Electronic Supplementary Information (ESI)

A New Labeling Agent for CE/MS N-Glycans Analysis

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Synthesis

Dextran standard were purchased from Sigma Aldrich (St. Louis, MO, USA), others chemical reagents and solvents for synthesis were purchased from Alfa Aesar(Ward Hill, MA, USA) and were used without further purification. HPLC grade methanol was purchased from Fisher Scientific (Fair Lawn, NJ, USA).
^{1}H NMR spectra were recorded on a Mercury300 MHz NMR (Varian Inc.) spectrometer at room temperature. All chemical shifts are relative to an internal standard of tetramethylsilane (δ = 0.000 ppm) and coupling constants are given in Hz. MS spectra were obtained on Agilent 6320 ion trap Mass spectrometer (Palo Alto, CA, USA).
The following abbreviations are used in this manuscript:
DIPEA: N, N-diisopropylethylamine,
DMSO: Dimethylsulfoxide, DIEA: Diethylamine.
All the three precursors were synthesized as mentioned1-3.
1mmol precursor was dissolved in 15ml acetone.5 00 μl DIPEA(3mmol) and 200 μl Hydrazine hydrate were added in sequence. The reaction was carried for further 3h under reflux. The reaction solution was pulled into crushed ice. The solution was extracted with diethyl ether twice, and concentrated.

	MS m/z	1H NMR
T-1	172([M+H]+)	1.573(s,2H), 3.974(s,6H), 6.479(s,1H)
T-2	213([M+H]+)	
T-3	254([M+H]+)	1.120,1.144,1.167(t,12H), 3.506,3.530,3.553,3.577(m,8H), 3.925(s,2H), 5.694(s,1H)

Labeling reaction
2-AB, sodium cyanoborohydride, acetic acid, dextran 1000 standard was purchased from Sigma Aldrich(St. Louis, MO, USA). APTS was provided from carbohydrate labeling and analysis kit (Beckman Coulter, Brea, CA, USA).
1 μg dextran 1000 standard was applied in the each labelling reaction.
2-AB and T-3 labeling process was as mentioned by Bigge et. al. 4
APTS labeling process was as mentioned by Guttman et. al. 5

All the labeled products were purified by GlycoClean S Cartridges purchased from Prozyme (Hayward, CA, USA) following the introduction. The purified labeled products was dried by N2 steam and reconditioned with 100 μl water .

CE/MS performance

CE/MS detection was carried on Beckman Coulter PA800 plus CE system (Brea, CA, USA) and Agilent 6320 ion trap Mass spectrometer (Palo Alto, CA, USA) with CESI CE/MS interface provided by Beckman Coulter, BGE: 20mM pH 3.8 NH4Ac with 30% methanol, injection: 10Psi×5s, separation voltage: 30kV, MS scan range: 300-2200, spray voltage: 1400V, Dry gas: 5l/min, temperature: 325℃

