Effective Stabilisation of α-Helical Structures in Short Peptides with Acetylenic Cross-Linking Agents

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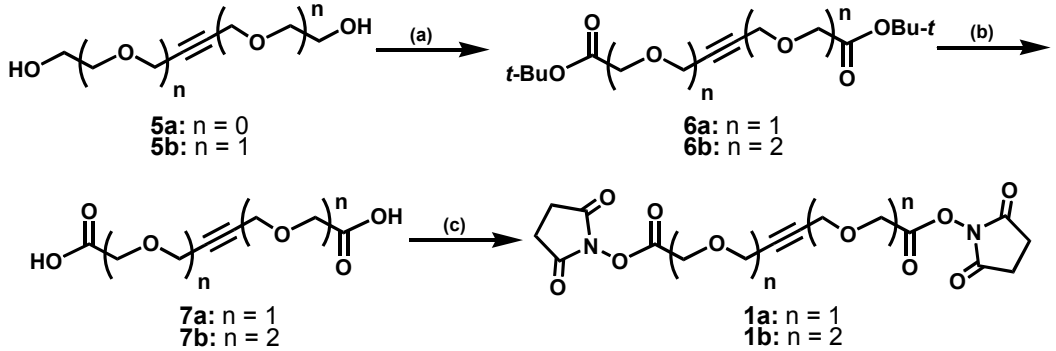
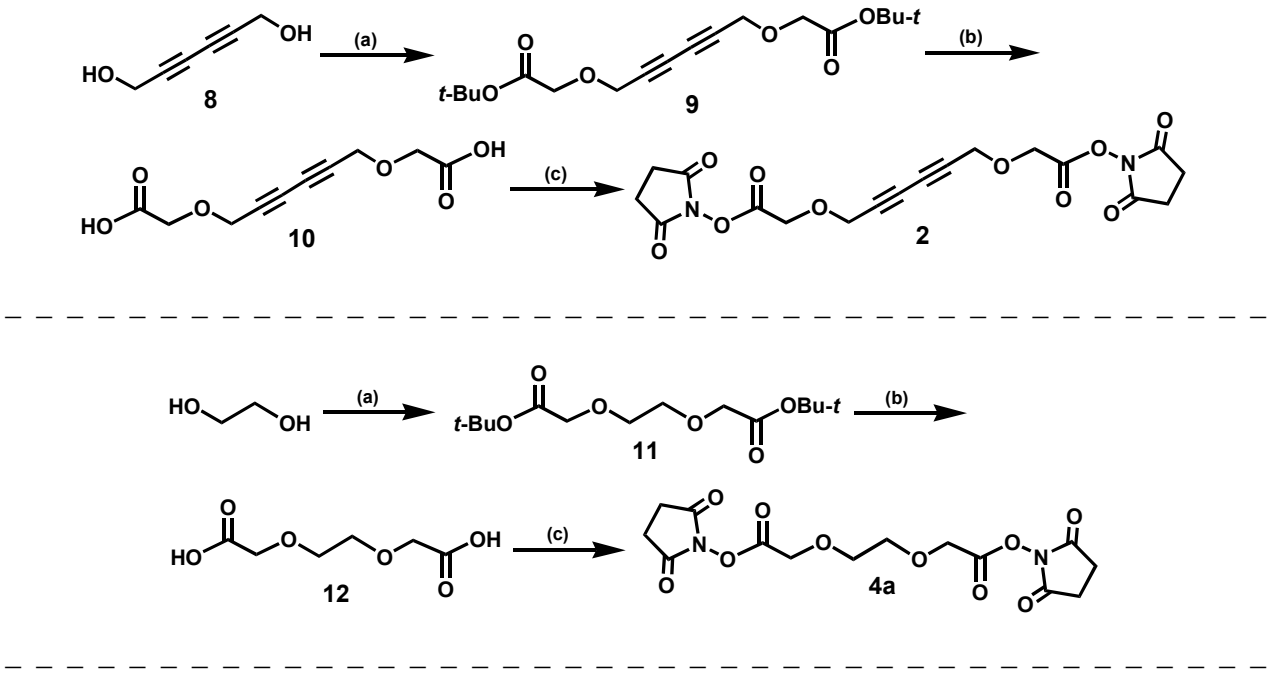
Supplementary Information

General Materials. The starting materials were all commercially available, and 1,4-bis(2-hydroxyethoxy)-2-butyne (**5b**)[1] and 2,4-hexadiyne-1,6-diol (**8**)[2] were prepared according to the literature procedures. The cross-linking agents **3a**[3], **3b**[4], and **4b**[5] were reported previously, however, the physical and spectroscopic data of **4b** were not described in the literature[5] and were presented in this Supplementary Information.

