Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

Synthesis of MUC1 *Neo*glycopeptides using Efficient Microwave-Enhanced Chaotrope-Assisted Click Chemistry

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Peptide synthesis

General

All solvents and reagents were used as supplied. *O*-(Benzotriazol-1-yl)-*N,N,N′,N′′*-tetramethyluronium-hexafluorophosphate (HBTU), *N*-hydroxy-benzotriazole (HOBt) were purchased from Advanced Chemtech (Louisville, KY). Dimethylformamide (DMF) (AR grade) and acetonitrile (HPLC grade) were purchased from Ajax Chemicals, diisopropylethylamine (DIPEA), piperidine, and triisopropylsilane (TIS) were purchased from Aldrich. *N*-methylpyrrolidine (NMP) was purchased from Fluka. Trifluoroacetic acid (TFA) was purchased from Halocarbons.

Fmoc-amino acids were purchased from CEM or Advanced Chemtech (Louisville, KY). Fmoc-Ala-HMPP-linker was purchased from PolyPeptide Group. Tris(2-carboxyethyl)phosphine (TCEP, 0.5 M solution) was purchased from Global Science & Technology Ltd. Aminomethyl resin was synthesized using literature methods.[1]

Peptide Synthesis

Solid phase peptide synthesis was performed using a Liberty Microwave Peptide Synthesiser (CEM Corporation, Mathews, NC) using the Fmoc/^{t}Bu strategy. The Fmoc group was deprotected with 20% v/v piperidine in DMF for 30 seconds followed by a second deprotection for 3 min using a microwave power of 60 W for both deprotections. The maximum temperature for both deprotections was set at 75°C.

The coupling step was performed with 5 equivalents of Fmoc protected amino acid in DMF (0.2 M), 4.5 equivalents of HBTU in DMF (0.45 M) and 10 equivalents of DIPEA in NMP (2 M). All couplings were performed for 5 min at 25 W at a maximum temperature of 75°C except for the following amino acids: Fmoc–Arg(Pbf)–OH was double coupled using a 25 min room temperature coupling followed by a 5 min period at 25 W; Fmoc-His(Trt)-OH coupling was performed for 5 min at 25 W at a maximum temperature of 50 ^{o}C.

Following completion of the sequence, the peptide was released from the resin with concomitant removal of protecting groups by treatment with TFA/TIS/H_2O (38/1/1, v/v/v) at room temperature for 2.5 h as required. The crude peptide was precipitated with cold diethyl ether, isolated by centrifugation, washed with cold diethyl ether, dissolved in 1:1 (v/v) acetonitrile:water containing 0.1% TFA and lyophilised.

The crude peptide products were analysed for purity by analytical RP-HPLC (Dionex P680 equipped with a 4 channel UV detector) at 210 and 254 nm using a Gemini C18 (5 μ; 2.0 × 50 mm) column (Phenomenex) at 0.2 ml/min using a linear gradient. The solvent system used was A (0.1% TFA in H_2O) and B (0.1% TFA in MeOH). Peptide masses were confirmed by LC-MS (Dionex Ultimate 3000 equipped with a Thermo Finnegan MSQ mass spectrometer) using ESI in the positive mode.

Masses of synthetic peptides were:

Peptide **8**: obs. 1882.0249 Da, calc. 1882.0373 Da.

Peptide **9**: obs. 1890.0356 Da, calc. 1890.0593 Da.

Peptide **10**: obs. 1876.0160 Da, calc. 1876.0325 Da.

Peptide **11**: obs. 1870.0086 Da, calc. 1870.0277 Da.

Peptide **12**: obs. 1892.0329 Da, calc. 1892.0765 Da.

Peptide **13**: obs. 1886.0313 Da, calc. 1886.0717 Da.

Purification of crude peptides was performed by semi-prep RP-HPLC (Dionex P680 equipped with a 4 channel UV detector) at 210 and 254 nm using a Gemini C18 (5 μ; 10 × 250 mm) column (Phenomenex) at 5.0 ml/min using a shallow gradient of increasing concentrations of solvent B. The solvent system used was A (0.1% TFA in H_2O) and B (0.1% TFA in MeOH). Fractions containing the pure target peptide were identified by analytical RP-HPLC, then combined and lyophilized.

