Supplementary Information for

Development of a traceable linker containing a thiol-responsive amino acid for the enrichment and selective labelling of target proteins

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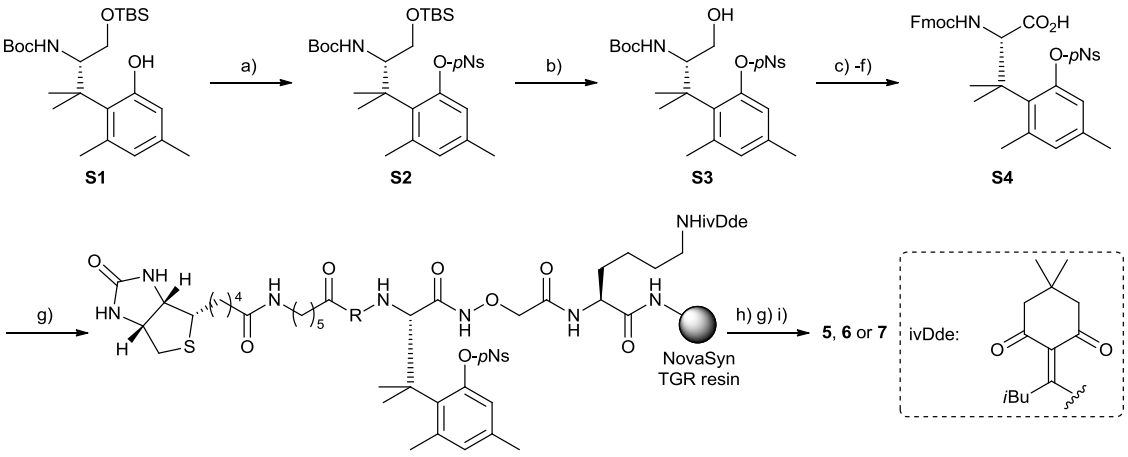


S5: R = -miniPEG-; **S6**: R = -$(miniPEG)_2$-; **S7**: R = -Pro_6-Gly-.

Scheme S1 Reagents and conditions: a) *p*NsCl, K_2CO_3, acetone, 86%; b) AcOH aq., THF, 92%; c) PDC, DMF; d) $NaClO_2$, 2-methyl-2-butene, NaH_2PO_4 aq., *tert*-BuOH, acetone; e) 4 M HCl, AcOEt; f) FmocOSu, Na_2CO_3 aq., MeCN, 81% (four steps); g) Fmoc SPPS; h) 2% (v/v) hydrazine monohydrate, DMF; i) TFA/TES/H_2O = 95:2.5:2.5 (v/v). (ivDde: 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl; *p*Ns: *p*-nitrobenzenesulfonyl; TES: triethylsilane)