Scheme S1 Synthesis of **1**, **2**, and **4a**.





(a) NaH, *t*-butyl bromoacetate, DMF; (b) conc. HCl, ether; (c) *N,N'*-disuccinimidyl carbonate, pyridine, CH_3CN.

General Procedure for (a) in Scheme S1. To a DMF (5 mL) suspension of NaH (400 mg, 10 mmol; commercial 60% dispersion was washed thoroughly with hexane prior to use) was added a DMF (15 mL) solution of diols (5 mmol) dropwise at 0 °C. After stirring at that temperature for 2 h, to the solution was added *t*-butyl bromoacetate (2.34 g, 12 mmol) dropwise at 0 °C. The reaction mixture was warmed to room temperature gradually and stirred at that temperature for an additional 3 h. After removal of the solvent by a rotary evaporator, the residue was poured into water and extracted with CH_2Cl_2. The CH_2Cl_2 extract was evaporated and chromatographed (alumina; eluent, hexane/AcOEt) to give corresponding di-*t*-butyl esters. All the di-*t*-butyl esters in Scheme S1 were synthesised according to this procedure.

3,8-Dioxa-5-decyne-1,10-dioic Acid Di-*tert*-butyl Ester (6a): yield 84% (1.32 g); oil; IR (KBr) 1835, 1730 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.34 (s, 4 H), 4.06 (s, 4 H), 1.49 (s, 18 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.8, 82.2, 81.8, 66.7, 58.3, 28.1; HRMS (ESI) *m/z* calcd for $C_{16}H_{26}NaO_6$ ($M + Na^+$) 337.1627, found 337.1632.

3,6,11,14-Tetraoxa-8-hexadecyne-1,16-dioic Acid Di-*t*-butyl Ester (6b): yield 68% (1.37 g); oil; IR (KBr) 1835, 1731 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 4.26 (s, 4 H), 4.02 (s, 4 H), 3.73 (m, 8 H), 1.48 (s, 18 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.4, 82.3, 81.6, 70.5, 69.1, 69.0, 58.7, 28.2; HRMS (ESI) *m/z* calcd for $C_{20}H_{34}NaO_8$ ($M + Na^+$) 425.2151, found 425.2151.

3,10-Dioxa-5,7-dodecadiyne-1,12-dioic Acid Di-*t*-butyl Ester (9): yield 69% (1.17 g); mp 92–94 °C; IR (KBr) 1834, 1731 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.38 (s, 4 H), 4.06 (s, 4 H), 1.49 (s, 18 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.6, 82.0, 74.7, 71.0, 66.8, 58.6, 28.2; HRMS (ESI) *m/z* calcd for $C_{18}H_{26}NaO_6$ ($M + Na^+$) 361.1627, found 361.1615.

3,6-Dioxaoctane-1,8-dioic Acid Di-*t*-butyl Ester (11): yield 64% (929 mg); mp 37–39 °C; IR (KBr) 1834, 1730 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.04 (s, 4 H), 3.76 (s, 4 H), 1.48 (s, 18 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.6, 81.5, 70.7, 69.0, 28.0; HRMS (ESI) *m/z* calcd for $C_{14}H_{26}NaO_6$ ($M + Na^+$) 313.1627, found 313.1638.

General Procedure for (b) in Scheme S1. An ether (2 mL) solution of di-*t*-butyl esters (1.0 mmol) and conc. HCl (1 mL) was stirred for 2 h at room temperature. A removal of the solvent gave corresponding diacids. All the diacids in Scheme S1 were synthesised according to this procedure.

