Development of a fluoride-responsive amide bond cleavage device that is potentially applicable to a traceable linker

Jun Yamamoto,^{a,†} Nami Maeda,^{a,†} Chiaki Komiya,^a Tomohiro Tanaka,^b Masaya Denda,^a Koji Ebisuno,^a Wataru Nomura,^b Hirokazu Tamamura,^b Youichi Sato,^c Aiko Yamauchi,^c Akira Shigenaga^{a,d,*} and Akira Otaka^{a,*}

^aDepartment of Bioorganic Synthetic Chemistry, Institute of Health Biosciences and Graduate School of Pharmaceutical Sciences, The University of Tokushima, Shomachi, Tokushima 770-8505, Japan

^bInstitute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Chiyoda-ku, Tokyo 101-0062, Japan

^cDepartment of Pharmaceutical Information Science, Institute of Health Biosciences and Graduate School of Pharmaceutical Sciences, The University of Tokushima, Shomachi, Tokushima 770-8505, Japan

^dJST, PRESTO, 4-1-8 Honcho, Kawaguchi, Saitama, 332-0012, Japan

[†]These authors contributed equally to this work.

ARTICLE INFO

ABSTRACT

Article history:

Received

Received in revised form

Accepted

Available online

Keywords:

Cleavable linker

Fluoride-responsive

Traceable linker

Stimulus-responsive amino acid

A fluoride-responsive (FR) amino acid that induces amide bond cleavage upon the addition of a fluoride was developed, and it was applied to a FR traceable linker. By the use of an alkyne-containing peptide as a model of an alkynylated target protein of a bioactive compound, introduction of the FR traceable linker onto the peptide was achieved. Subsequent fluoride induced cleavage of the linker followed by labeling of the released peptide derivative was also conducted to examine the potential applicability of the FR traceable linker to the enrichment and labeling of alkynylated target molecules.

2009 Elsevier Ltd. All rights reserved.

1. Introduction

A wide variety of molecules including natural products, peptides, and synthetic small compounds exhibit their biological activities through specific interactions with target biomacromolecules. Proteins including enzymes, receptors, and ion channels represent the major group of these targets. Identification of unknown protein targets that interact with biologically active ligands has become indispensable in the fields of chemical biology and drug development; however, this research approach is time-consuming and laborious. The target identification comprises a sequence of processes: (1) fishing a target using a biologically active ligand as bait; (2) enrichment of the hooked target; and (3) sequence analysis of the target by Edman degradation or mass spectrometry (MS).¹ For the first step, photo-affinity labeling which allows bait to be covalently bounded to the corresponding target upon photo-irradiation has significant use, because of the potential applicability to low affinity ligand-target pairs.^{1a,b,2} The hooked target is then linked with a biotinylated linker molecule for facile purification by streptavidin beads using the biotin-streptavidin interaction.^{1,3} The immobilized target is subsequently released from the beads for

sequence analysis by attenuating the biotin-streptavidin interaction. The high affinity of the biotin-streptavidin interaction ($K_d = 10^{-15}$ M),⁴ however, hampers liberation of the target from the beads. An alternative to liberate the target is the use of a cleavable linker between the bait and biotin.⁵ This approach enables efficient elution of the target protein from the beads via the linker cleavage, but contamination owing to the presence of non-target proteins sometimes hampers identification of the target.⁶ The cleavage under mild conditions and generation of an orthogonal functional group not seen in proteins, therefore, has been desired in this procedure. The orthogonal functional group enables chemoselective labeling of the target protein by an isotopic or fluorescent tag. That facilitates discrimination of the target from contaminated proteins by MS using isotopic tag or SDS-PAGE using fluorescent tag.

We previously developed a traceable linker as an advanced cleavable linker that enables selective labeling of the target protein after elution from the streptavidin beads via the linker cleavage (Figure 1a).^{7,8} A key component of the traceable linker is a stimulus-responsive amino acid that possesses a stimulus-removable protective group (PG) on the phenolic hydroxyl group (Figure 1b).⁹ The stimulus-responsive amino acid induces amide

* Corresponding authors. Tel.: +81-88-633-9534; fax: +81-88-633-9505; e-mail: shigenaga.akira@tokushima-u.ac.jp (A. Shigenaga), aotaka@tokushima-u.ac.jp (A. Otaka)

bond cleavage after stimulus-induced removal of the PG and subsequent lactonization of a trimethyl lock moiety.¹⁰ In a previous report, the traceable linker composed of a thiol-responsive amino acid, in which the PG is a *p*-nitrobenzenesulfonyl (*p*Ns) group, was presented (Figure 1a). The traceable linker was introduced onto an alkynylated protein by click chemistry and then adsorbed onto the streptavidin beads. Subsequent addition of a thiol triggered the cleavage of the linker to release the protein possessing an aminooxy group. Since the aminooxy group can react with an aldehyde chemoselectively,¹¹ the eluted target protein was selectively labeled with an aldehyde-containing labeling reagent even when contaminated non-target proteins co-existed. As the thiol was used as the cleavage inducer in this system, it is preferable to remove endogenous thiols such as glutathione before the use of the thiol-responsive traceable linker.¹² To avoid the risk of unintentional cleavage of the traceable linker, in this study, we have developed a fluoride-responsive (FR) traceable linker, because there are few fluoride ions present in a living body.¹³

Figure 1. Molecular design. (a) Purification and selective labeling of an alkynylated protein using a thiol-responsive traceable linker (*p*Ns: *p*-nitrobenzenesulfonyl group). (b) A stimulus-responsive amide bond cleavage system (PG: protective group that is removable by appropriate stimulus).