SI-1

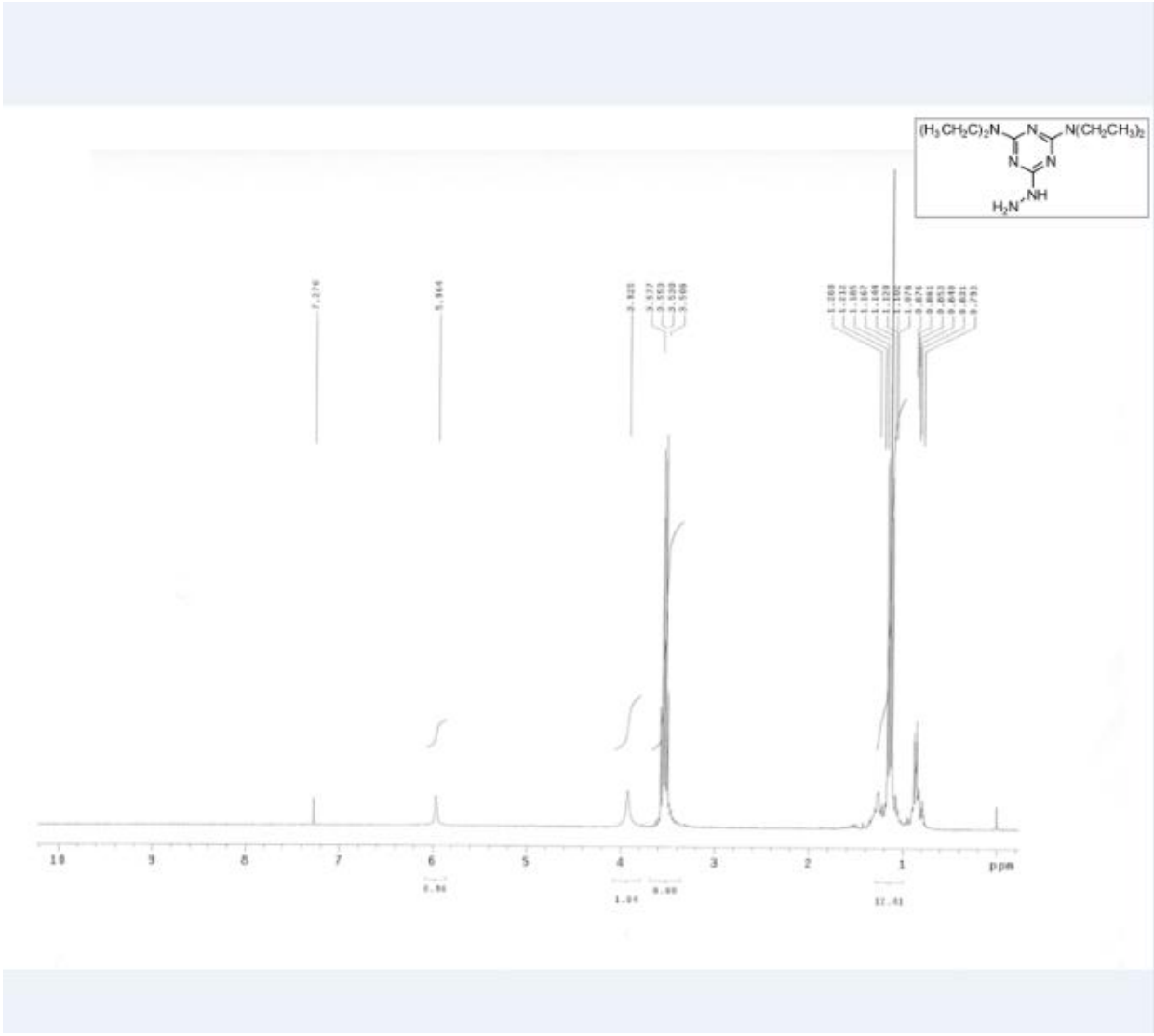
Where N is theoretical plate number, R_s is resolution, μ_a is apparent mobility, l is the total length of capillary, E is the electric field strength, D is the diffusion coefficient of the analytes, and $\frac{\Delta \upsilon_{ep}}{\upsilon_{ep}}$ is the relative velocity difference between two analytes.