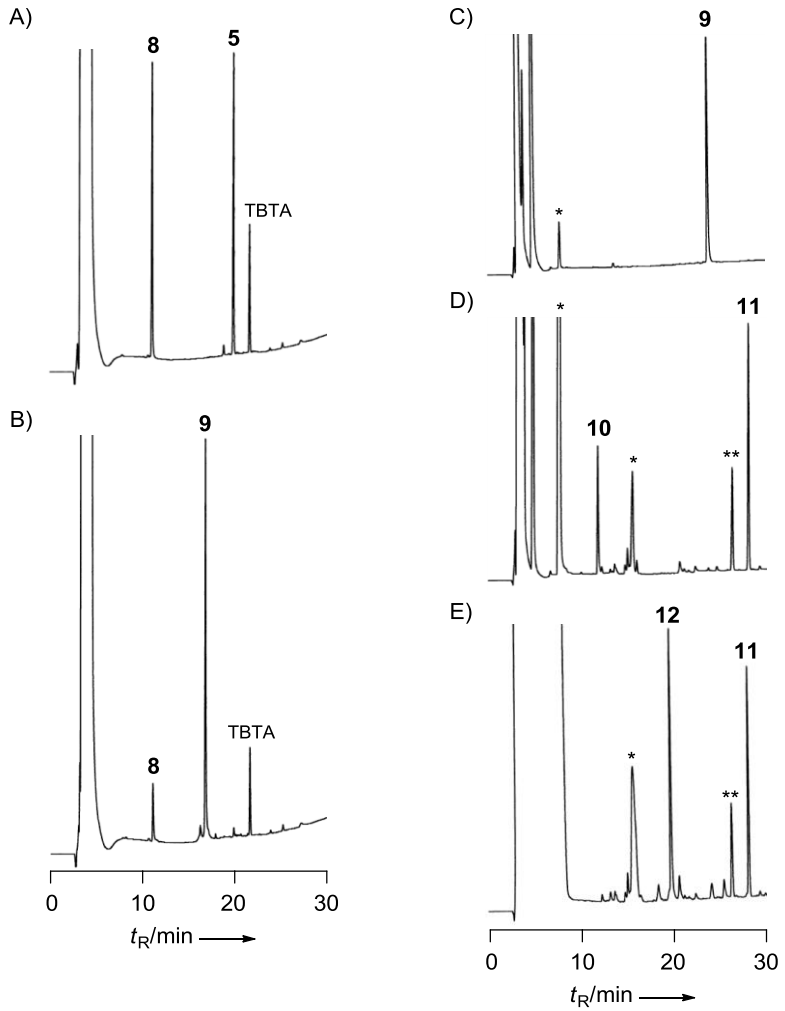


Fig. S1 HPLC monitoring of the reactions as shown in Scheme 1. A) Before the click chemistry (without $CuSO_4$ and Na ascorbate). B) After the click chemistry (reaction time = 1 h). C) After the linker cleavage (reaction time < 5 min). D) After the linker cleavage (reaction time = 24 h). E) After treatment with *o*-bromobenzaldehyde and aniline. Analytical HPLC conditions: linear gradient of 0.1% (v/v) TFA/MeCN in 0.1% (v/v) TFA aq., 5 to 90% over 30 min for A and B, 10 to 50% over 30 min for C, D, and E. *Peaks observed when 2-mercaptoethanol was incubated in Na phosphate buffer with DMSO. **Derivative of *p*Ns group.

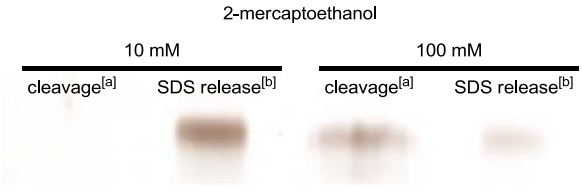


Fig. S2 Effect of concentration of 2-mercaptoethanol on the thiol-induced linker cleavage. Enolase-traceable linker **5** conjugate was prepared and adsorbed on streptavidin beads as similar to that described in footnote of Fig. 4. The obtained beads were reacted with 2-mercaptoethanol (10 or 100 mM) and NP40 (1% (v/v)) in Na phosphate buffer (10 mM, pH 7.8) at 37 °C for 24 h. All proteins were visualized by silver staining. [a] Eluted proteins by the thiol-treatment. After centrifugation, the obtained supernatant was analyzed. [b] Proteins remaining on streptavidin beads after the thiol treatment. The beads after centrifugation followed by removal of the supernatant was suspended in SDS-PAGE sample loading buffer, and the resulting mixture was heated at 100 °C for 5 min. After centrifugation, the supernatant was analyzed.

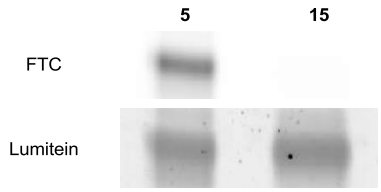


Fig. S3 Examination of orthogonal nature of the aminooxy group generated on the enolase following the cleavage of the linker. Click chemistry of the alknylated enolase with **5** or **15** followed by a reaction of the resulting product with 2-mercaptoethanol generated a conjugate, which was reacted with labelling reagent **14**. Following purification by SDS-PAGE, the labelled products or all proteins were visualized by fluorimetry without staining (FTC: λ_{ex} = 488 nm, λ_{em} = 530 nm) or Lumitein staining (Lumitein), respectively. The reaction conditions used were similar to those described in the footnote of Fig. 4.

General Methods

All reactions of small molecules were carried out under a positive pressure of argon. For column chromatography, silica gel (KANTO KAGAKU N-60) was employed. Mass spectra were recorded on a Waters MICROMASS® LCT PREMIER™ (ESI-TOF) or a Bruker Esquire2000T (ESI-Ion Trap). NMR spectra were recorded using a Bruker AV400N. For HPLC separations, a Cosmosil $5C_{18}$-AR-II analytical column (Nacalai Tesque, 4.6 × 250 mm, flow rate 1.0 mL/min) or a Cosmosil $5C_{18}$-AR-II preparative column (Nacalai Tesque, 20 × 250 mm, flow rate 10.0 mL/min) was employed, and eluting products were detected by UV at 220 nm. For HPLC elution, linear gradient of 0.1% TFA (v/v) in MeCN in 0.1% (v/v) TFA in H_2O over 30 min was used. Optical rotations were measured using a JASCO P-2200 polarimeter (concentration in g/100 mL). ECL signals from the western blot analysis were detected using a LAS-4000mini (Fujifilm). A Molecular Imager FX Pro and a Quantity One 1-D Analysis Software (Bio-Rad Laboratories) were employed for fluorescence gel images and its analyses, respectively.

Synthesis of Chiral Thiol-responsive Amino Acid Derivative **S4**

Starting from chiral intermediate **S1**,[S1] thiol-responsive amino acid derivative **S4** was prepared according to the literature.[S2] ^{1}H NMR spectra were identical to those reported previously.

(*S*)-2-*tert*-Butoxycarbonylamino-3,3-dimethyl-3-[2,4-dimethyl-6-(nitrobenzene-4-sulfonyloxy)-phenyl] propanol *tert*-butyldimethylsilyl ether (S2).

