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Electronic Supplementary Information
Deuterated Hydrazino-*s*-triazine as highly-efficient labeling reagent for N-Glycans Relative Quantification Analysis by using Electrospray Ionization Mass Spectrometry

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Contents

Experimental Section

Synthesis of labelling reagents.

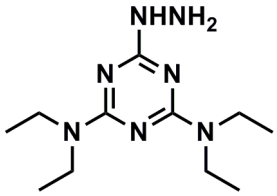
General

^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AVANCE III 500 NMR spectrometer at 500 and 125 MHz, respectively. Chemical shifts (δ) were reported in ppm and respectively referenced to internal standard tetramethylsilane (TMS) and solvent signals (TMS, 0 ppm for ^{1}H NMR and $CDCl_3$, 77.0 ppm for ^{13}C NMR). HRMS data were recorded on Thermo Q-Exactive mass spectrometer. Silica gel (200-300 mesh) was used for flash column chromatography, eluting with ethyl acetate/ petroleum ether mixture.

Materials

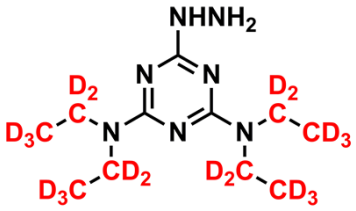
Cyanuric chloride (98%) was purchased from Alfa Aesar. Diisopropylethylamine (99%) was purchased from J&K Chemicals. Diethylamine (A. R.), hydrochloric acid (A. R.), hydrazine hydrate (85%, A. R.), petroleum ethe r(bp: 60~90 ^{o}C, A. R.), ethyl acetate (A. R.), tetrahydrofuran (A. R.), dichloromethane (A. R.), sodium chloride (A. R.), potassium hydroxide (A. R.) and sodium sulfate anhydrous (A. R.) were all purchased from Beijing Tong Guang Fine Chemicals Company. D_{11}-diethyamine (isotopic purity 99.6%) was purchased from CDN isotopes (Quebec, Canada).

Synthesis of 2-hydrazino-4,6-bis-(diethylamino)-*s*-triazine (denoted as HDEAT)[1]



Diisopropylethylamine (5.16 mL, 29.81 mmol) and diethylamine (3.07 mL, 29.81 mmol) in dichloromethane (10 mL) was added dropwise to a suspension of cyanuric chloride (2.5 g, 13.55 mmol) in dichloromethane (50 mL) under ice-cooling for 1 h. The reaction mixture was stirred for 24 h and slowly returned to room temperature. Then, it was washed with 2 mol/L HCl and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum to afford crude product, which was chromatographed on silica gel with petroleum ether-ethyl acetate (20:1) to obtain pure intermediate. 85% hydrazine hydrate (4 mL) was added dropwise to intermediate solution in THF (50 mL). The reaction mixture was heated to reflux overnight. After cooling, KOH (2.1 g) in H_2O (20 mL) was added and stirred for 15 min. Then, it was extracted with dichloromethane. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum to afford a crude product, which was recrystallized from a mixture of petroleum ether and diethyl ether to give pure product. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.88 (s, 1H), 3.91 (s, 2H), 3.54 (q, *J*=7.0 Hz, 8H), 1.14 (t, *J*=7.0Hz, 12H). ^{13}C NMR (125 MHz, CDCl3) δ: 169.0, 164.5, 41.0, 13.4. HRMS-ESI calcd for $[M+H]^+$ 254.2088; found: 254.2081

Synthesis of 2-hydrazino-4,6-bis-(d_{10}-diethylamino)-*s*-triazine (denoted as d_{20}-HDEAT)



The synthetic route of d_{20}-HDEAT was the same as HDEAT except the replacement of diethylamine by d_{11}-diethylamine. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.77 (s, 1H), 3.91 (s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.96, 164.51, 40.14, 12.33. HRMS-ESI calcd for $[M+H]^+$ 274.3343; found: 274.3341

Tables and Figures

Table S1 The intergrated peak areas of HDEAT and d_{20}-HDEAT labelled glycans from human serum and glycan abundance ratios.