3,8-Dioxa-5-decyne-1,10-dioic Acid (7a): yield 94% (190 mg); mp 112–113 °C; IR (KBr) 1834, 1733 cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6) δ 12.73 (s, 2 H), 4.29 (s, 4 H), 4.06 (s, 4 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.8, 82.4, 65.7, 57.4; HRMS (ESI) *m/z* calcd for $C_8H_8NaO_6$ ($M - 2H^+ + Na^+$) 223.0219, found 223.0223.

3,6,11,14-Tetraoxa-8-hexadecyne-1,16-dioic Acid (7b): yield 98% (284 mg); oil; IR (KBr) 1834, 1731 cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6) δ 12.59 (s, 2 H), 4.22 (s, 4 H), 4.02 (s, 4 H), 3.58 (m, 8 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 174.5, 82.2, 70.7, 68.9, 68.2, 58.5; HRMS (ESI) *m/z* calcd for $C_{12}H_{18}NaO_8$ ($M + Na^+$) 313.0899, found 313.0912.

3,10-Dioxa-5,7-dodecadiyne-1,12-dioic Acid (10): yield 85% (192 mg); mp 128–131 °C; IR (KBr) 1834, 1736 cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6) δ 12.78 (s, 2 H), 4.39 (s, 4 H), 4.07 (s, 4 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.9, 76.1, 69.7, 66.1, 57.9; HRMS (ESI) *m/z* calcd for $C_8H_8NaO_6$ ($M - 2H^+ + Na^+$) 247.0219, found 247.0212.

3,6-Dioxaoctane-1,8-dioic Acid (12): yield 100% (178 mg); mp 68–71 °C; IR (KBr) 1834, 1731 cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6) δ 12.62 (s, 2 H), 4.03 (s, 4 H), 3.60 (s, 4 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.5, 69.7, 67.5; HRMS (ESI) *m/z* calcd for $C_{12}H_{18}KO_{12}$ ($2M - 2H^+ + K^+$) 393.0435, found 393.0422.

General Procedure for (c) in Scheme S1. A CH_3CN (3 mL) solution of diacids (1.0 mmol), pyridine (162 μL, 2.0 mmol), and *N,N'*-disuccinimidyl carbonate (512 mg, 2.0 mmol) was stirred at room temperature. After 3 h, the solvent was removed by a rotary evaporator. The residue was poured into a 1N-HCl aqueous solution and extracted with CH_2Cl_2. The CH_2Cl_2 extract was evaporated to give corresponding disuccinimidyl esters. All the cross-linking agents in Scheme S1, **3c**, and **4b** were synthesised according to this procedure.

3,8-Dioxa-5-decyne-1,10-dioic Acid Bis(2,5-dioxopyrrolidin-1-yl) Ester (1a): yield 89% (353 mg); mp 122–125 °C; IR (KBr) 1835, 1731 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.55 (s, 4 H), 4.43 (s, 4 H), 2.86 (s, 8 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.7, 165.5, 82.5, 64.1, 58.9, 25.5; HRMS (ESI) *m/z* calcd for $C_{16}H_{16}N_2NaO_{10}$ ($M + Na^+$) 419.0703, found 419.0713.

3,6,11,14-Tetraoxa-8-hexadecyne-1,16-dioic Acid Bis(2,5-dioxopyrrolidin-1-yl) Ester (1b): yield 85% (412 mg); oil; IR (KBr) 1835, 1727 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 4.52 (s, 4 H), 4.26 (s, 4 H), 3.82–3.76 (m, 4 H), 3.75–3.72 (m, 4 H), 2.86 (s, 8 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.6, 165.8, 82.3, 71.1, 69.0, 66.5, 58.7, 25.7; HRMS (ESI) *m/z* calcd for $C_{20}H_{24}N_2NaO_{12}$ ($M + Na^+$) 507.1227, found 507.1221.

3,10-Dioxa-5,7-dodecadiyne-1,12-dioic Acid Bis(2,5-dioxopyrrolidin-1-yl) Ester (2): yield 94% (395 mg); oil; IR (KBr) 1835, 1728 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.56 (s, 4 H), 4.45 (s, 4 H), 2.87 (s, 8 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.6, 165.2, 74.1, 71.8, 64.2, 59.2, 25.6; HRMS (ESI) *m/z* calcd for $C_{18}H_{16}N_2NaO_{10}$ ($M + Na^+$) 443.0703, found 443.0712.