A pale yellow oil; 86% yield; $[\alpha]^{20}_D$ –35.6 (*c* 1.22, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = –0.07 (6H, s), 0.83 (9H, s), 1.30 (3H, s), 1.37 (9H, s), 1.44 (3H, s), 2.21 (3H, s), 2.62 (3H, s), 3.38 (1H, dd, *J* = 10.8 and 4.7 Hz), 3.43 (1H, dd, *J* = 10.8 and 4.5 Hz), 4.26 (1H, ddd, *J* = 9.8, 4.7 and 4.5 Hz), 4.73 (1H, d, *J* = 9.8 Hz), 6.89 (1H, s), 6.94 (1H, s), 8.24 (2H, d, *J* = 8.7 Hz), 8.42 (2H, d, *J* = 8.7 Hz).

(*S*)-2-*tert*-Butoxycarbonylamino-3,3-dimethyl-3-[2,4-dimethyl-6-(nitrobenzene-4-sulfonyloxy)-phenyl]propanol (S3).

A white powder; 92% yield; $[\alpha]^{20}_D$ –38.3 (*c* 1.13, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 1.33 (3H, s), 1.37 (9H, s), 1.42 (3H, s), 2.17 (3H, s), 2.61 (3H, s), 3.42 (1H, dd, *J* = 11.2 and 7.6 Hz), 3.49 (1H, dd, *J* = 11.2 and 2.6 Hz), 4.40 (1H, m), 4.85 (1H, d, *J* = 9.0 Hz), 6.78 (1H, s), 6.89 (1H, s), 8.22 (2H, d, *J* = 8.8 Hz), 8.42 (2H, d, *J* = 8.8 Hz).

(*S*)-3,3-Dimethyl-3-[2,4-dimethyl-6-(nitrobenzene-4-sulfonyloxy)phenyl]-2-(9-fluorenylmethyl-carbonylamino)propionic acid (S4).

A pale yellow powder; 81% yield over four steps; $[\alpha]^{20}_{D}$ –19.6 (*c* 1.02, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 1.53 (3H, s), 1.56 (3H, s), 2.14 (3H, s), 2.64 (3H, s), 4.16 (1H, t, *J* = 7.2 Hz), 4.27 (1H, dd, *J* = 10.5 and 7.2 Hz), 4.35 (1H, dd, *J* = 10.5 and 7.2 Hz), 5.20 (1H, d, *J* = 9.0 Hz), 5.73 (1H, d, *J* = 9.0 Hz), 6.66 (1H, s), 6.91 (1H, s), 7.23–7.31 (2H, m), 7.39 (2H, t, *J* = 7.5 Hz), 7.49 (1H, d, *J* = 7.5 Hz), 7.56 (1H, d, *J* = 7.5 Hz), 7.75 (2H, d, *J* = 7.5 Hz), 8.18 (2H, d, *J* = 8.6 Hz), 8.40 (2H, d, *J* = 8.8 Hz).

Preparation of Traceable Linkers **5**, **6** and **7**, and negative control **15**

General Procedure: The peptides were synthesized using Fmoc-based solid phase peptide synthesis (Fmoc SPPS). Building blocks were coupled on NovaSyn TGR resin (0.22 mmol amine/g). Reagents and solvents are listed below. All coupling reactions were performed for 2 h.

building block	reagents	solvent
S4 (2 eq.)	HATU (1.9 eq.), DIPEA (1.9 eq.)	DMF
(+)-biotin (5 eq.)	DIC (5 eq.), HOBt·H_2O (5 eq.)	DMSO/DMF = 1/1 (v/v)
$N_3(CH_2)_4CO_2H$[S3] (5 eq.)	DIC (5.3 eq.), Oxyma Pure[S4] (5 eq.)	DMF
Others (3 eq.) [S5]	DIC (3.2 eq.), Oxima Pure (3 eq.)	DMF

Abbreviations. DIC: *N*,*N*'-diisopropylcarbodiimide; DIPEA: *N*,*N*-diisopropylethylamine; HATU: 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate; HOBt: 1-hydroxybenzotriazole; Oxyma pure: ethyl cyanoglyoxylate-2-oxime.

For removal of an ivDde (1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl) group, the peptide resin was treated with 2% (v/v) hydrazine hydrate in DMF (twice for 2 h followed by once overnight). Following to completion of the peptide elongation, the resin was subjected to global deprotection using TFA/triethylsilane (TES)/H_2O (95:2.5:2.5 (v/v)) for 2 h at room temperature. After the resin was filtered off, cooled diethyl ether was added to the filtrate. The resulting precipitate was collected by centrifugation, washed with diethyl ether, and then purified by a preparative HPLC. When an amount of the precipitate had not been sufficient, the filtrate after the global deprotection was concentrated using N_2 flow and neutralized by addition of $NaHCO_3$. Then it was purified by a preparative HPLC without the precipitation step.