HPLC chromatograms and mass spectra of propargylated peptides 8-13

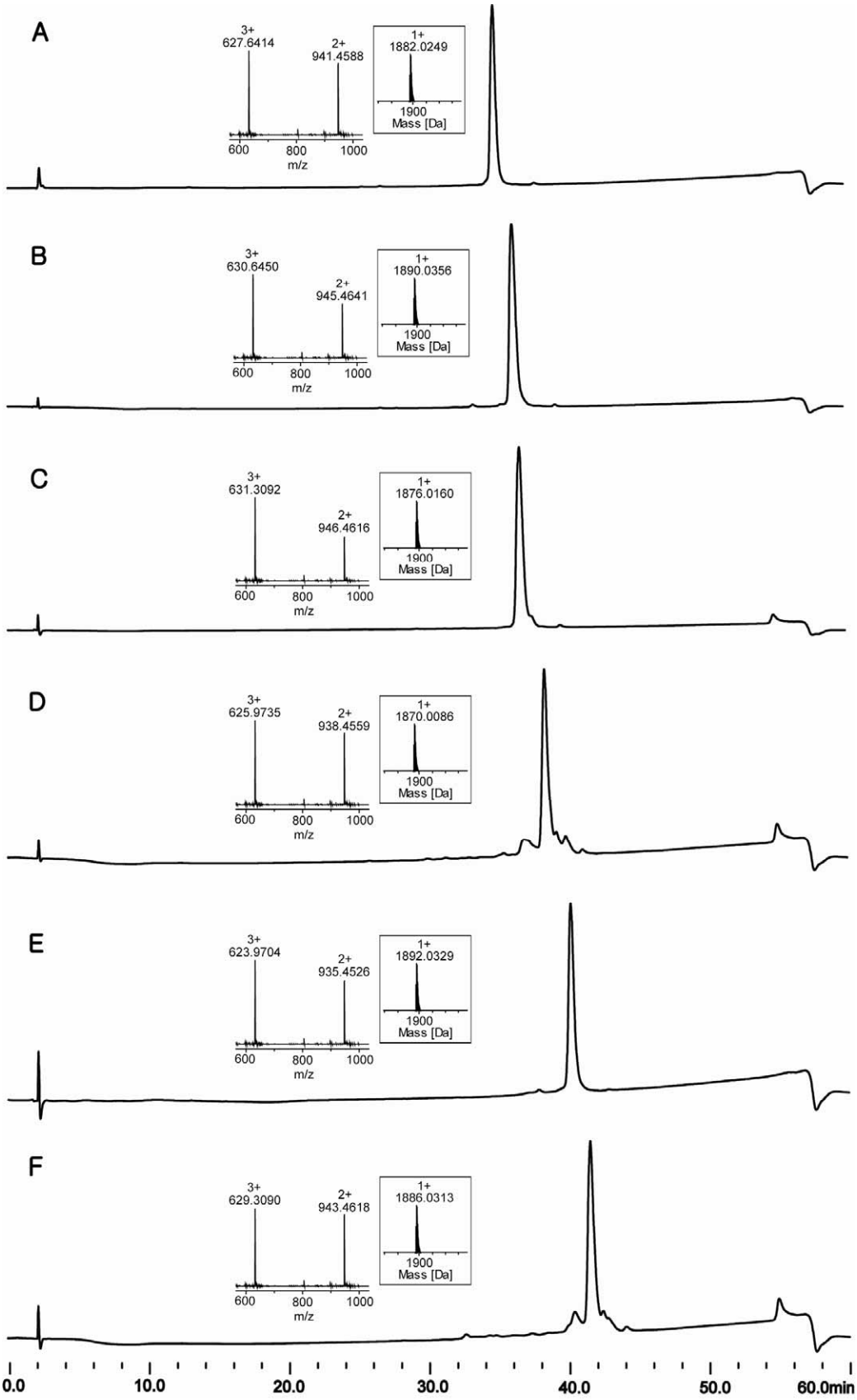

Figure S1. Purified propargylated peptides (RP-HPLC gradient ran at 1%B min^{-1} from 1%B to 60%B): (A) Peptide **8**; (B) Peptide **9**; (C) Peptide **10**; (D) Peptide **11**; (E) Peptide **12**; (F) Peptide **13**.

Examples of unsuccessful click reactions using $CuSO_4$/NaAsc system

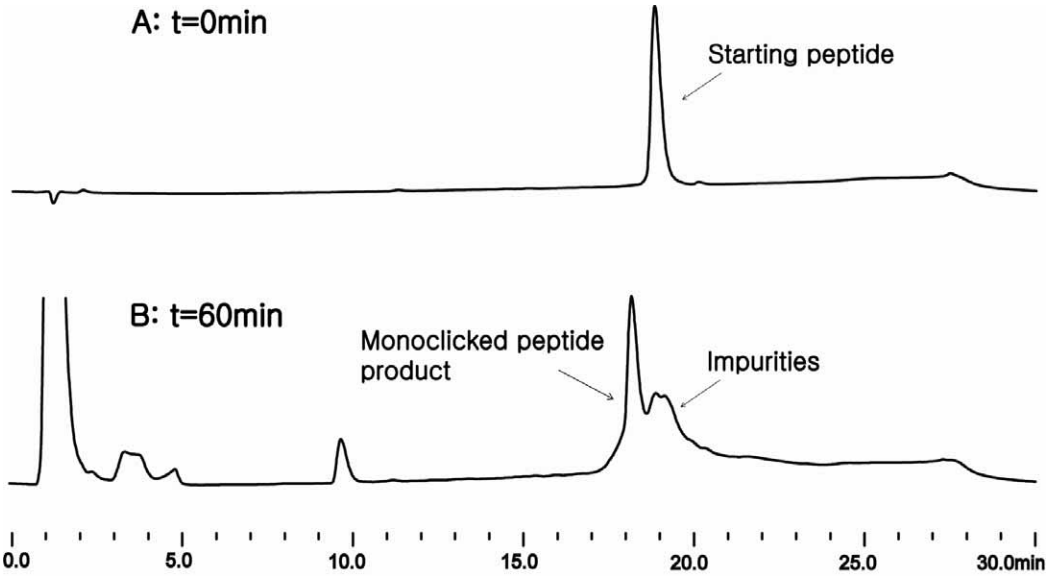

Figure S2. Click reaction using peptide **8**. Concentrations of 3 mM of the peptide and 20 mM of $CuSO_4$ and NaASc were used. After 60 min at room temperature (B), the desired mono-clicked *neo*glycopeptide was observed, with significant amount of unwanted side products.