Dodecanedioic Acid Bis(2,5-dioxopyrrolidin-1-yl) Ester (3c): yield 90% (382 mg); mp 155–157 °C; IR (KBr) 1835, 1750 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.84 (s, 8 H), 2.60 (t, J = 12.0 Hz, 4 H), 1.79–1.69 (m, 4 H), 1.40–1.30 (m, 12 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.1, 168.5, 31.0, 29.2, 29.04, 28.8, 25.7, 24.6; HRMS (ESI) *m/z* calcd for $C_{20}H_{28}N_2NaO_8$ ($M + Na^+$) 447.1743, found 447.1711.

3,6-Dioxaoctane-1,8-dioic Acid Bis(2,5-dioxopyrrolidin-1-yl) Ester (4a): yield 94% (350 mg); oil; IR (KBr) 1835, 1729 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.53 (s, 4 H), 3.86 (s, 4 H), 2.86 (s, 8 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.8, 165.9, 71.4, 66.6, 25.5; HRMS (ESI) *m/z* calcd for $C_{14}H_{16}N_2NaO_{10}$ ($M + Na^+$) 395.0703, found 395.0716.

3,6,9,12-Tetraoxatetradecane-1,14-dioic Acid Bis(2,5-dioxopyrrolidin-1-yl) Ester (4b): oil; IR (KBr) 1835, 1727 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.54 (s, 4 H), 3.82–3.79 (m, 4 H), 3.73–3.70 (m, 4 H), 3.67 (s, 4 H), 2.85 (s, 4 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.8, 166.0, 71.2, 70.6, 70.6, 66.5, 25.6; HRMS (ESI) *m/z* calcd for $C_{18}H_{24}N_2NaO_{12}$ ($M + Na^+$) 483.1227, found 483.1216.

Solid Phase Peptide Synthesis (SPPS). All peptides were synthesised by an automated peptide synthesiser using the standard Fmoc chemistry. Peptides were constructed on an Fmoc-NH-SAL resin (capacity 0.62 mmol/g). After the automated SPPS, *N*-terminal amino groups were acetylated with 5% Ac_2O in NMP over 10 min at room temperature. Peptide cleavage and side-chain deprotection of amino acids were carried out by treating with TFA-ethanedithiol-thioanisole-2-metylindole (90 : 5 : 5 : 0.1) over 2 h at room temperature.

Peptide Purification. Peptides synthesised above were purified by reversed-phase HPLC (column; COSMOSIL $5C_{18}$-AR-300 nakalai tesque, 10 × 150 mm) and eluted with 0.1 % TFA buffer and a 10–40 % CH_3CN (including 0.1% TFA) linear gradient (0–30 min) at a flow rate of 2.0 mL/min. The fractions of the peptides were monitored at 220 nm by a UV detector, and identified with ESI-MS. MS (ESI) *m/z* Peptide **A**: calcd for $C_{72}H_{113}N_{20}O_{25}$ ($M + H^+$) 1657.8, found 1657.6, Peptide **B**: calcd for $C_{72}H_{113}N_{20}O_{25}$ ($M + H^+$) 1657.8, found 1657.6.

Cross-linking Reactions. To a peptide solution (50 μL; 1.0×10^{-4} M in phospate buffer (pH 7.0)) was added a cross-linking agent (50 μL; 5.0×10^{-4} M in DMSO). The reaction mixture was incubated at 25 °C in a thermo-mixer. A solution of Lys monomer (5 μL; 0.5 M in H_2O) was added to the reaction mixture after 15, 30, 45, or 60 min in order to quench the reaction. The all cross-linked peptides were identified by ESI-MS ($M + Na^+$). The spectra of the cross-linked peptides by the acetylenic agents are presented in Fig. S2.

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(4) M. Kondo, Y. Shimizu, and A. Murata, *Agric. Biol. Chem.*, 1982, **46**, 913.