5: A white lyophilized powder; 13% yield; Analytical HPLC conditions: 10 to 90%. Retention time = 18.8 min; Preparative HPLC conditions: 37 to 47%; LRMS (ESI-Ion Trap) *m/z* calcd for $[M + H]^+$ 1377.6, found 1378.0.

6: A white lyophilized powder; 13% yield; Analytical HPLC conditions: 10 to 90%. Retention time = 18.6 min; Preparative HPLC conditions: 35 to 45%; LRMS (ESI-TOF) *m/z* calcd for $[M + 2H]^{2+}$ 761.9, found 761.7.

7: A white lyophilized powder; 14% yield; Analytical HPLC conditions: 10 to 90%. Retention time = 18.0 min; Preparative HPLC conditions: 35 to 45%; LRMS (ESI-TOF) *m/z* calcd for $[M + 2H]^{2+}$ 936.5, found 936.2.

15: A white lyophilized powder; 33% yield; Analytical HPLC conditions: 10 to 90%. Retention time = 13.2 min; Preparative HPLC conditions: 22 to 32%; LRMS (ESI-Ion Trap) *m/z* calcd for $[M + H]^{+}$ 1136.6, found 1137.1.

Model Reactions Using Alkynylated Peptide **8**

Preparation of Model Peptide 8: The peptide was synthesized on Nova Syn TGR resin (0.22 mmol amine/g) using Fmoc SPPS. Fmoc protected amino acids (3 eq.) were coupled at room temperature for 2 h by using DIC (3.2 eq.) and Oxyma Pure (3 eq.) in DMF. After treatment with TFA/TES/H_2O (95:2.5:2.5 (v/v)) for 1.5 h at room temperature, the resin was filtered off and cooled diethyl ether was added to the filtrate. The resulting precipitate was collected by centrifugation, washed with diethyl ether, and then purified by a preparative HPLC.

8: A white lyophilized powder; 67% yield; Analytical HPLC conditions: 5 to 30%. Retention time = 16.7 min; Preparative HPLC conditions: 15 to 25%; LRMS (ESI-TOF) *m/z* calcd for $[M + H]^{+}$ 849.5, found 849.2.

Click Chemistry: Traceable linker **5** in DMSO (6.0 mM, 66.6 μL, final concn. 0.20 mM), peptide **8** in PBS (1.25 mM, 400 μL, final concn. 0.25 mM), TBTA in 20% (v/v) DMSO/*tert*-BuOH (1.7 mM, 118 μL, final concn. 0.10 mM), $CuSO_4$ in water (50 mM, 40.0 μL, final concn. 1.0 mM), sodium ascorbate in water (25 mM, 40.0 μL, final concn. 0.50 mM), and PBS (416 μL) were added to 1.00 mL of water. After 1 h of the reaction at room temperature, reaction mixture was injected into a preparative HPLC to yield conjugate **9**.

9: A white lyophilized powder; 56% yield; Analytical HPLC conditions: 5 to 90%. Retention time = 16.7 min; Preparative HPLC conditions: 30 to 39%; LRMS (ESI-TOF) *m/z* calcd for $[M + 3H]^{3+}$ 742.7, found 742.6.

Thiol-induced cleavage: To sodium phosphate buffer (10 mM, pH 7.8, 137 μL) were added conjugate **9** in DMSO (6.0 mM, 2.37 μL, final concn. 0.10 mM), 2-mercaptoethanol (0.99 μL, final concn. 100 mM), and NP40 (1.42 μL, final concn. 1% (v/v)). After incubation at 37 °C for 24 h under argon, completion of cleavage of the linker was confirmed using HPLC and the products were characterized by MS analyses.

10: Analytical HPLC conditions: 10 to 50%. Retention time = 11.9 min; LRMS (ESI-TOF) *m*/*z* calcd for $[M + 2H]^{2+}$ 669.4, found 669.3.

11: Analytical HPLC conditions: 10 to 50%. Retention time = 28.2 min; LRMS (ESI-TOF) *m*/*z* calcd for $[M + H]^{+}$ 704.4, found 704.2.

Labelling with *o*-bromobenzaldehyde: To the reaction mixture after the linker cleavage (72.0 μL) were added 10 mM sodium phosphate buffer (pH 7.8, 634 μL), *o*-bromobenzaldehyde in DMSO (10 mM, 7.20 μL, final concn. 0.10 mM), and aniline (6.56 μL, final concn. 100 mM). The reaction was performed at room temperature for 1 h, and labelled product **12** was characterized using MS analysis.

12: Analytical HPLC conditions: 10 to 50%. Retention time = 19.8 min; LRMS (ESI-TOF) *m*/*z* calcd for $[M + 2H]^{2+}$ 752.3 (^{79}Br deriv.) and 753.3 (^{81}Br deriv.), found 752.2 and 753.2.