2. Results and discussion

2.1. Synthesis of a FR amino acid

Preparation of the FR amino acid possessing a fluoride-removable protective group as the PG of the stimulus-responsive amino acid was attempted. A *tert*-butyldiphenylsilyl (TBDPS) group was chosen as the fluoride-removable PG because acid treatment is unavoidable for the synthesis of the traceable linker and the TBDPS group is relatively acid tolerant compared with other trisubstituted silyl protections.¹⁴ In this study, Fmoc protected derivatives were designed for Fmoc-based solid phase peptide synthesis (Fmoc SPPS). We first attempted to synthesize

silyl ether **1**, but introduction of the TBDPS group onto the phenolic hydroxyl group of **2**¹⁵ did not proceed (Figure 2). In these reactions, recovery of the starting material, removal of the Boc group and/or removal of the *tert*-butyldimethylsilyl (TBS) group were observed. We speculated that the direct introduction of the TBDPS group onto the phenol is sterically unfavorable and steric crowding around the phenol was observed in an energy minimized structure of substrate **2** using an MM2 calculation (Figure S1). Therefore, preparation of **3** possessing a sterically less demanding siloxybenzyl unit, which can be removed via fluoride-induced cleavage of the silyl group followed by release of the quinone methide, onto the phenol was next examined (Scheme 1).¹⁶ Starting from phenol **2**, it was subjected to the modified Mitsunobu reaction with the TBDPS derivative **4**¹⁷ using *N,N,N',N'*-tetramethylazodicarboxamide (TMAD).¹⁸ The TBS group of **5** was then removed under acidic conditions to yield alcohol **6**. After stepwise oxidation of the alcohol of **6**, the Boc group was removed by the use of Ohfuné's protocol,¹⁹ because cleavage of the *p*-siloxybenzyl group was observed when trifluoroacetic acid (TFA) or hydrogen chloride was employed. The obtained amine was finally protected with an Fmoc group to yield FR amino acid **3**.

Figure 2. Synthetic attempts to prepare FR amino acid **1**. (LiHMDS: lithium hexamethyldisilazide; TBDPSOTf: *tert*-butyldiphenylsilyl trifluoromethanesulfonate)

Scheme 1. Reagents and conditions: (i) **4**, TMAD, *n*-Bu₃P, toluene, 98%; (ii) AcOH, THF, H₂O, quant.; (iii) oxalyl chloride, DMSO, Et₃N, THF; (iv) NaClO₂, NaH₂PO₄, 2-methyl-2-butene, *tert*-BuOH, acetone, H₂O; (v) *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf), 2,6-lutidine, CH₂Cl₂; (vi) FmocOSu, Na₂CO₃, acetonitrile (MeCN), H₂O, 43% (four steps).

Scheme 2. Reagents and conditions: (i) **8**, HOBT-H₂O, DIC, DMF; (ii) 20% (v/v) piperidine in DMF; (iii) **9**, HOBT-H₂O, DIC, DMF; (iv) **3**, HATU, DIEA, DMF; (v) **10**, HOBT-H₂O, DIC, DMF; (vi) biotin, HOBT-H₂O, DIC, DMF; (vii) TFE/AcOH/CH₂Cl₂ = 1/1/3 (v/v).

2.2. Preparation of a **FR** traceable linker

FR traceable linker **7** was prepared using Fmoc SPPS (Scheme 2). Release of the linker from the resin using standard TFA conditions was not compatible because of acid lability of the siloxybenzyl unit. A 2-chlorotrityl resin from which the product can be released by treatment with a weak acid was therefore suitable for this synthesis and a commercially available amino acid-preloaded resin was employed to avoid laborious attachment of the first amino acid on the resin. Starting from the glycine-preloaded 2-chlorotrityl resin, azide derivative **8**²⁰ and aminoxy derivative **9**²¹ were incorporated by standard Fmoc SPPS conditions using *N,N*-diisopropylcarbodiimide (DIC)/1-hydroxybenzotriazole (HOBt) system. After coupling of **FR** amino acid **3** in the presence of *O*-(7-azabenzotriazol-1-yl)-

N,N,N'-tetramethyluronium hexafluorophosphate (HATU) and *N,N*-diisopropylethylamine (DIEA), miniPEG unit **10** and biotin were incorporated using the DIC/HOBt system. The resin was finally treated with a 2,2,2-trifluoroethanol (TFE)/AcOH/CH₂Cl₂ cocktail to generate **FR** traceable linker **7** without accompanying the deprotection of the siloxybenzyl unit (12% all over yield. An HPLC chart of the product is shown in Figure S2 in the Supporting Information).