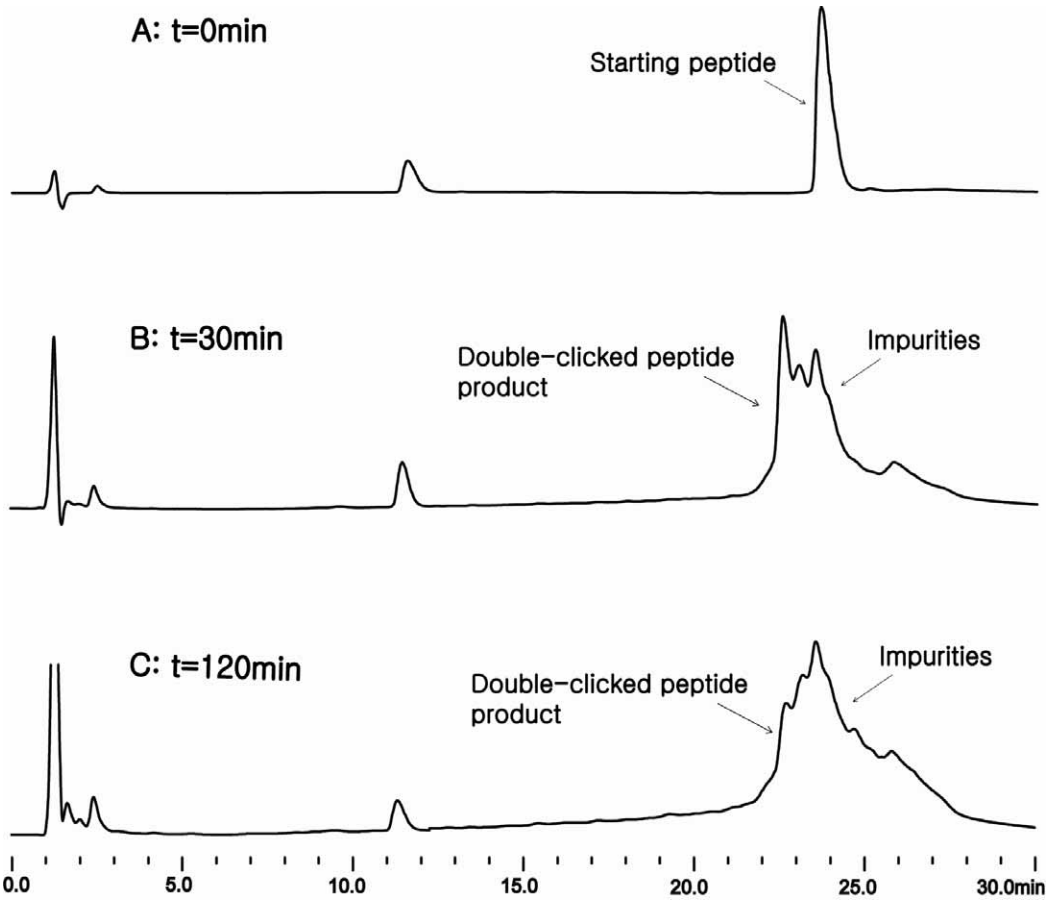

Figure S3. Click reaction using peptide **10**. Concentrations of 3 mM of the peptide and 40 mM of $CuSO_4$ and NaASc were used. After 30 min at room temperature (B), the desired di-clicked *neo*glycopeptide was observed with impurities. The increased amount of impurities over time (C) significantly decreased product yield and hindered purification.

HPLC chromatograms of $CuSO_4$/TCEP in phosphate buffers with/without 6 M GnHCl.