Preparation of Alkynylated Enolase

Starting from a commercially available enolase (7.5 mg), the alkynylated enolase was prepared according to the literature.[S6] After the reaction, unreacted small molecules were removed by dialysis (Slide-A-Lyzer® G2 Dialysis Cassette, Thermo SCIENTIFIC) with PBS instead of gel filtration. Volume of the resulting solution was adjusted to 1.8 to 2.0 mL, and it was used as 4.2 g/L to 3.8 g/L solution of the alkynylated enolase in PBS.

Introduction of Traceable Linker onto Alkynylated Enolase

Click Chemistry: To a mixture of PBS (550 μL) and water (447 μL) were added the alkynylated enolase in PBS (4.2 g/L, 180 μL, final concn. 0.50 g/L), traceable linker **5**, **6**, **7**, or negative control **15** in DMSO (6 mM, 25.0 μL, final concn. 0.10 mM), TBTA in 20% (v/v) DMSO/*tert*-BuOH (1.7 mM, 88.0 μL, final concn. 0.10 mM), $CuSO_4$ aq. (50 mM, 30.0 μL, final concn. 1.0 mM), sodium ascorbate aq. (25 mM, 30.0 μL, final concn. 0.50 mM), and SDS aq. (10% (w/v), 150 μL, final concn. 1% (w/v)). After the reaction at room temperature for 1 h, small molecules were removed by

dialysis (Slide-A-Lyzer® G2 Dialysis Cassette, Thermo SCIENTIFIC) with PBS.

SDS-PAGE: After addition of 5 × non-reducing SDS-PAGE sample loading buffer (5 × SDS-PAGE sample loading buffer without 2-mercaptoethanol) followed by heating at 100 °C for 5 min, the reaction mixture was analyzed using SDS-PAGE in 12% polyacrylamide gels. For the chemiluminescence imaging of the biotinylated proteins, the proteins were transferred to Amersham Hybond-P PVDF Membrane (GE Healthcare) and detected with a streptavidin-horseradish peroxidase conjugate (SAv-HRP, GE Healthcare) and ECL plus Western Blotting Detection System (GE Healthcare). For fluorescence imaging of all proteins, proteins in a gel were stained with Lumitein™ Protein Gel Stain (Nacalai Tesque).

Adsorption on Streptavidin Beads, Linker Cleavage, and Labelling of Enolase Conjugate

Adsorption on Streptavidin Beads: After the click chemistry, Pierce® Streptavidin UltraLink® Resin (35 μL, Thermo SCIENTIFIC) was added to the reaction mixture containing ca. 100 μg enolase and its derivatives. After the adsorption at room temperature for 24 h, the resulting resin was washed with PBS five times and it was subjected to subsequent reactions.

Linker Cleavage: To the resulting streptavidin beads was added a cleavage cocktail consisting of 2-mercaptoethanol (1.40 μL, final concn. 100 mM), NP40 (2.00 μL, final concn. 1% (v/v)), and sodium phosphate buffer (197 μL, 10 mM, pH 7.8). The reaction was conducted at 37 °C for 24 h under N_2. After centrifugation of the resulting mixture (2000 rpm, 2 min), supernatant was collected and the precipitate was suspended in 100 μL PBS. The suspension was subjected to centrifugation (2000 rpm, 2 min) again and the obtained supernatant was combined with the first one.

Labelling: To the obtained supernatant were added fluorophore **14**[S7] (final concn. 0.10 mM) and aniline (final concn. 100 mM), and the mixture was stirred at room temperature for 24 h. After concentration using Amicon® Ultra-0.5, Ultracel-10 Membrane, 10 kDa (Merk Millipore) (14,000 × g, 15 min), addition of PBS and the concentration was repeated four times to remove the excess of the non-reacted fluorophore. Then the obtained mixture was subjected to the SDS-PAGE as mentioned above. In this case, 5 × SDS-PAGE sample loading buffer was used instead of the non-reducing SDS-PAGE sample loading buffer. The labelled enolase was detected by fluorescence imaging (λ_{ex} = 488 nm, λ_{em} = 530 nm) without staining or the use of a western blot analysis.

Elution of Proteins Remaining on Streptavidin Beads After Thiol-induced Cleavage: The resin obtained after the linker cleavage as mentioned above was suspended in 2 × SDS-PAGE sample

loading buffer (25 μL) and water (25 μL), and the mixture was heated at 100 °C for 5 min. After centrifugation as mentioned in the section "Linker Cleavage", the combined supernatant was concentrated and analyzed using SDS-PAGE as similar to those described in section "Labelling".

Examination of Orthogonality of Aminooxy Group on Enolase

After click chemistry of the alkynylated enolase with the linkers followed by treatment with 2-mercaptoethanol, the product was treated with fluorophore **14**. The obtained mixture was analyzed using SDS-PAGE followed by fluorimetric detection. Reaction conditions as similar to that described in section "Introduction of Traceable Linker onto Alkynylated Enolase" and "Adsorption on Streptavidin Beads, Linker Cleavage, and Labelling of Enolase Conjugate" were employed.

Enrichment and Selective Labelling of Enolase in Protein Mixture

As a protein mixture, solution of the alkynylated enolase, bovine serum albumin (BSA), and ovalbumin (1/1/1 (w/v)) was used.