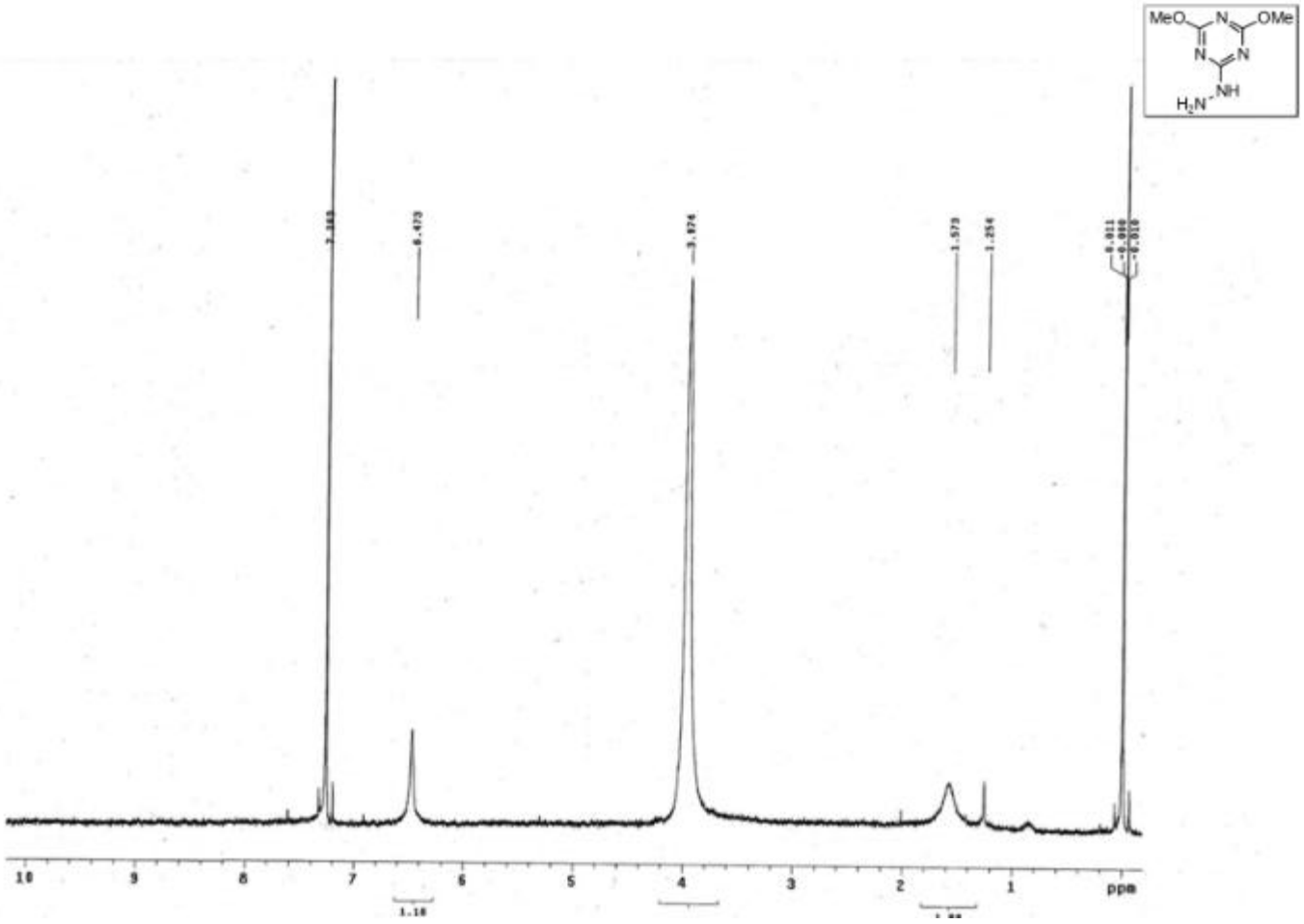
The EOF in neutral coated can be ignored and the above equation becomes:

The theoretical plate number and resolution can be transferred to be

$$R_s = 0.144\sqrt{\frac{FzlE}{\eta \pi rD}}\left(\frac{r_2D_2 - r_1D_1}{r_2D_2 + r_1D}\right)$$

Where F is Faraday constant, η is the viscosity of the solution, r is radius of ions, z is the charges number of analytes.