(5) V. Wittmann, S. Takayama, K. W. Gong, G. Weitz-Schmidt, and C.-H. Wong, *J. Org. Chem.*, 1998, **63**, 5137.

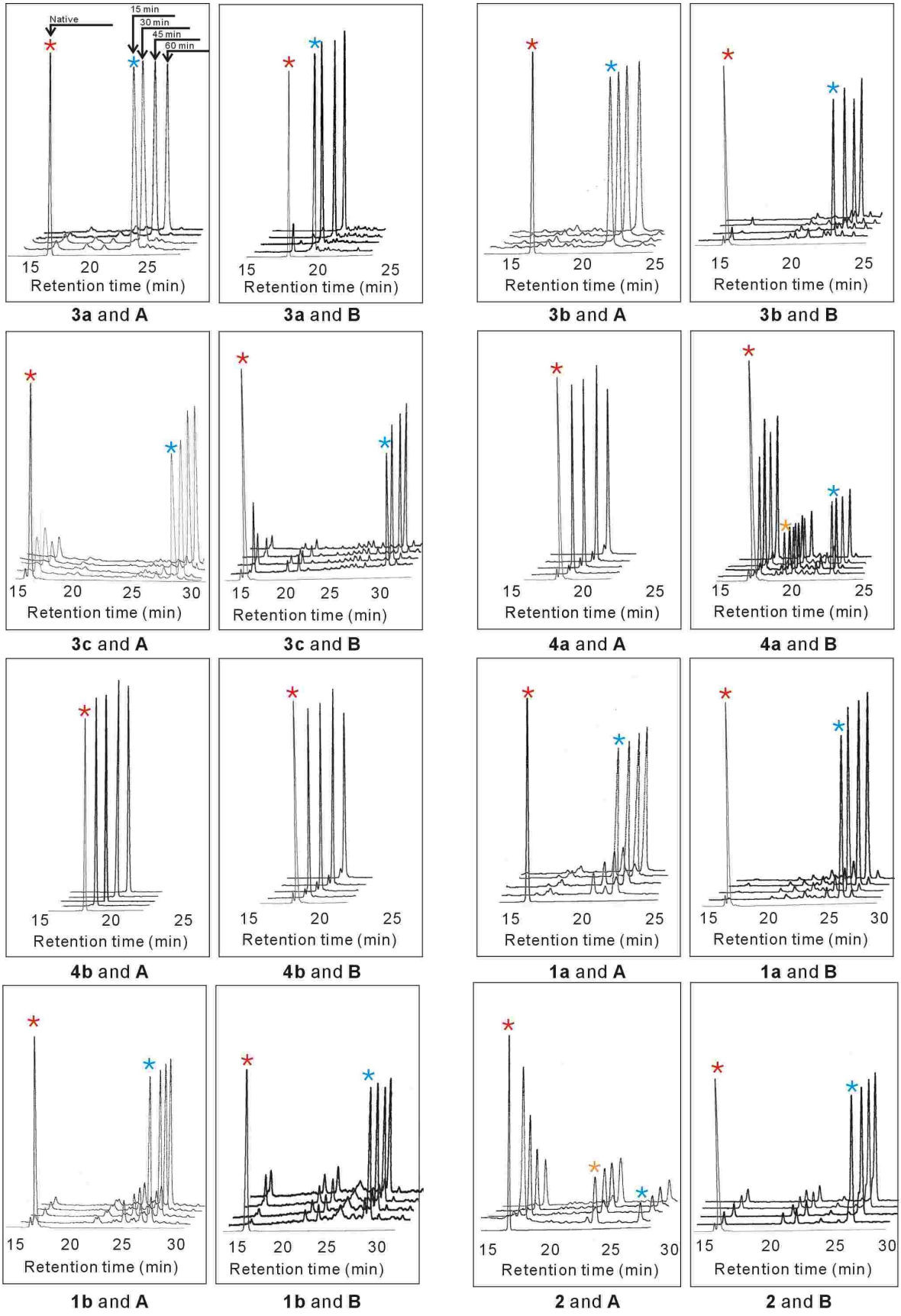

Fig. S1 HPLC profiles of the native and the cross-linked peptides; the native peptide (red star), the half-linked one (orange star), the cross-linked one (blue star) .