Glycan Compositions	Peak Area of HDEAT Labelled Glycans	Peak area of d_{20}-HDEAT labelled Glycans	Glycan Abundance Ratio	Glycan Abundance Ratio (Corrected by Internal Standard)
Hex_7 (internal standard)	3.98E+06	3.68E+06	1.08	1.00
$Hex_3HexNac_3$	1.18E+06	1.06E+06	1.11	1.03
$Hex_3HexNac_4$	1.80E+06	1.69E+06	1.07	0.99
$Hex_3HexNac_5$	7.98E+06	7.86E+06	1.02	0.94
$Hex_3HexNac_3Fuc_1$	8.88E+06	8.10E+06	1.10	1.01
$Hex_3HexNac_4Fuc_1$	1.37E+08	1.27E+08	1.08	1.00
$Hex_3HexNac_5Fuc_1$	3.72E+07	3.22E+07	1.16	1.07
$Hex_4HexNac_2$	6.75E+05	6.71E+05	1.01	0.93
$Hex_4HexNac_3$	2.48E+06	2.30E+06	1.08	1.00
$Hex_4HexNac_4$	1.68E+07	1.54E+07	1.09	1.01
$Hex_4HexNac_5$	9.35E+06	9.32E+06	1.00	0.93
$Hex_4HexNac_3Fuc_1$	1.77E+06	1.58E+06	1.12	1.04
$Hex_4HexNac_4Fuc_1$	3.02E+08	2.65E+08	1.14	1.05
$Hex_4HexNac_5Fuc_1$	7.68E+07	6.60E+07	1.16	1.08
$Hex_4HexNac_3NeuAc_1$	5.98E+05	5.52E+05	1.08	1.00
$Hex_4HexNac_4NeuAc_1$	1.44E+06	1.31E+06	1.10	1.02
$Hex_4HexNac_5NeuAc_1$	2.94E+05	2.85E+05	1.03	0.95
$Hex_4HexNac_3Fuc_1NeuAc_1$	1.68E+05	1.60E+05	1.05	0.97
$Hex_5HexNac_2$	6.20E+07	5.33E+07	1.16	1.08
$Hex_5HexNac_3$	4.34E+06	4.36E+06	1.00	0.92
$Hex_5HexNac_4$	2.22E+07	2.04E+07	1.09	1.01
$Hex_5HexNac_5$	4.79E+06	4.10E+06	1.17	1.08
$Hex_5HexNac_3Fuc_1$	2.98E+06	2.61E+06	1.14	1.06
$Hex_5HexNac_4Fuc_1$	2.09E+08	1.89E+08	1.11	1.03
$Hex_5HexNac_4Fuc_2$	1.21E+07	1.16E+07	1.04	0.96
$Hex_5HexNac_5Fuc_1$	3.07E+07	2.65E+07	1.16	1.07
$Hex_5HexNac_5Fuc_2$	2.47E+06	2.39E+06	1.03	0.96
$Hex_5HexNac_6Fuc_1$	5.11E+04	4.79E+04	1.07	0.99
$Hex_5HexNac_3NeuAc_1$	5.25E+05	5.01E+05	1.05	0.97
$Hex_5HexNac_4NeuAc_1$	1.28E+07	1.14E+07	1.12	1.04
$Hex_5HexNac_5NeuAc_1$	1.07E+06	1.01E+06	1.06	0.98
$Hex_5HexNac_4Fuc_1NeuAc_1$	1.84E+06	1.73E+06	1.06	0.99
$Hex_5HexNac_5Fuc_1NeuAc_1$	6.74E+05	6.54E+05	1.03	0.95
$Hex_6HexNac_2$	4.61E+07	4.43E+07	1.04	0.96

$Hex_6HexNac_3$	3.73E+06	3.60E+06	1.04	0.96
$Hex_6HexNac_5$	5.05E+05	4.62E+05	1.09	1.01
$Hex_6HexNac_3Fuc_1$	8.75E+05	8.48E+05	1.03	0.96
$Hex_6HexNac_3Fuc_2$	3.65E+05	3.41E+05	1.07	0.99
$Hex_6HexNac_4Fuc_1$	7.83E+05	7.14E+05	1.10	1.01
$Hex_6HexNac_5Fuc_1$	7.88E+05	7.72E+05	1.02	0.95
$Hex_6HexNac_6Fuc_1$	9.26E+04	8.88E+04	1.04	0.97
$Hex_6HexNac_3NeuAc_1$	3.80E+05	3.62E+05	1.05	0.97
$Hex_6HexNac_4NeuAc_1$	5.07E+04	4.93E+04	1.03	0.95
$Hex_6HexNac_5NeuAc_1$	1.70E+05	1.58E+05	1.08	1.00
$Hex_7HexNac_2$	5.48E+06	4.93E+06	1.11	1.03
$Hex_7HexNac_3Fuc_1$	7.97E+05	7.32E+05	1.09	1.01
$Hex_8HexNac_2$	2.81E+06	2.77E+06	1.01	0.94
$Hex_9HexNac_2$	2.10E+06	1.99E+06	1.06	0.98
$Hex_{10}HexNac_2$	9.52E+04	8.46E+04	1.12	1.04