(H3CH2C)2N
N
N(CH2CH3)2
N
N
H2N
NH
7.376
5.944
3.925
3.577
3.553
3.539
3.508
1.208
1.192
1.165
1.147
1.129
1.102
1.076
0.876
0.861
0.849
0.833
0.793
10
9
8
7
6
5
4
3
2
1
ppm
6.00
1.04
6.00
12.41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OMe
N
N
NH
H_2N
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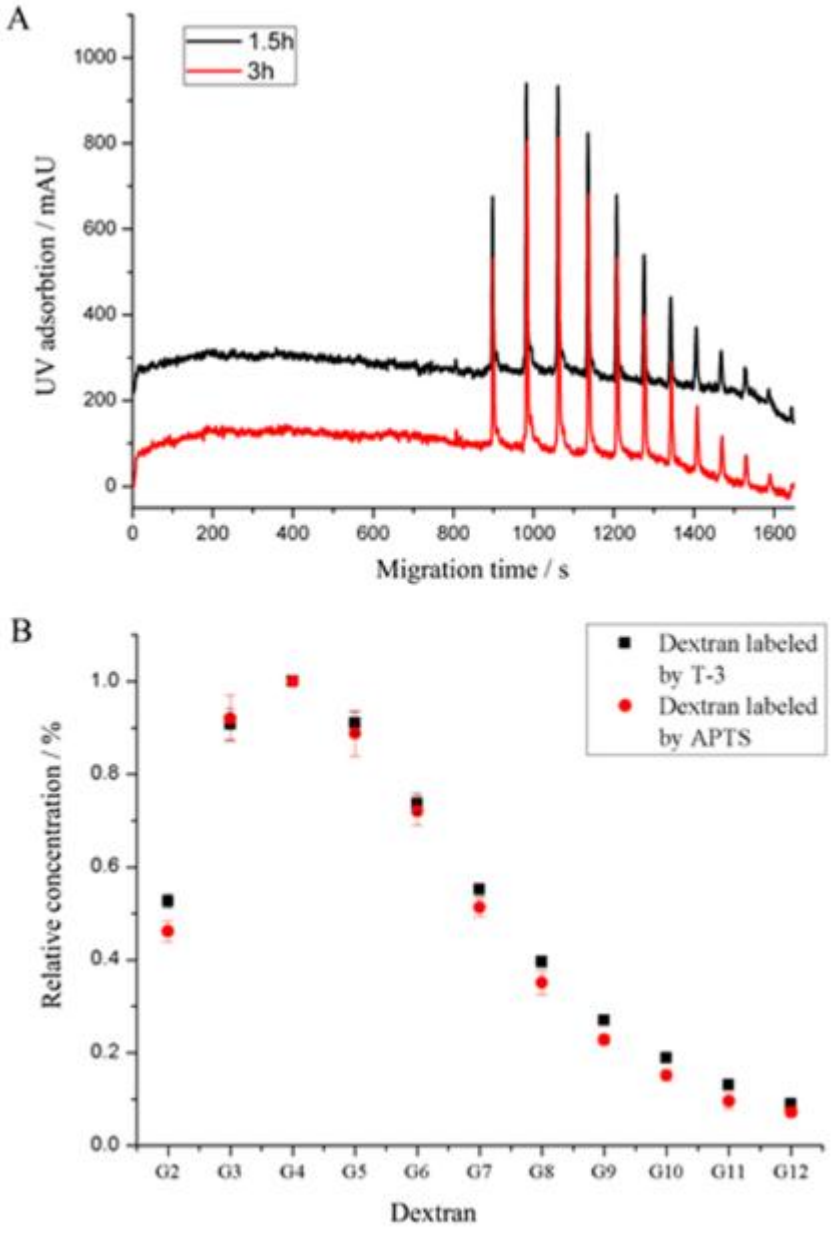
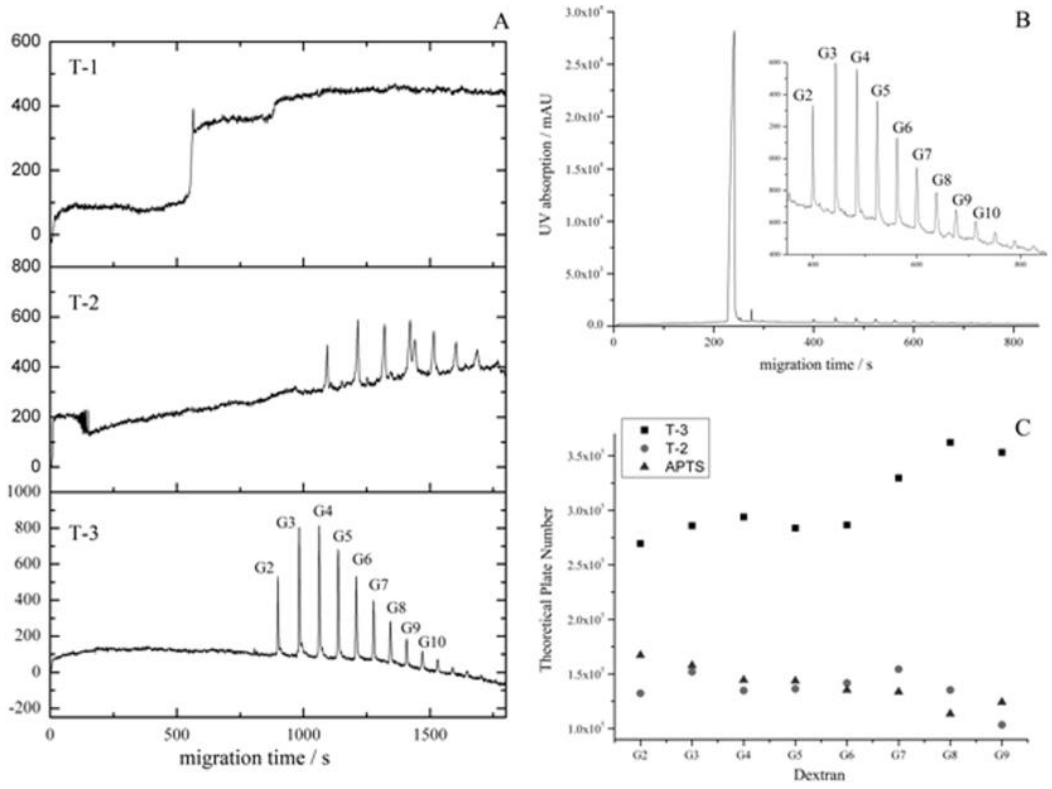
6.473
3.974
1.573
1.254
0.911
0.888
0.870
10
9
8
7
6
5
4
3
2
1
ppm
1.18