2.3. Click chemistry, fluoride-induced cleavage, and selective labeling of the **FR** traceable linker with a model peptide

In this study, alkyne-containing peptide **11**⁷ was employed as a model of the alkynylated target protein because of ease of handling and characterization of products (Figure 3). *tert*-Butanol was used as a cosolvent to dissolve the traceable linker. Traceable linker **7** and model peptide **11** in an aqueous *tert*-butanol solution were treated with CuSO₄ and sodium ascorbate. Following a reaction time of 1 h, completion of the coupling and production of conjugate **12** in high purity were confirmed by HPLC monitoring.

Figure 3. Click chemistry of the traceable linker. (a) Reaction of traceable linker **7** with model peptide **11**. (b) HPLC monitoring of the click chemistry. HPLC conditions: Cosmosil 5C₁₈-AR-II analytical column, linear gradient of 0.1% (v/v) TFA/MeCN in 0.1% (v/v) TFA aq., 5–90% over 30 min. *Non-peptidic compound.

Fluoride-induced cleavage of the linker moiety of peptide conjugate **12** followed by the labeling reaction was next examined (Figure 4). Conjugate **12** was dissolved in a phosphate buffer containing 0.1 M KF, 100 eq. of 2-mercaptoethanesulfonic acid sodium salt (MESNa) as a scavenger of quinone methide generated via removal of the siloxybenzyl unit, 6 M guanidine hydrochloride, and 0.05 mM EDTA (0.2 M phosphate, pH 7.6), and the reaction mixture was incubated at 37 °C. The silyl group was completely removed after 2 h of the reaction. Following the additional 10 h of incubation, intermediate **13** was cleaved to generate biotin derivative **14** and aminoxy derivative **15**. These results suggest that a rate determining step of the reaction is the removal of the quinone methide. The reaction mixture was then subjected to subsequent labeling without purification. We used 3-bromobenzaldehyde as the labeling reagent, because the labeled compounds can easily be distinguished based on an isotope pattern of the MS.^{6b,22} To the reaction mixture was added 3-bromobenzaldehyde and the labeling was accomplished within 5 min. Although **15** possesses a free amino group at the N-terminus of the peptide, incorporation of two aldehydes was not observed. This result demonstrates that the selective labeling of the aminoxy group with the aldehyde was achieved.²³

Figure 4

3. Conclusion

Preparation of the FR amino acid and its application to the FR traceable linker were reported. The traceable linker was successfully introduced onto an alkyne-containing model peptide using click chemistry, and fluoride-induced cleavage followed by selective labeling of the obtained traceable linker-model peptide conjugate was achieved. Its application to the isolation and selective labeling of alkynylated target proteins is currently underway in our laboratory.

4. Experimental section

4.1. General methods

All reactions of small molecules were carried out under a positive pressure of argon at room temperature unless otherwise noted. For column chromatography, silica gel (KANTO KAGAKU N-60) was employed. Mass spectra were recorded on a Waters MICROMASS[®] LCT PREMIER[™] or a Bruker Esquire200T. NMR spectra were measured using a JEOL GSX400 or a JEOL GSX300 spectrometer. For HPLC separations, a Cosmosil 5C₁₈-AR-II analytical column (Nacalai Tesque, 4.6 × 250 mm, flow rate 1.0 mL/min) or a semi-preparative column (10 × 250 mm, flow rate 3 mL/min) was employed, and eluting products were detected by UV at 220 nm. A solvent system consisting of 0.1% (v/v) TFA in H₂O (solvent A) and 0.1% TFA (v/v) in MeCN (solvent B) was used for HPLC elution. IR spectra and optical rotations were measured using a JASCO FT-IR 6200 and a JASCO P-2200 polarimeter (concentration in g/100 mL), respectively. A melting point was determined on a YAMATO-MODEL 20 melting point apparatus and was uncorrected. An elemental analysis was performed using a J-SCIENCE LAB JM10.