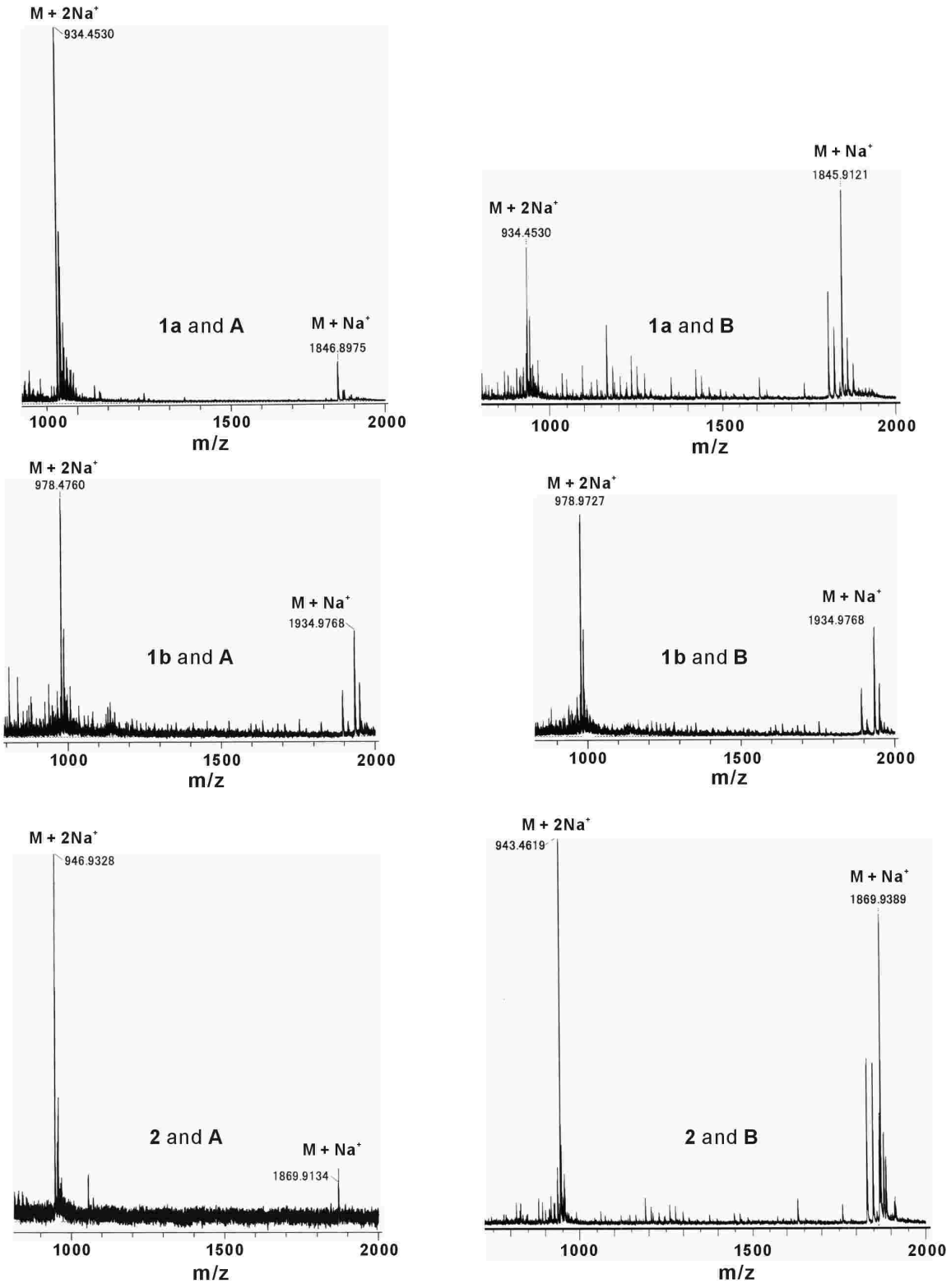

Fig. S2 ESI-MS spectra of the cross-linked peptides by acetylenic agents.