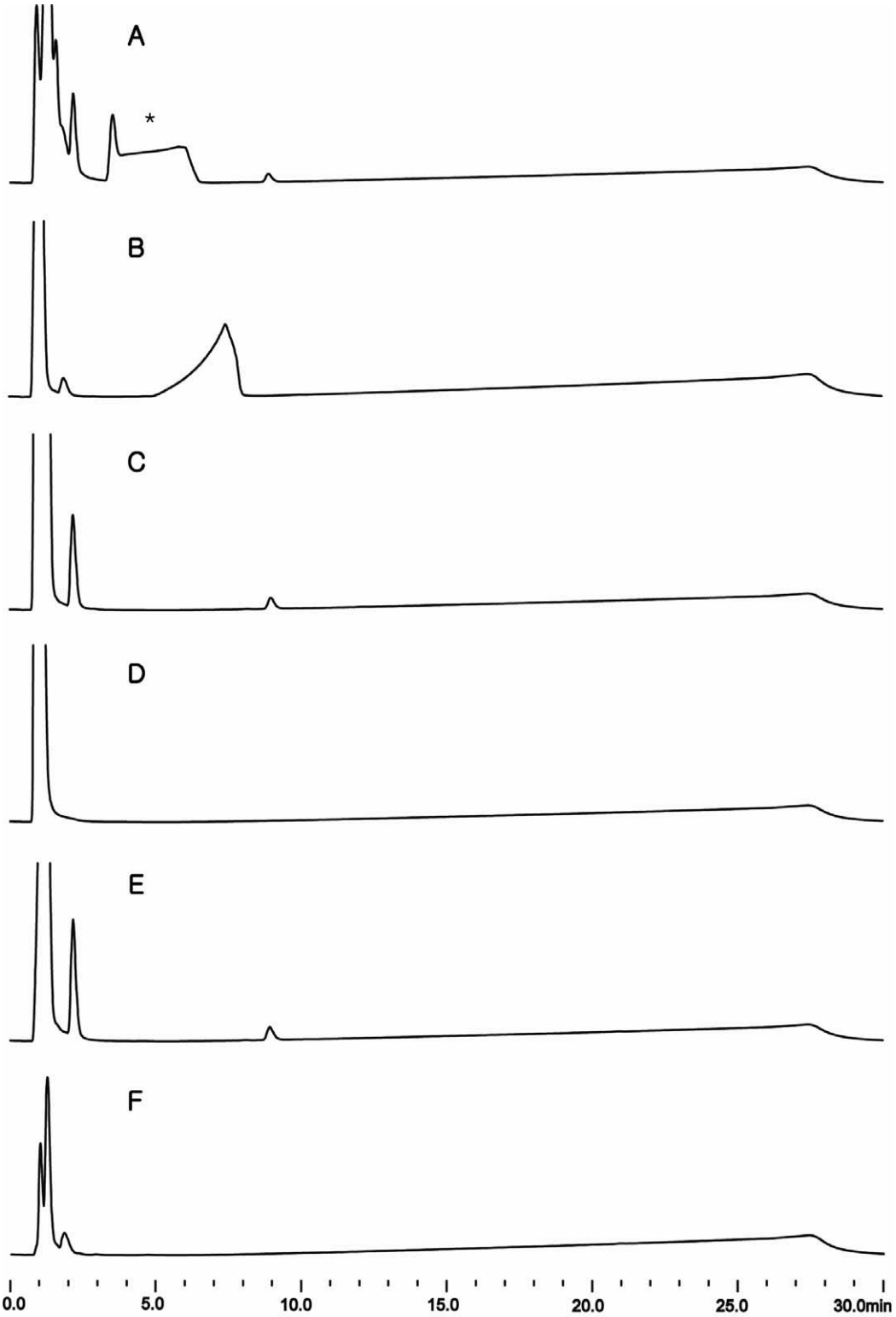

Figure S4. (A) $CuSO_4$/TCEP in 6 M GnHCl/phosphate buffer. The broad peak pattern (*) was observed in successful click reactions. (B) $CuSO_4$/TCEP in phosphate buffer only. (C) $CuSO_4$ in 6 M GnHCl/phosphate buffer. (D) $CuSO_4$ in phosphate buffer only. (E) TCEP in 6 M GnHCl/phosphate buffer. (F) TCEP in phosphate buffer only. In C-F, the broad peak was not observed. This strongly suggests that it the peak may correspond to product formed from the reduction of $CuSO_4$ by TCEP, possibly Cu^{1+} species. In all successful click reactions, the broad peak observed in (A) was present.

Successful click reactions with HPLC chromatograms and mass spectra

General procedure of click reaction

Peptide (3 mM) and $CuSO_4$/TCEP (20 mM per 3 mM of propargyl group) were mixed in a degassed solution of 6 M GnHCl/0.2 M Na_2HPO_4 buffer. After 30 min, 3´-Azido-1´-propyl-2-acetamido-2-deoxy-α-D-galactopyranoside **6** (1.5 equiv. per propargyl group) was added and the reaction was carried out under argon with microwave irradiation (25 W). The reaction progress was monitored by analytical RP-HPLC, and upon completion of the reaction the crude product was isolated by solid phase extraction then lyophilized. The crude product was purified by semi-prep RP-HPLC to afford the desired clicked *neo*glycopeptide products.