Click Chemistry: The alkynylated enolase in PBS (3.9 g/L, 127 μL, final concn. 0.50 g/L), BSA in PBS (3.3 g/L, 150 μL, final concn. 0.50 g/L), ovalbumin in PBS (3.3 g/L, 150 μL, final concn. 0.50 g/L), the traceable linker in DMSO (6.0 mM, 17 μL, final concn. 0.10 mM), TBTA in 20% (v/v) DMSO/*tert*-BuOH (1.7 mM, 59 μL, final concn. 0.10 mM), $CuSO_4$ aq. (50 mM, 20 μL, final concn. 1.0 mM), sodium ascorbate aq. (25 mM, 20 μL, final concn. 0.50 mM), SDS aq. (10% (w/v), 100 μL, final concn. 1% (w/v)), PBS (100 μL), and water (257 μL) were mixed. Following to reaction at room temperature for 1 h, the resulting solution was dialyzed using Slide-A-Lyzer® G2 Dialysis Cassette (Thermo SCIENTIFIC) with PBS.

Adsorption on Streptavidin Beads, Cleavage of Linker, and Labelling: It was performed as similar to that described in a section "Adsorption on Streptavidin Beads, Linker Cleavage, and Labelling of Enolase Conjugate".

Enrichment Without Use of Thiol-induced Linker Cleavage: After the adsorption of the protein mixture on streptavidin beads, the proteins on the beads were eluted and analyzed as mentioned in a section "Elution of Proteins Remaining on Streptavidin Beads After Thiol-induced Cleavage".

References

S1 A. Shigenaga, J. Yamamoto, N. Nishioka and A. Otaka, *Tetrahedron*, 2010, **66**, 7367–7372.

S2 A. Shigenaga, J. Yamamoto, H. Hirakawa, K. Ogura, N. Maeda, K. Morishita and A. Otaka, *Tetrahedron Lett.*, 2010, **51**, 2525–2528.

S3 W. Shi, S. Dolai, S. Averick, S. S. Fernando, J. A. Saltos, W. L'Amoreaux, P. Banerjee and K. Raja, *Bioconjugate Chem.*, 2009, **20**, 1595–1601.

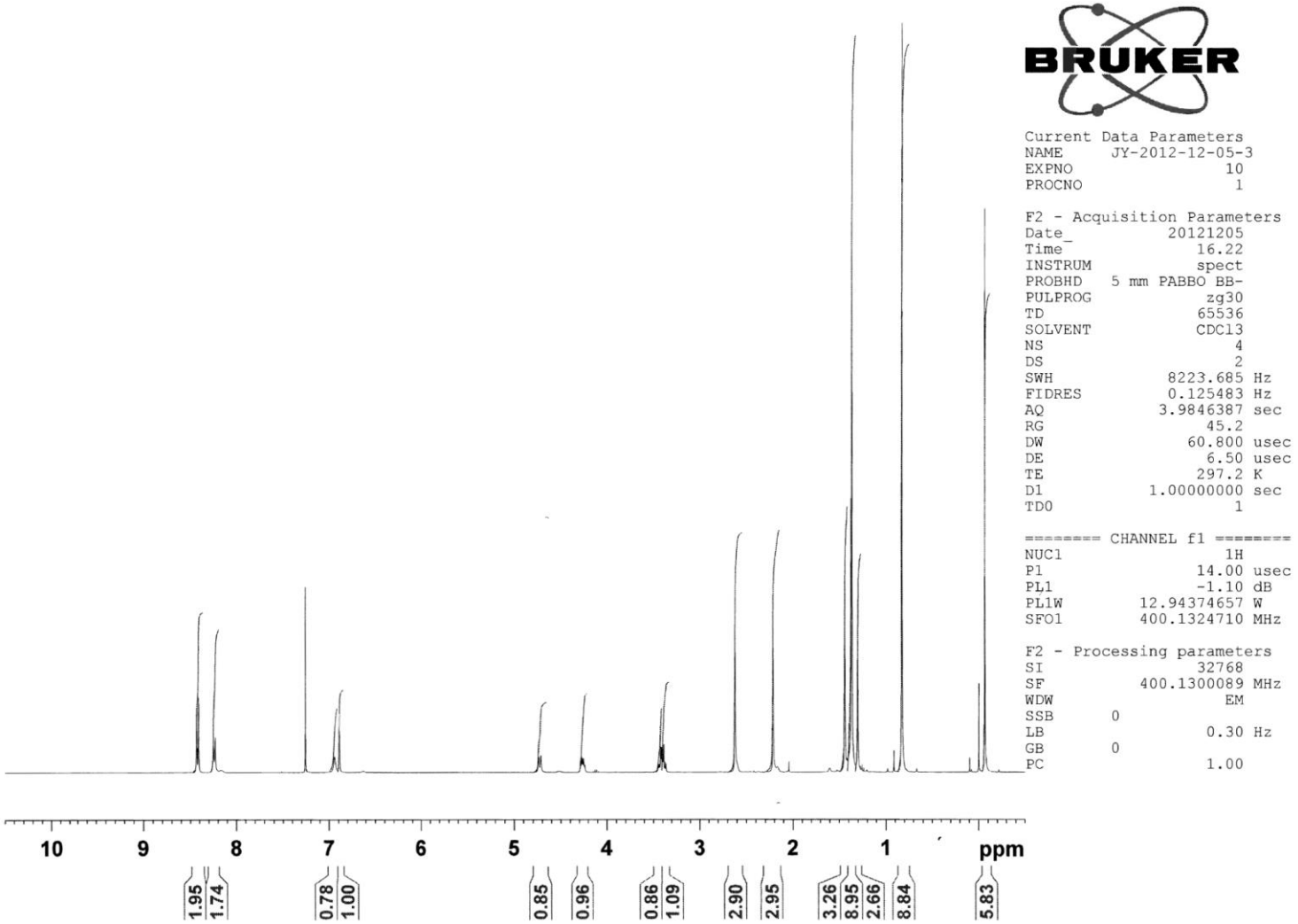
S4 R. Subiros-Funosas, S. N. Khattab, L. Nieto-Rodriguez, A. El-Faham and F. Albericio, *Aldrichimica Acta*, 2013, **46**, 21–40.