4.2. Synthesis of FR amino acid derivatives

4.2.1. (S)-tert-butyl [1-((tert-butyl)dimethylsilyl)oxy]-3-{2-([4-((tert-butyl)dimethylsilyl)oxy}benzyl)oxy}-4,6-dimethylphenyl]-3-methylbutan-2-yl]carbamate (**5**)

To a stirred solution of phenol **2**¹⁵ (200 mg, 0.457 mmol), benzyl alcohol **4**¹⁷ (250 mg, 0.690 mmol) and TMAD (236 mg, 1.37 mmol) in toluene (4.6 mL) was added tri-*n*-butylphosphine (342 μL, 1.37 mmol) at 0 °C. After being stirred at same temperature for 30 min, the resulting mixture was additionally stirred at room temperature for 3 h. Following to the addition of water, the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na₂SO₄, and concentrated in vacuo. The crude product was purified by column chromatography (hexanes/EtOAc = 20/1 (v/v)) and 349 mg of ether **5** (0.446 mmol, 98%) was obtained as a colorless oil: [α]_D²⁰ −22.3 (c 1.29, CHCl₃); ¹H NMR (CDCl₃, 400 MHz) δ = −0.06 (3H, s), −0.04 (3H, s), 0.86 (9H, s), 1.13 (9H, s), 1.40 (9H, s), 1.49 (3H, s), 1.50 (3H, s), 2.20 (3H, s), 2.55 (3H, s), 3.47 (1H, dd, *J* = 10.6 and 5.0 Hz), 3.56 (1H, dd, *J* = 10.6 and 4.0 Hz), 4.54 (1H, ddd, *J* = 10.0, 5.0 and 4.0 Hz), 4.85 (1H, d, *J* = 10.0 Hz), 4.94 (1H, d, *J* = 11.6 Hz), 4.99 (1H, d, *J* = 11.6 Hz), 6.55 (1H, s), 6.57 (1H, s), 6.78 (2H, d, *J* = 8.5 Hz), 7.21 (2H, d, *J* = 8.5 Hz), 7.35–7.47 (6H, m), 7.71–7.77 (4H, m); ¹³C NMR (CDCl₃, 75 MHz) δ = −5.6, −5.5, 18.1, 19.5, 20.7, 25.8, 25.8, 26.5, 27.7, 28.4, 29.3, 44.5, 56.7, 63.6, 70.7, 78.3, 112.7, 119.7, 127.4, 127.7, 128.6, 129.8, 130.0, 131.0, 132.9, 135.5, 136.0, 138.5, 155.1, 156.0, 158.7; IR (neat) 701, 835, 919, 1113, 1172, 1255, 1511, 1610, 1700, 1721, 2858, 2930, 2957 cm^{−1}; HRMS (ESI-TOF) *m/z* calcd for C₄₇H₆₈NO₅Si₂ ([M + H]⁺) 782.4636, found 782.4610.

4.2.2. (S)-tert-butyl [3-{2-([4-((tert-butyl)dimethylsilyl)oxy}benzyl)oxy}-4,6-dimethylphenyl]-1-hydroxy-3-methylbutan-2-yl]carbamate (**6**)

Glacial acetic acid (6.0 mL) and H₂O (2.1 mL) were added to a solution of silyl ether **5** (298 mg, 0.381 mmol) in THF (2.1 mL). The reaction mixture was stirred for 9 h. After addition of water followed by extraction with EtOAc, the organic layer was washed with water and brine, dried over Na₂SO₄, and concentrated in vacuo. The crude product was purified by column chromatography (hexanes/EtOAc = 4/1 then 2/1 (v/v)) and 258 mg of alcohol **6** (0.386 mmol, quant.) was obtained as a white amorphousness: [α]_D²¹ −6.10 (c 1.04, CHCl₃); ¹H NMR (CDCl₃, 400 MHz) δ = 1.11 (9H, s), 1.36 (9H, s), 1.45 (6H, s), 2.20 (3H, s), 2.49 (3H, s), 3.45 (1H, dd, *J* = 9.7 and 7.8 Hz), 3.59 (1H, d, *J* = 9.7 Hz), 4.35 (1H, dd, *J* = 8.5 and 7.8 Hz), 4.89 (1H, d, *J* = 11.7 Hz), 4.93 (1H, d, *J* = 11.7 Hz), 5.05 (1H, d, *J* = 8.5 Hz), 6.56 (1H, s), 6.60 (1H, s), 6.77 (2H, d, *J* = 8.3 Hz), 7.17 (2H, d, *J* = 8.3 Hz), 7.34–7.46 (6H, m), 7.70–7.74 (4H, m); ¹³C NMR (CDCl₃, 75 MHz) δ = 19.4, 20.7, 25.9, 26.5, 28.3, 29.2, 43.5, 59.4, 64.5, 70.9, 79.2, 112.6, 119.9, 127.6, 127.7, 129.1, 129.3, 129.9, 130.3, 132.8, 135.5, 136.4, 138.3, 155.4, 157.3, 158.5; IR (KBr) 701, 823, 921, 1171, 1253, 1511, 1695, 2860, 2931, 2961 cm^{−1}; HRMS (ESI-TOF) *m/z* calcd for C₄₁H₅₄NO₅Si ([M + H]⁺) 668.3771, found 668.3797.