Table S2 The exact mass of the typical glycans presented in the research and their theoretical m/z of corresponding derivatives

Glycan Compositions	Exact Mass	Theoritical m/z after HDEAT lablled	Theoritical m/z after d_{20}-HDEAT labelled	Origins
maltoheptaose	1152.3803	1387.5803	1407.5803	Standard Glycan
$Hex_4HexNAc_2$	1072.3806	1307.5806	1327.5806	Ovalbumin
$Hex_3HexNAc_3$	1113.4072	1348.6072	1368.6072	Ovalbumin
$Hex_5HexNAc_2$	1234.4334	1469.6334	1489.6334	Ovalbumin
$Hex_4HexNAc_3$	1275.4600	1510.6600	1530.6600	Ovalbumin
$Hex_6HexNAc_2$	1396.4863	1631.6863	1651.6863	Ovalbumin, Human Serum
$Hex_5HexNAc_4$	1640.5922	1875.7922	1895.7922	Human Serum
$Hex_5HexNAc_4Fuc_1$	1786.6501	2021.8501	2041.8501	Human Serum
$Hex_5HexNAc_4NeuAc_1$	1931.6876	2166.8876	2186.8876	Human Serum

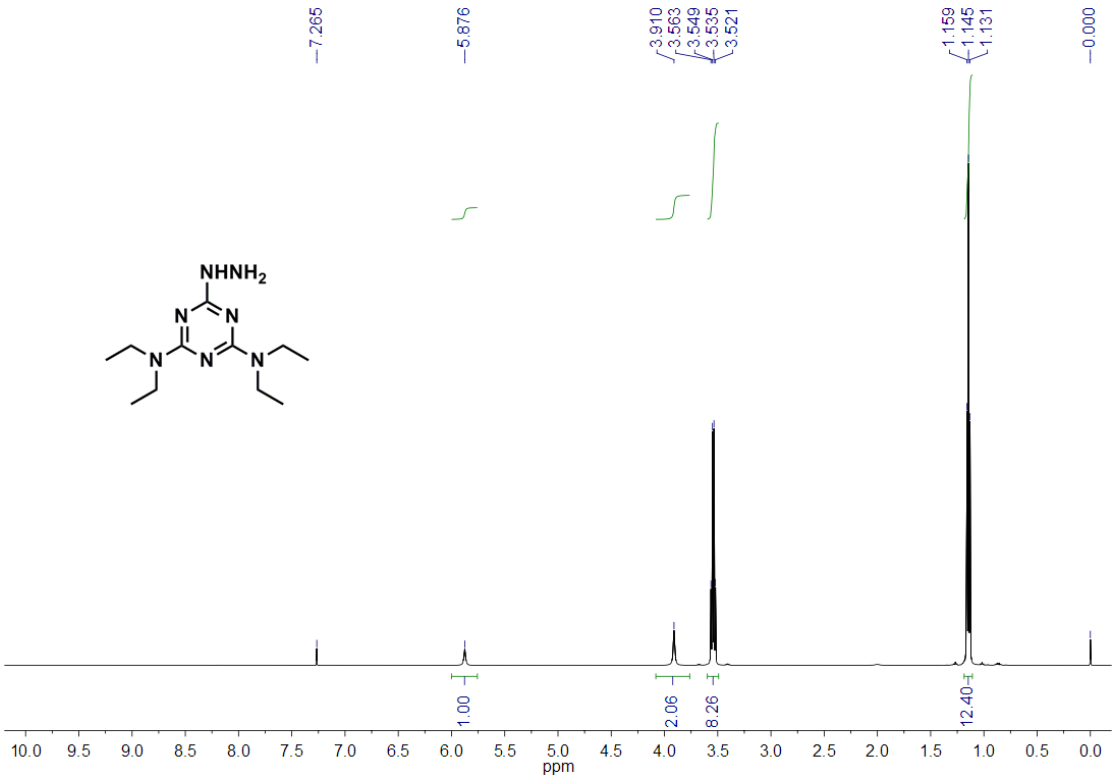


Fig. S1a. ^{1}H NMR spectrum of HDEAT[1]