SI-2 Labeling reaction efficiency of new labelling reagent T-3

(A) Results of CE separation of dextran with different labelling time

(B) Comparison between the new labelling reagent T-3 and APTS in optimized conditions



SI-3 The influence of the charges carried by labeling reagents on CE separation of glycans

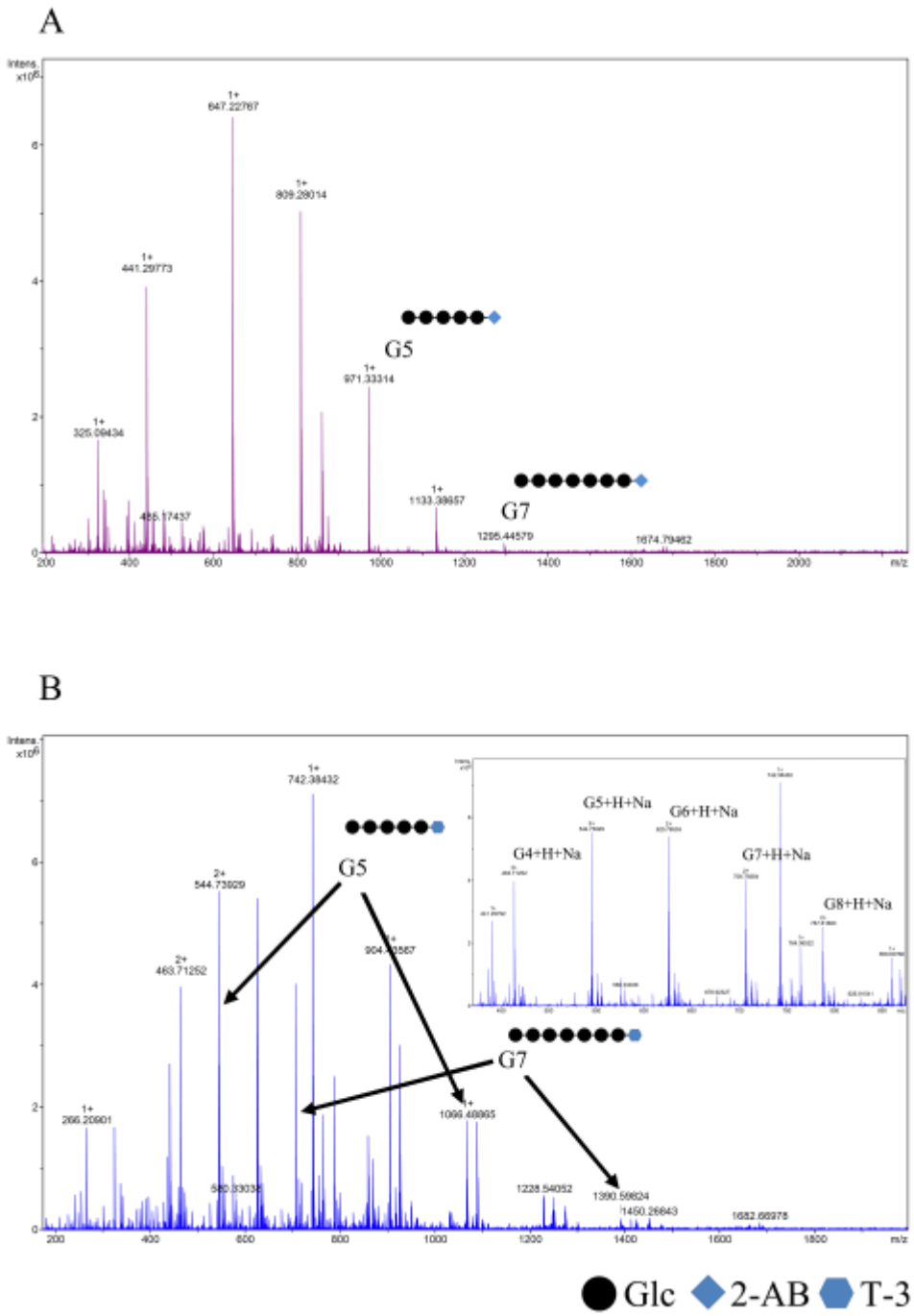
All the CE separation was performed on Beckman Coulter PA 800 plus CE system. All the glycans samples were purified by the GlycoClean S cartridge.

(A) CE separation of labeled dextran with T-1/2/3CE condition: 60 cm total length NHO-coated capillary provided by Beckman Coulter, BGE: 20mM pH 3.8 NH4Ac purchased from Sigma-Aldrich (St. Louis, MO, USA), 3 s injection under 0.5Psi, separation voltage 30kV, detection wave length: 254nm

(B) CE separation of APTS labeled dextran

CE condition: 60 cm total length NHO-coated capillary, BGE: 20mM pH 7 NH4Ac, 3s injection under 0.5Psi, separation voltage 30kV, detection wave length: 214nm

(C) Comparison of the separation efficiency for T-2/3 or APTS labeled dextran



SI-4 The mass spectra of 2-AB/T-3 labeled dextran (A/B) by high resolution ESI-FTICR mass spectrometer

The HR-MS was performed on Bruker APEX IV FTICR- MS (Billerica, MA, USA). All the samples were in the same concentration and diluted 10folds with methanol prior to MS analysis. In section B, double charged G4 to G8 could be found in the zoomed spectrum.

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3. M. M. Naseer, D.-X. Wang, L. Zhao, Z.-T. Huang and M.-X. Wang, *The Journal of Organic Chemistry*, 2011, **76**, 1804-1813.
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