S5 Preparation of FmocNHOCH$_2$CO$_2$H: L. Cipolla, M. Rescigno, A. Leone, F. Peri, B. L. Ferla and F. Nicotra, *Bioorg. Med. Chem.*, 2002, **10**, 1639–1646.

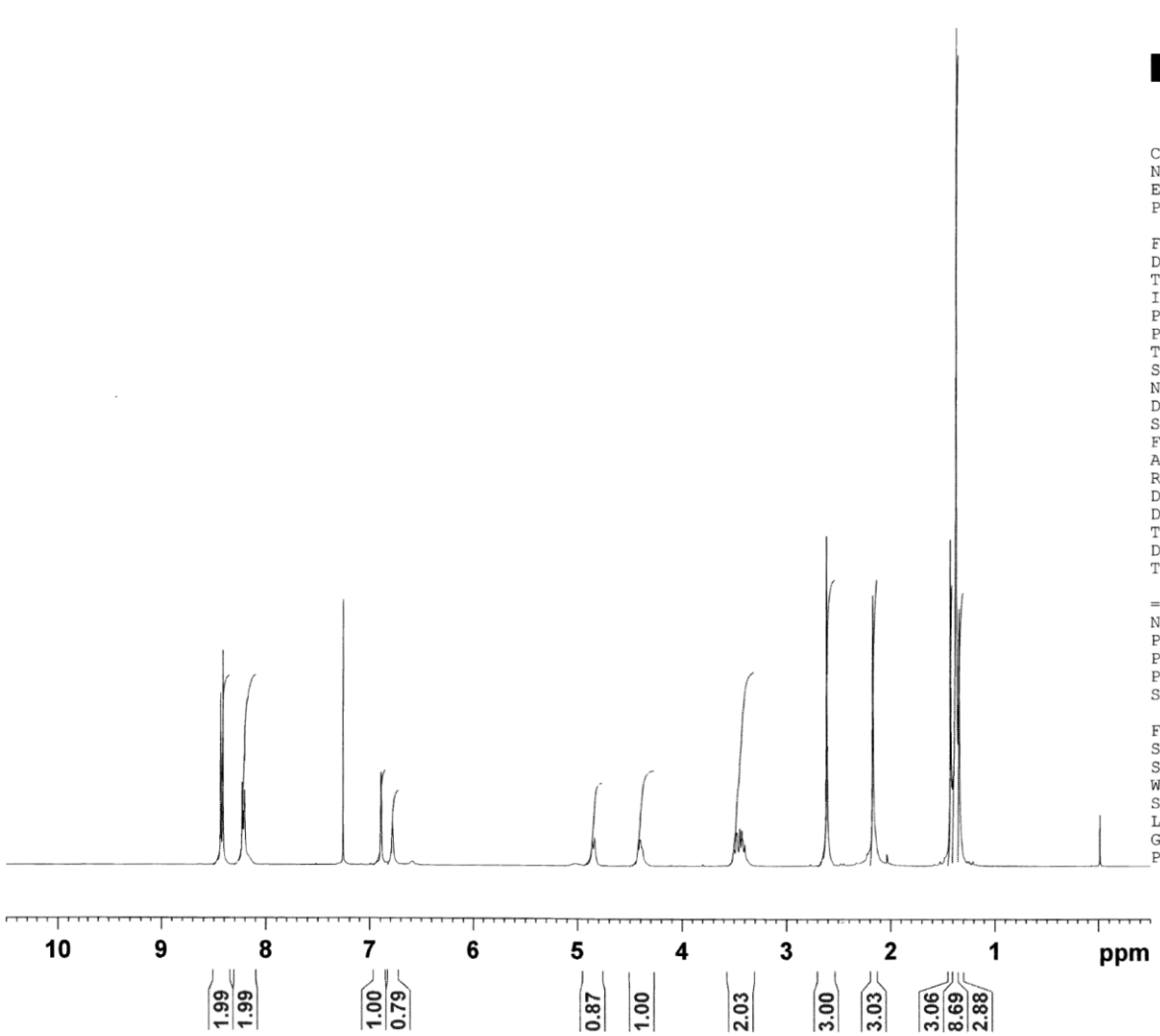
S6 K. D. Park, R. Liu and H. Kohn, *Chem. Biol.*, 2009, **16**, 763–772.