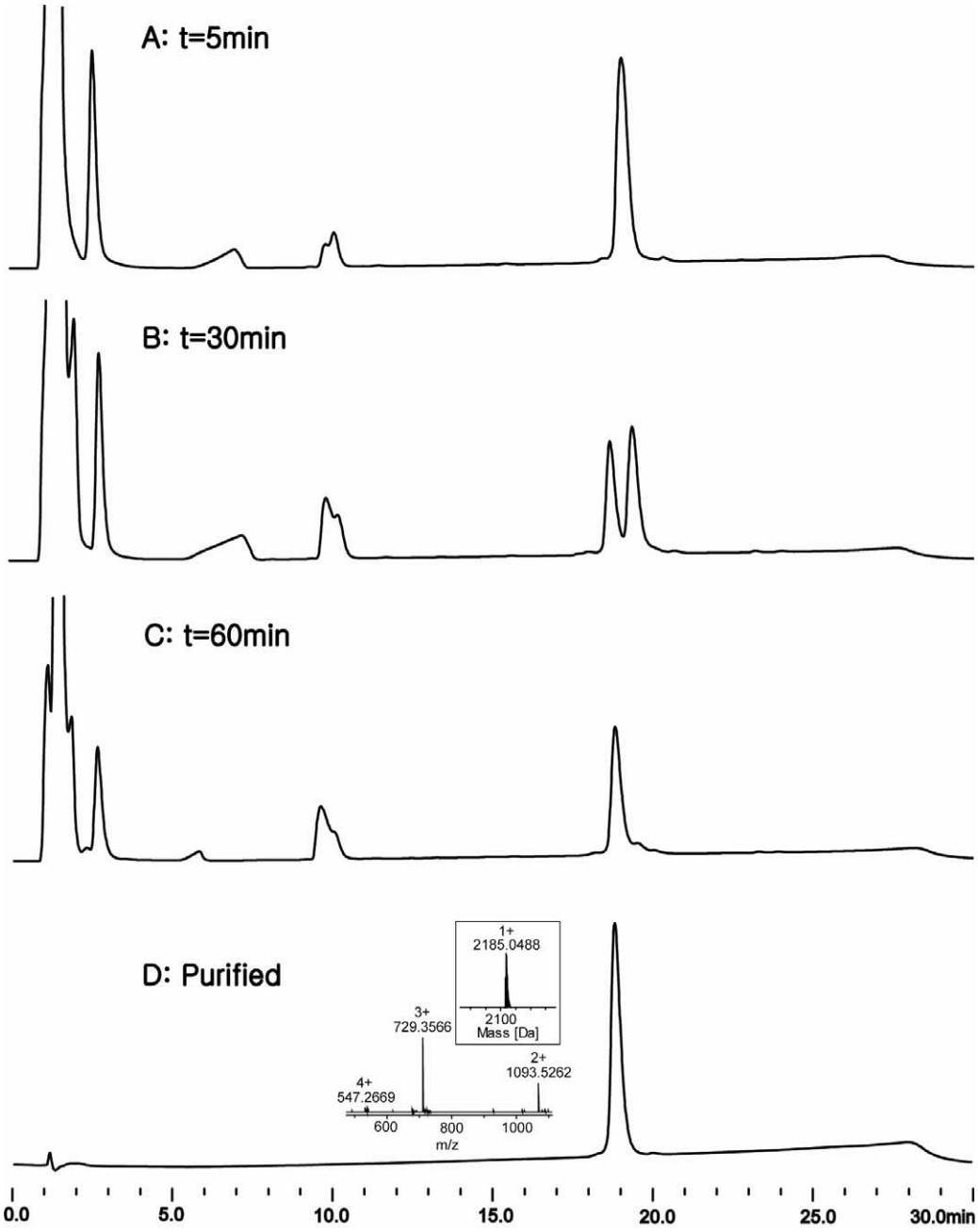

Figure S5. Click reaction using peptide **8**. Solvent: 6 M GnHCl/0.2 M Na_2HPO_4 buffer. Peptide conc.: 3 mM. $CuSO_4$/TCEP conc.: 20 mM. Microwave: 25 W. The mono-clicked *neo*glycopeptide product was observed at t=30min (B) and the reaction was essentially complete at t=60min. The crude product was purified by RP-HPLC to give the pure mono-clicked *neo*glycopeptide product (D): obs. 2185.0488 Da, calc. 2185.3293 Da.