4.2.3. (S)-2-([{9H-fluoren-9-yl)methoxy}carbonyl]amino)-3-{2-([4-((tert-butyl)dimethylsilyl)oxy}benzyl)oxy}-4,6-dimethylphenyl]-3-methylbutanoic acid (**3**)

To a solution of oxalyl chloride (38.0 μL, 0.444 mmol) in CH₂Cl₂ (3.6 mL) were added DMSO (63.1 μL, 0.888 mmol) and alcohol **6** (198 mg, 0.296 mmol) in CH₂Cl₂ (660 μL) slowly at −78 °C, and the resulting solution was stirred at −40 °C for 30 min. After addition of triethylamine (206 μL, 1.48 mmol) followed by stirring for 30 min at the same temperature, the reaction mixture was stirred at room temperature for an additional 30 min. Then the reaction was quenched by the addition of water and the obtained mixture was extracted with CH₂Cl₂. The organic layer was washed with sat. NH₄Cl aq., dried over Na₂SO₄, and concentrated in vacuo. To a solution of the crude product in

acetone/*tert*-BuOH/water (6/4/1 (v/v), 10 mL) were added 2-methyl-2-butene (212 μ L, 2.00 mmol), NaH_2PO_4 (53.3 mg, 0.444 mmol) and NaClO_2 (176 mg, 1.55 mmol). The resulting mixture was stirred for 2.5 h. Following to the addition of sat. NH_4Cl aq., the mixture was extracted with EtOAc. The resulting organic layer was dried over Na_2SO_4 and concentrated in vacuo. To the crude product in CH_2Cl_2 (6.3 mL) were added 2,6-lutidine (207 μ L, 1.78 mmol) and TBSOTf (272 μ L, 1.18 mmol), and the reaction mixture was stirred at room temperature for 2 h. The resulting mixture was concentrated in vacuo, and the obtained residue was dissolved in MeCN/10% (w/v) Na_2CO_3 aq. (3/1 (v/v), 6.3 mL). To the resulting solution was added FmocOSu (120 mg, 0.355 mmol), and the reaction mixture was stirred at room temperature overnight. After the addition of 5% (w/v) KHSO_4 aq., the mixture was extracted with diethyl ether. The obtained organic layer was washed with brine, dried over Na_2SO_4 , and concentrated in vacuo. The crude material was purified by column chromatography (CHCl_3) and 103 mg of Fmoc derivative **3** (0.128 mmol, 43%) was obtained as a beige amorphousness: $[\alpha]_D^{25}$ -5.58 (c 0.64, CHCl_3); ^1H NMR (CDCl_3 , 400 MHz) δ = 1.06 (9H, s), 1.57 (6H, s), 2.15 (3H, s), 2.47 (3H, s), 4.05–4.13 (1H, m), 4.15 (1H, dd, J = 10.5 and 6.8 Hz), 4.28 (1H, dd, J = 10.5 and 6.8 Hz), 4.93 (2H, s), 5.28 (1H, d, J = 9.5 Hz), 5.56 (1H, d, J = 9.5 Hz), 6.51 (1H, s), 6.57 (1H, s), 6.74 (2H, d, J = 8.0 Hz), 7.18 (2H, d, J = 8.0 Hz), 7.20–7.44 (10H, m), 7.48 (1H, d, J = 7.5 Hz), 7.67 (4H, d, J = 6.8 Hz), 7.70 (2H, d, J = 7.5 Hz); ^{13}C NMR (CDCl_3 , 75 MHz) δ = 19.4, 20.8, 25.7, 26.5, 28.3, 28.8, 44.1, 47.1, 60.2, 66.8, 70.7, 112.5, 119.8, 119.9, 119.9, 125.1, 125.1, 127.0, 127.6, 127.7, 128.3, 129.0, 129.4, 129.8, 132.8, 135.5, 136.9, 137.9, 141.2, 143.9, 155.3, 156.0, 158.5, 175.6; IR (KBr) 706, 742, 757, 823, 918, 1255, 1511, 1717, 2858, 2934, 3028 cm^{-1} ; HRMS (ESI-TOF) m/z calcd for $\text{C}_{51}\text{H}_{53}\text{NO}_6\text{NaSi}$ ($[\text{M} + \text{Na}]^+$) 826.3540, found 826.3577.