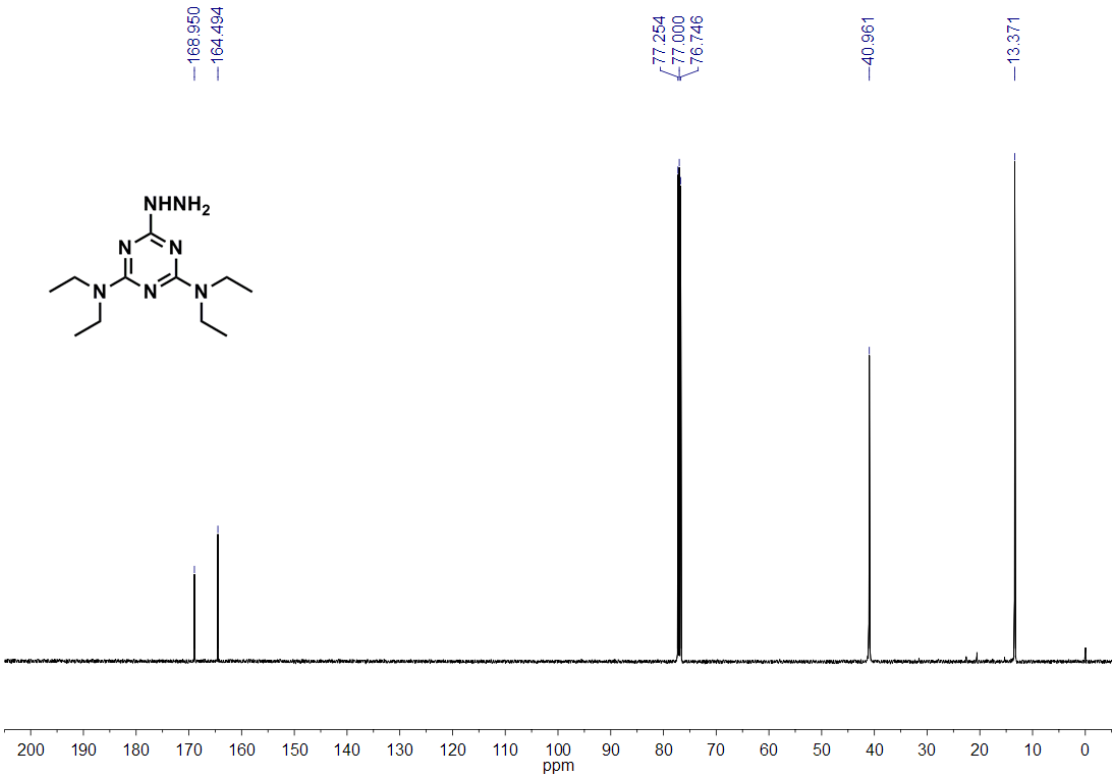


Fig. S1b. ^{13}C NMR spectrum of HDEAT[1]