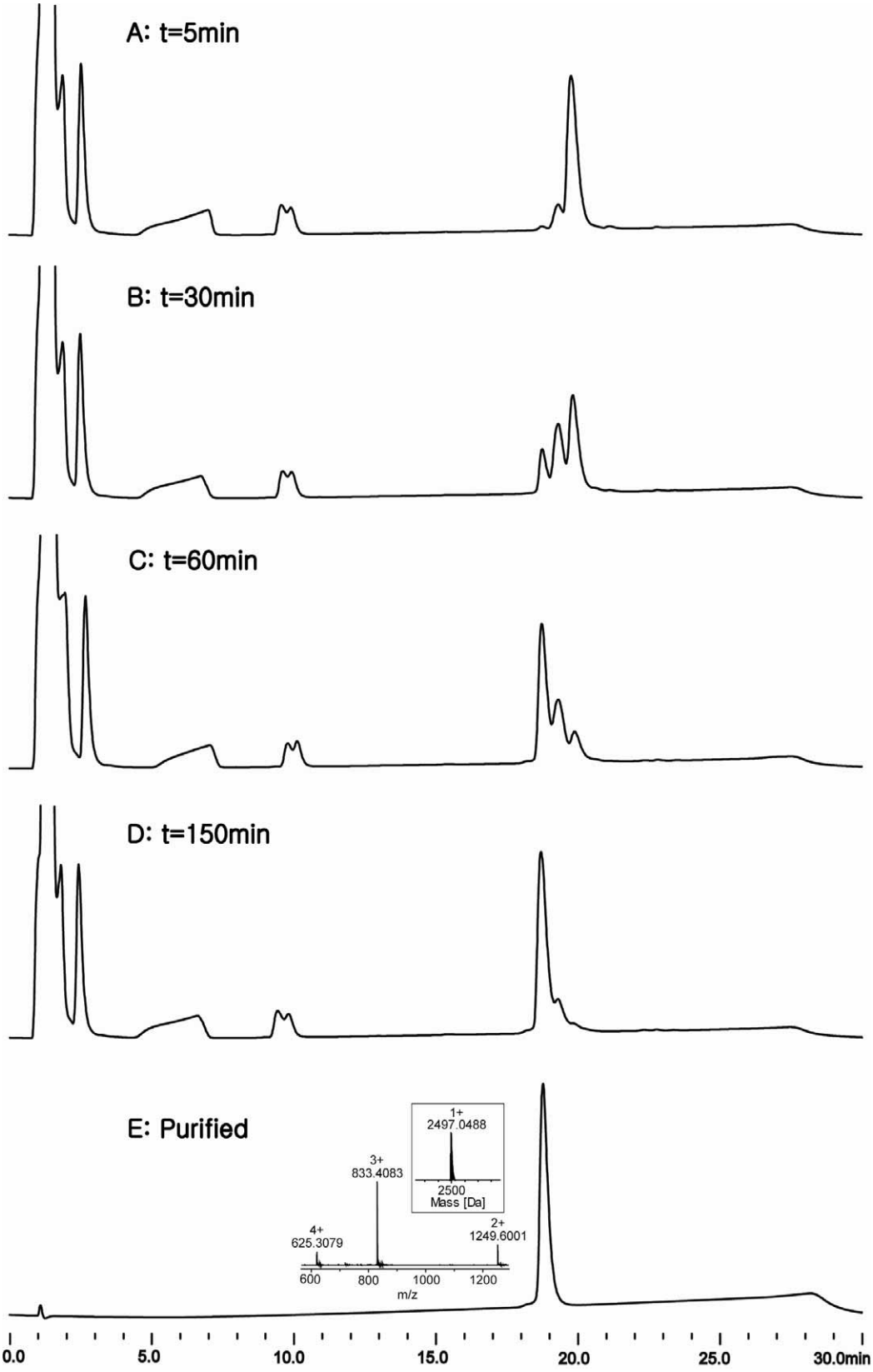

Figure S6. Click reaction using peptide **9**. Solvent: 6 M GnHCl/0.2 M Na_2HPO_4 buffer. Peptide conc.: 3 mM. $CuSO_4$/TCEP conc.: 40 mM. Microwave: 25 W. The mono-clicked intermediates and the di-clicked *neo*glycopeptide product were observed at t=30min (B) and the reaction was essentially complete at t=150min (D). The crude product was purified by RP-HPLC to give the pure di-clicked *neo*glycopeptide product (E): obs. 2497.0488 Da, calc. 2497.6513 Da.