4.3. Preparation of the FR traceable linker (7)

The traceable linker was constructed on glycine preloaded 2-chlorotriptyl resin (0.87 mmol amine/g, 20 mg, 17 μ mol). Coupling conditions for **8**,²⁰ **9**,²¹ **10**, and biotin: 3 eq. building block, 3 eq. DIC, and 3 eq. HOBT· H_2O in DMF, 2 h, room temperature. Coupling conditions for **3**: 1.2 eq. **3**, 1.2 eq. HATU, 1.2 eq. DIEA in DMF (preactivated for 1 min), 2 h, room temperature. Removal of Fmoc group: 20% (v/v) piperidine in DMF, 2 min first treatment followed by washing and subsequent second treatment for 8 min, room temperature. Cleavage from resin (20 mg): TFE/AcOH/ CH_2Cl_2 (1/1/3 (v/v)), 1 mL, 2 h, room temperature. Work-up: After removal of solvent under vacuo, MeCN/ H_2O (1/1 (v/v)) was added to the resulting mixture. Following the removal of the resin by filtration, the solution was subjected to HPLC purification to yield traceable linker **7** as a white lyophilized powder (2.6 mg, 12%). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 50 to 90% over 30 min. Retention time = 24.3 min. Semi-preparative HPLC conditions: linear gradient of solvent B in solvent A, 50 to 90% over 30 min. LRMS (ESI-Ion Trap) m/z calcd for $\text{C}_{62}\text{H}_{85}\text{N}_{10}\text{O}_{13}\text{SSi}$ ($[\text{M} + \text{H}]^+$) 1237.6, found 1237.2.

4.4. Click chemistry of traceable linker with model peptide (12)

To a solution of traceable linker **7** (0.1 μ mol) and peptide **11** (0.1 μ mol) in $\text{H}_2\text{O}/\text{tert}$ -BuOH (1/1 (v/v), 100 μ L) were added $\text{CuSO}_4\cdot\text{H}_2\text{O}$ (0.06 μ mol) and sodium ascorbate (0.5 μ mol), and the reaction mixture was shaken at room temperature for 1 h. Reaction progress was monitored by analytical HPLC. Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5 to 90% over 30 min. Conjugate **12**: retention time = 23.1 min. LRMS (ESI-Ion Trap) m/z calcd for $\text{C}_{101}\text{H}_{142}\text{N}_{24}\text{O}_{21}\text{SSi}$ ($[\text{M} + 2\text{H}]^{2+}$) 1043.5, found 1043.2.

4.5. Fluoride-induced cleavage of the traceable linker followed by labeling (13–16)

Peptide-traceable linker conjugate **12** (0.1 μ mol) in phosphate buffer containing 6 M guanidine hydrochloride and 0.05 mM EDTA (200 mM phosphate, pH 7.6, 100 μ L) was treated with KF (10 μ mol) and MESNa (10 μ mol). After incubation at 37 $^\circ\text{C}$ for 12 h, 3-bromobenzaldehyde (0.1 μ mol) was added to the reaction mixture. Progress of the reaction was monitored by HPLC. Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5 to 90% over 30 min. Intermediate **13**: retention time = 16.5 min, LRMS (ESI-Ion Trap) m/z calcd for $\text{C}_{85}\text{H}_{123}\text{N}_{24}\text{O}_{21}\text{S}$ ($[\text{M} + \text{H}]^+$) 1847.9, found 1847.3. Biotin derivative **14**: retention time = 20.2 min, LRMS (ESI-Ion Trap) m/z calcd for $\text{C}_{29}\text{H}_{43}\text{N}_4\text{O}_7\text{S}$ ($[\text{M} + \text{H}]^+$) 591.3, found 591.1. Aminooxy derivative **15**: retention time = 11.2 min, LRMS (ESI-Ion Trap) m/z calcd for $\text{C}_{49}\text{H}_{75}\text{N}_{20}\text{O}_{13}$ ($[\text{M} + \text{H}]^+$) 1151.6, found 1151.2. Labeled derivative **16**: retention time = 15.4 min, LRMS (ESI-Ion Trap) m/z calcd for $\text{C}_{56}\text{H}_{78}\text{BrN}_{20}\text{O}_{13}$ ($[\text{M} + \text{H}]^+$) 1317.5 (^{71}Br derivative) and 1319.3 (^{81}Br derivative), found 1317.1 and 1319.3.

4.6. Preparation of siloxybenzyl chloride 17

Scheme 3. Reagents and conditions: (i) SOCl_2 , CH_2Cl_2 , 70%.

Thionyl chloride (0.80 mL, 11 mmol) was slowly added to a solution of alcohol **4**¹⁷ (2.0 g, 5.2 mmol) in CH_2Cl_2 (12 mL) at 0 $^\circ\text{C}$, and the solution stirred for 2 h at room temperature. After the addition of ice, the reaction mixture was extracted with EtOAc. The organic layer was washed with saturated aqueous solution of NaHCO_3 followed by brine, dried over MgSO_4 , and concentrated in vacuo. Chloride **17** was obtained as a white solid (1.4 g, 70%) and was used without further purification. mp 77–79 $^\circ\text{C}$; ^1H NMR (CDCl_3 , 400 MHz) δ = 1.09 (9H, s), 4.48 (2H, s), 6.73 (2H, d, J = 8.5 Hz), 7.11 (2H, d, J = 8.5 Hz), 7.34–7.45 (6H, m), 7.68–7.72 (4H, m); ^{13}C NMR (CDCl_3 , 75 MHz) δ = 19.4, 26.2, 26.4, 46.2, 119.8, 127.8, 129.7, 129.9, 132.6, 135.4, 155.6; IR (KBr) 704, 919, 1115, 1258, 1510, 1605, 2858, 2932, 2960, 3070 cm^{-1} ; HRMS (ESI-TOF) m/z calcd for $\text{C}_{23}\text{H}_{26}\text{OSiCl}$ ($[\text{M} + \text{H}]^+$) 381.1441, found 381.1457; Anal. calcd for $\text{C}_{23}\text{H}_{25}\text{OSiCl}$: C, 6.61; H, 72.51. Found: C, 6.63; H, 72.39.

