, 131.8, 129.9, 128.2, 126.2, 122.0 (q, J_{CF}=274.7 Hz), 118.9, 109.7 (t, J_{CD}=31.2 Hz), 108.7, 55.6, 28.4 (q, $^2J_{CF}$=40.8 Hz), MS (EI) 321, 323, HR-MS (EI) Calcd for $C_{11}H_7DBrF_3N_2O$ (M^+) 320.9834, Found 320.9840.

General Procedure for the Synthesis of Diazirinyl (*Z*)-*β*-Bromostyrene (5) To a stirred solution of compound **2** (23.8 mg, 0.06 mmol), in dry benzene (0.5 ml), was added $Pd(PPh_3)_4$ (4 mol%) and $(n\text{-Bu})_3SnH$ (2.3 eq), successively, and the mixture was stirred at room temperature for 50 min. After the reaction was completed, it was diluted with hexane and washed with water and brine. The hexane extract was dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified with silica gel column chromatography (hexane) to afford colorless oil.

(*Z*)-*β*-Bromo-4-(3-trifluoromethyl-3*H*-diazirin-3-yl)styrene (**5a**): Yield 87%, ^1H-NMR ($CDCl_3$) δ: 7.71 (d, 1H, J=7.9 Hz), 7.19 (d, 1H, J=7.9 Hz), 7.06 (d, 1H, J=8.3 Hz), 6.52 (d, 1H, J=8.3 Hz). ^{13}C-NMR ($CDCl_3$) δ: 136.2, 131.2, 129.2, 128.9, 126.2, 122.0 (d, J_{CF}=274.7 Hz), 108.3, 28.4 (d, $^2J_{CF}$=39.6 Hz), MS (EI) 290, 292, HR-MS (EI) Calcd for $C_{10}H_6BrF_3N_2$ (M^+) 289.9666, Found 289.9660.

(*Z*)-*β*-Bromo-2-methoxy-4-(3-trifluoromethyl-3*H*-diazirin-3-yl)styrene (**5b**): Yield 83%, ^1H-NMR ($CDCl_3$) δ: 7.90 (d, 1H, J=8.0 Hz), 7.22 (d, 1H, J=8.0 Hz), 6.82 (d, 1H, J=8.0 Hz), 6.52 (d, 1H, J=8.0 Hz), 6.61 (s, 1H), 3.84 (3H, s). ^{13}C-NMR ($CDCl_3$) δ: 156.96, 130.29, 129.83, 126.95, 125.49, 122.05 (q, J_{CF}=274.7 Hz), 118.23, 108.66, 108.21, 55.63, 28.4 (q, $^2J_{CF}$=40.8 Hz), MS (EI) 320, 322, HR-MS (EI) Calcd for $C_{11}H_8BrF_3N_2O$ (M^+) 319.9772, Found 319.9780.

(*Z*)-*β*-Bromo-*β*-deutrium-4-(3-trifluoromethyl-3*H*-diazirin-3-yl)styrene (**5c**): Yield 70%, ^1H-NMR ($CDCl_3$) δ: 7.71 (d, 2H, J=8.3 Hz), 7.19 (d, 2H, J=8.3 Hz), 7.06 (s, 1H, J=8.3 Hz), ^{13}C-NMR ($CDCl_3$) δ: 136.2, 131.2, 129.2, 128.9, 126.2, 122.0 (d, J_{CF}=274.7 Hz), 108.09 (t, J_{CD}=30.5 Hz), 28.4 (d, $^2J_{CF}$=39.6 Hz), MS (EI) 291, 293, HR-MS (EI) Calcd for $C_{10}H_5DBrF_3N_2$ (M^+) 290.9728, Found 290.9725.

(*Z*)-*β*-Bromo-*β*-deutrium-2-methoxy-4-(3-trifluoromethyl-3*H*-diazirin-3-yl)styrene (**5d**): Yield 64%, ^1H-NMR ($CDCl_3$) δ: 7.91 (d, 1H, J=8.0 Hz), 7.22 (1H, s), 6.82 (d, 1H, J=8.0 Hz), 6.61 (1H, s), 3.84 (3H, s), ^{13}C-NMR ($CDCl_3$) δ: 157.0, 130.3, 129.8, 126.8, 125.5, 122.1 (q, J_{CF}=274.7 Hz), 118.2, 108.4 (t, J_{CD}=28.2 Hz), 108.20, 55.63, 28.4 (q, $^2J_{CF}$=40.8 Hz) MS (EI) 321, 323, HR-MS (EI) Calcd for $C_{11}H_7DBrF_3N_2O$ (M^+) 320.9834, Found 320.9830.

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