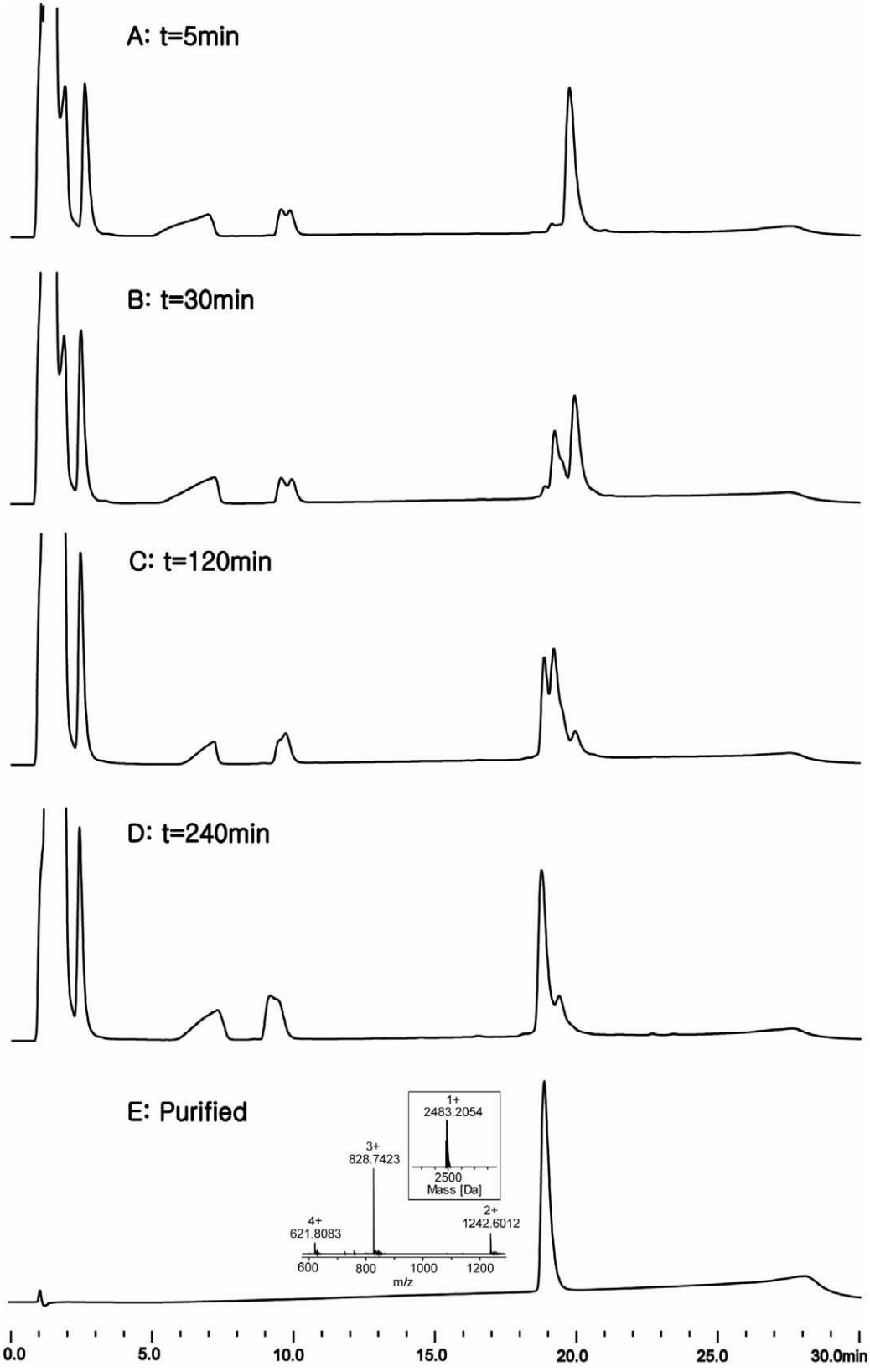

Figure S7. Click reaction using peptide **10**. Solvent: 6 M GnHCl/0.2 M Na_2HPO_4 buffer. Peptide conc.: 3 mM. $CuSO_4$/TCEP conc.: 40 mM. Microwave: 25 W. The mono-clicked intermediates and the di-clicked *neo*glycopeptide product were observed at t=120min (C) and the reaction was essentially complete at t=240min (D). The crude product was purified by RP-HPLC to give the pure di-clicked *neo*glycopeptide product (E): obs. 2483.2054 Da, calc. 2483.6245 Da.

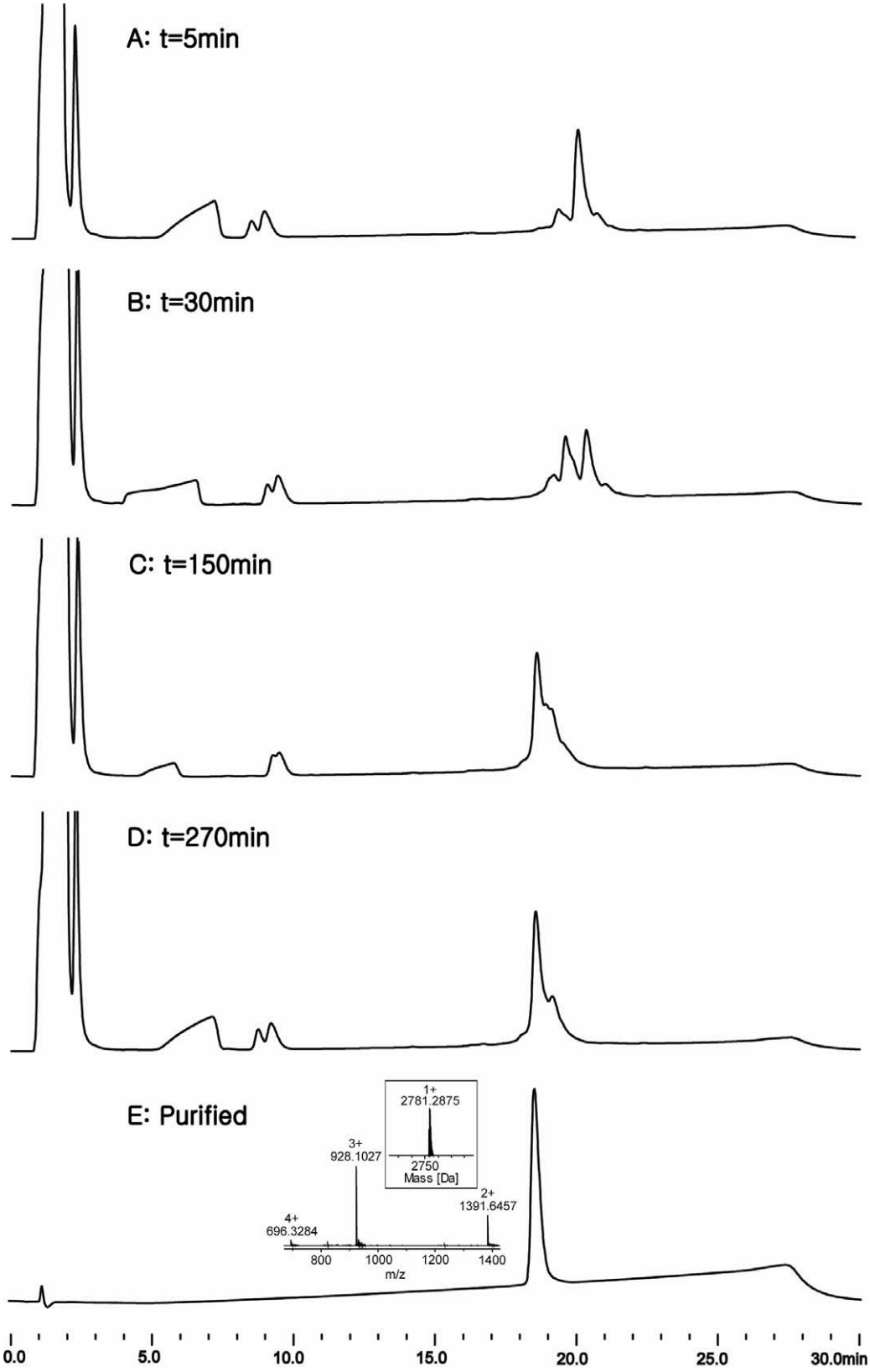

Figure S8. Click reaction using peptide **11**. Solvent: 6 M GnHCl/0.2 M Na_2HPO_4 buffer. Peptide conc.: 3 mM. $CuSO_4$/TCEP conc.: 60 mM. Microwave: 25 W. The mono- and di-clicked intermediates were observed at t=30min (B). The tri-clicked *neo*glycopeptide product was observed at t=150min (C) and the reaction was essentially complete at t=270min (D). The crude product was purified by RP-HPLC to give the pure tri-clicked *neo*glycopeptide product (E): obs. 2781.2875 Da, calc. 2781.9197 Da.