Acknowledgement

This research was supported in part by PRESTO, Japan Science and Technology Agency (JST) and a Grant-in-Aid for Scientific Research (KAKENHI) including Innovative Areas “Fusion Material” and “ChemBioChem”. Takeda Pharmaceutical Company and Astellas Foundation for Research on Metabolic Disorders are also acknowledged. JY and MD are grateful for JSPS fellowships. A propargyl glycine used in this study was a gift from Nagase & Co., Ltd.

References

- Recent reviews: (a) Lapinsky, D. J. *Bioorg. Med. Chem.* **2012**, *20*, 6237–6247; (b) Dubinsky, L.; Krom, B. P.; Meijler, M. M. *Bioorg. Med. Chem.* **2012**, *20*, 554–570; (c) Li, N.; Overkleeft, H. S.; Florea, B. I. *Curr. Opin. Chem. Biol.* **2012**, *16*, 227–233; (d) Wang, K.; Yang, T.; Wu, Q.; Zhao, X.; Nice, E. C.; Huang, C. *Exp. Rev. Proteomics* **2012**, *9*, 293–310; (e) Cravatt, B. F.; Wright, A. T.; Kozarich, J. W. *Annu. Rev. Biochem.* **2008**, *77*, 383–414.

2. (a) Kotzyba-Hibert, F.; Kapfer, I.; Goeldner, M. *Angew. Chem. Int. Ed.* **1995**, *34*, 1296–1312; (b) Fleming, S. A. *Tetrahedron* **1995**, *51*, 12479–12520.
3. (a) Savage, M. D. *BioMethods* **1996**, *7*, 1–29; (b) Hofmann, K.; Kiso, Y. *Proc. Natl. Acad. Sci. USA* **1976**, *73*, 3516–3518.
4. Green, N. M. *Adv. Protein Chem.* **1975**, *29*, 85–133.
5. Recent reviews: (a) Bielski, R.; Witczak, Z. *Chem. Rev.* **2013**, *113*, 2205–2243; (b) Leriche, G.; Chisholm, L.; Wagner, A. *Bioorg. Med. Chem.* **2012**, *20*, 571–582.
6. (a) Verhelst, S. H. L.; Fonovic, M.; Bogoy, M. *Angew. Chem. Int. Ed.* **2007**, *46*, 1284–1286; (b) Paulick, M. G.; Hart, K. M.; Brinner, K. M.; Tjandra, M.; Charych, D. H.; Zuckermann, R. N. *J. Comb. Chem.* **2006**, *8*, 417–426; (c) van der Veken, P.; Dirksen, E. H. C.; Ruijter, E.; Elgersma, R. C.; Heck, A. J. R.; Rijkers, D. T. S.; Slijper, M.; Liskamp, R. M. J. *ChemBioChem* **2005**, *6*, 2271–2280.
7. Yamamoto, J.; Denda, M.; Maeda, N.; Kita, M.; Komiya, C.; Tanaka, T.; Nomura, W.; Tamamura, H.; Sato, Y.; Yamauchi, A.; Shigenaga, A.; Otaka, A. *Org. Biomol. Chem.* **2014**, *12*, 3821–3826.
8. Equilibrium-based hydrazone type linkers designed for purification and labeling of the target protein as similar to the traceable linker, see (a) Dirksen, A.; Yegneswaran, S.; Dawson, P. E. *Angew. Chem. Int. Ed.* **2010**, *49*, 2023–2027; (b) Park, K. D.; Liu, R.; Kohn, H. *Chem. Biol.* **2009**, *16*, 763–772.
9. (a) Shigenaga, A.; Ogura, K.; Hirakawa, H.; Yamamoto, J.; Ebisuno, K.; Miyamoto, L.; Ishizawa, K.; Tsuchiya, K.; Otaka, A. *ChemBioChem* **2012**, *13*, 968–971; (b) Shigenaga, A.; Hirakawa, H.; Yamamoto, J.; Ogura, K.; Denda, M.; Yamaguchi, K.; Tsuji, D.; Itoh, K.; Otaka, A. *Tetrahedron* **2011**, *67*, 3984–3990; (c) Shigenaga, A.; Yamamoto, J.; Sumikawa, Y.; Furuta, T.; Otaka, A. *Tetrahedron Lett.* **2010**, *51*, 2868–2871; (d) Shigenaga, A.; Yamamoto, J.; Hirakawa, H.; Ogura, K.; Maeda, N.; Morishita, K.; Otaka, A. *Tetrahedron Lett.* **2010**, *51*, 2525–2528; (e) Shigenaga, A.; Tsuji, D.; Nishioka, N.; Tsuda, S.; Itoh, K.; Otaka, A. *ChemBioChem* **2007**, *8*, 1929–1931.
10. (a) Milstien, S.; Cohen, L. A. *Proc. Natl. Acad. Sci. USA* **1970**, *67*, 1143–1147; (b) Levine, M. N.; Raines, R. T. *Chem. Sci.* **2012**, *3*, 2412–2420 and references therein.