S7 E. Trevisiol, E. Defrancq, J. Lhomme, A. Laayoun and P. Cros, *Eur. J. Org. Chem.*, 2000, 211–217.

^{1}H NMR spectrum of **S2** (400 MHz, $CDCl_3$)



^{1}H NMR spectrum of **S3** (400 MHz, $CDCl_3$)

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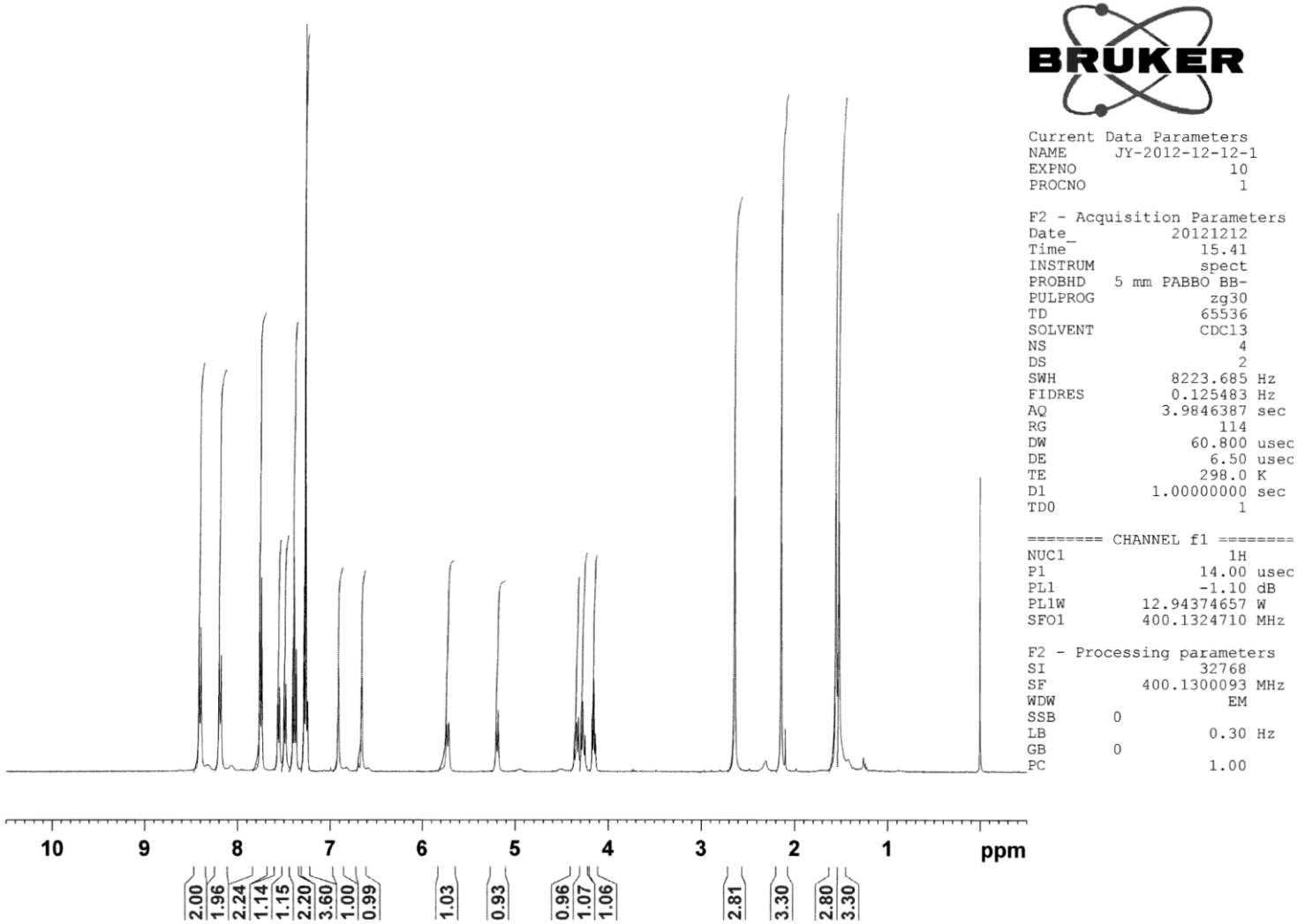
^{1}H NMR spectrum of **S4** (400 MHz, $CDCl_3$)

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