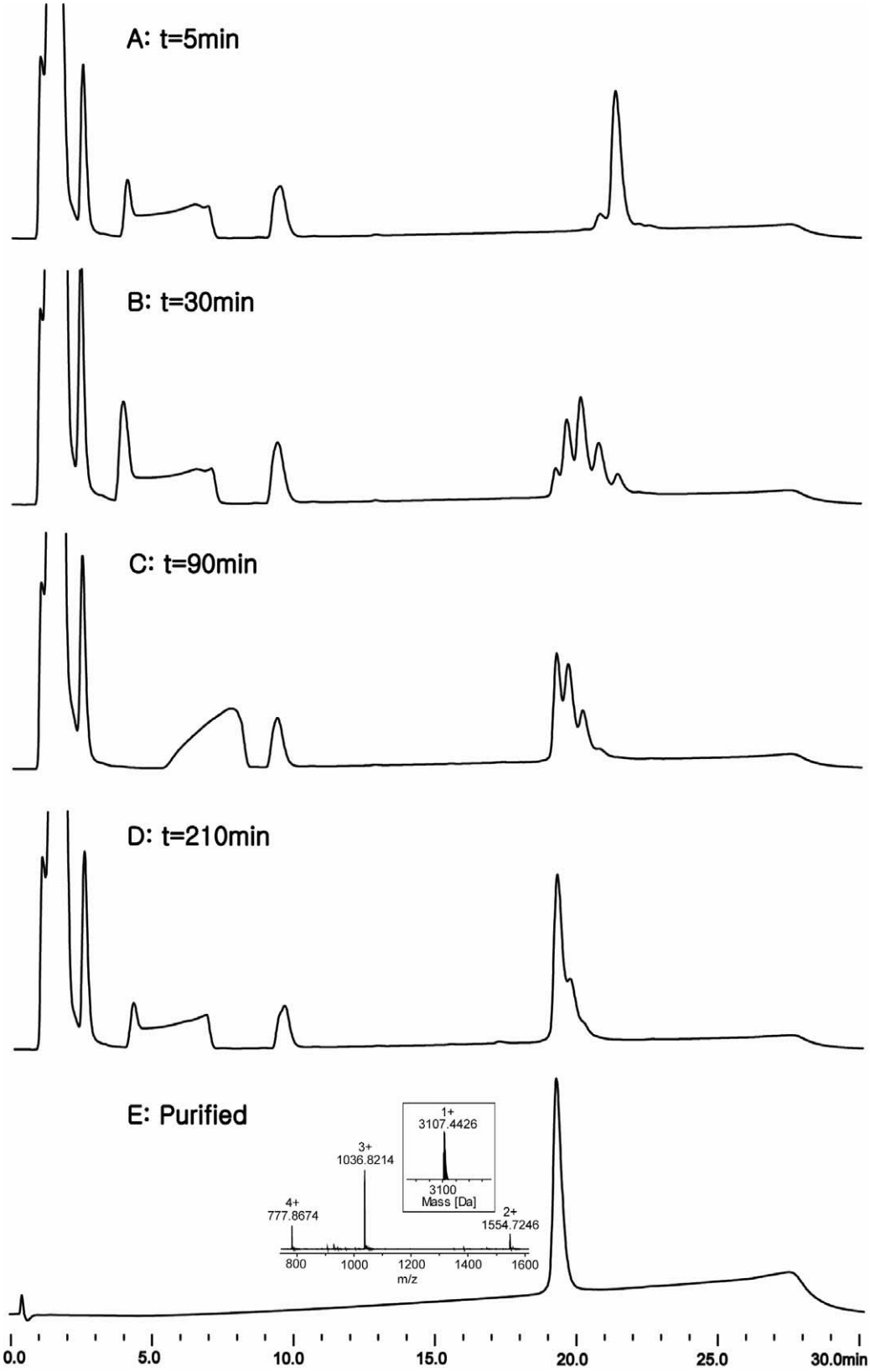

Figure S9. Click reaction using peptide **12**. Solvent: 6 M GnHCl/0.2 M Na_2HPO_4 buffer. Peptide conc.: 3 mM. $CuSO_4$/TCEP conc.: 80 mM. Microwave: 25 W. The mono-, di-, and tri-clicked intermediates were observed at t=30min (B). The tetra-clicked *neo*glycopeptide product was observed at t=90min (C) and the reaction was essentially complete at t=210min (D). The crude product was purified by RP-HPLC to give the pure tetra-clicked *neo*glycopeptide product (E): obs. 3107.4426 Da, calc. 3108.2685 Da.

References

1. A. R. Mitchell, S. B. H. Kent, M. Engelhard and R. B. Merrifield, *J. Org. Chem.*, 1978, **43**, 2845-2852.