11. Ulrich, S.; Boturn, D.; Marra, A.; Renaudet, O.; Dumy, P. *Chem. Eur. J.* **2014**, *20*, 34–41.
12. (a) Anderson, E. *Chem. Biol. Interact.* **1998**, *112*, 1–14 and references therein; (b) Jones, D. P.; Carlson, J. L.; Samiec, P. S.; Sternberg, P.; Mody, V. C.; Reed, R. L.; Brown, L. A. S. *Clin. Chim. Acta* **1998**, *275*, 175–184.
13. Itai, K.; Onoda, T.; Nohara, M.; Ohsawa, M.; Tanno, K.; Sato, T.; Kuribayashi, T.; Okayama, A. *Clin. Chim. Acta* **2010**, *411*, 263–266, and references therein.
14. Wuts, P. G. M.; Greene, T. W. *Greene's Protective Group in Organic Synthesis*, 4th Ed.; John Wiley & Sons, Inc.: Hoboken, NJ, 2007.
15. Shigenaga, A.; Yamamoto, J.; Nishioka, N.; Otaka, A. *Tetrahedron* **2010**, *66*, 7367–7372.
16. A review of fluoride sensors including the use of the siloxybenzyl unit, see: Zhou, Y.; Zhang, J. F.; Yoon, J. *Chem. Rev.* in press (doi: 10.1021/cr400352m).
17. Pettit, G. R.; Grealish, M. P.; Jung, M. K.; Hamel, E.; Pettit, R. K.; Chapuis, J.-C.; Schmit, J. M. *J. Med. Chem.* **2002**, *45*, 2534–2542.
18. Tsunoda, T.; Otsuka, J.; Yamamiya, Y.; Ito, S. *Chem. Lett.* **1994**, *23*, 539–542.
19. Sakaitani, M.; Ohfune, Y. *J. Org. Chem.* **1990**, *55*, 870–876.
20. Katayama, H.; Hojo, H.; Ohira, T.; Nakahara, Y. *Tetrahedron Lett.* **2008**, *49*, 5492–5494.
21. Cipolla, L.; Rescigno, M.; Leone, A.; Peri, F.; Ferla, B. L.; Nicotra, F. *Bioorg. Med. Chem.* **2002**, *10*, 1639–1646.
22. Recent examples of MS analyses utilizing isotope pattern of bromine: (a) Liu, H.; Lichti, C. F.; Mirfattah, B.; Frahm, J.; Nilsson, C. L. *J. Proteome Res.* **2013**, *12*, 4248–4254; (b) Hudson, S. R.; Chadbourne, F. L.; Helliwell, P. A.; Pflimlin, E.; Thomas-Oates, J. E.; Routledge, A. *ACS Comb. Sci.* **2012**, *14*, 97–100.
23. Peptide **11** was treated with KF, MESNa and 3-bromobenzaldehyde in Na phosphate buffer (pH 7.6) containing guanidine hydrochloride and EDTA, but no reaction was observed (Figure S3 in the Supporting Information). This result also supports that the aldehyde was incorporated onto the aminoxy group but not onto the amino group.
24. It was confirmed by co-injecting the reaction mixture of 4-(*tert*-butyldiphenylsiloxy)benzyl chloride **17** with KF and MESNa in phosphate buffer. Preparation of **17** is described in Scheme 3 of the experimental section.

Figure 4. Fluoride-induced cleavage of the traceable linker followed by selective labeling. (a) Treatment of the traceable linker-peptide conjugate **12** with KF followed by labeling with 3-bromobenzaldehyde. (b) HPLC monitoring of the reactions. HPLC conditions: Cosmosil 5C₁₈-AR-II analytical column, linear gradient of 0.1% (v/v) TFA/MeCN in 0.1% (v/v) TFA aq., 5–90% over 30 min. *Non-peptidic compound derived from the quinone methide. **3-Bromobenzaldehyde.

Graphical Abstract

To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

Development of a fluoride-responsive amide bond cleavage device that is potentially applicable to a traceable linker

Leave this area blank for abstract info.

Jun Yamamoto, Nami Maeda, Chiaki Komiya, Tomohiro Tanaka, Masaya Denda, Koji Ebisuno, Wataru Nomura, Hirokazu Tamamura, Youichi Sato, Aiko Yamauchi, Akira Shigenaga* and Akira Otaka*