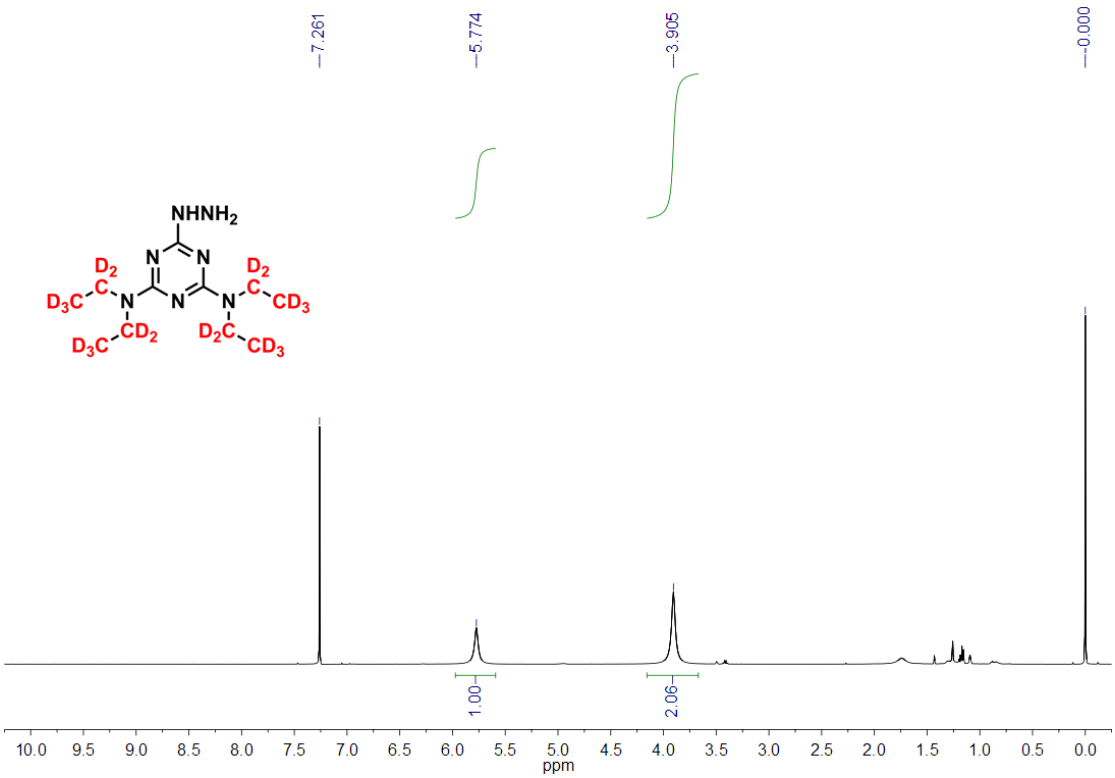


Fig. S2a. ^{1}H NMR spectrum of d_{20}-HDEAT

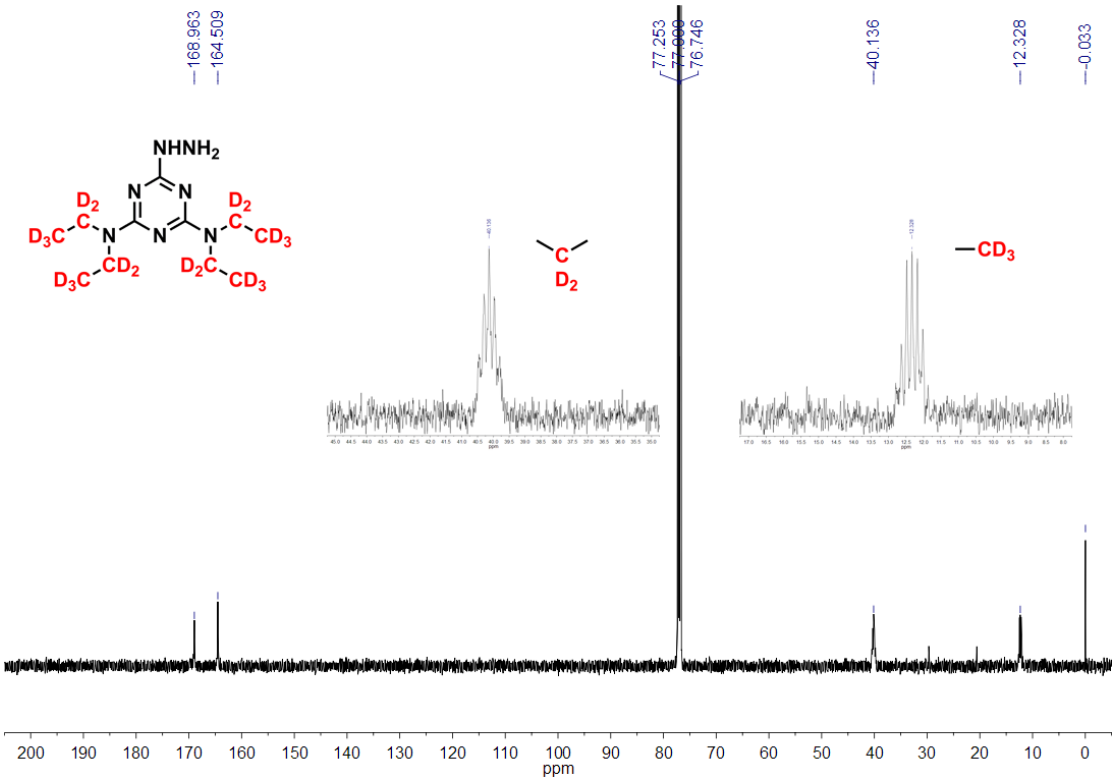


Fig. S2b. ^{13}C NMR spectrum of d_{20}-HDEAT

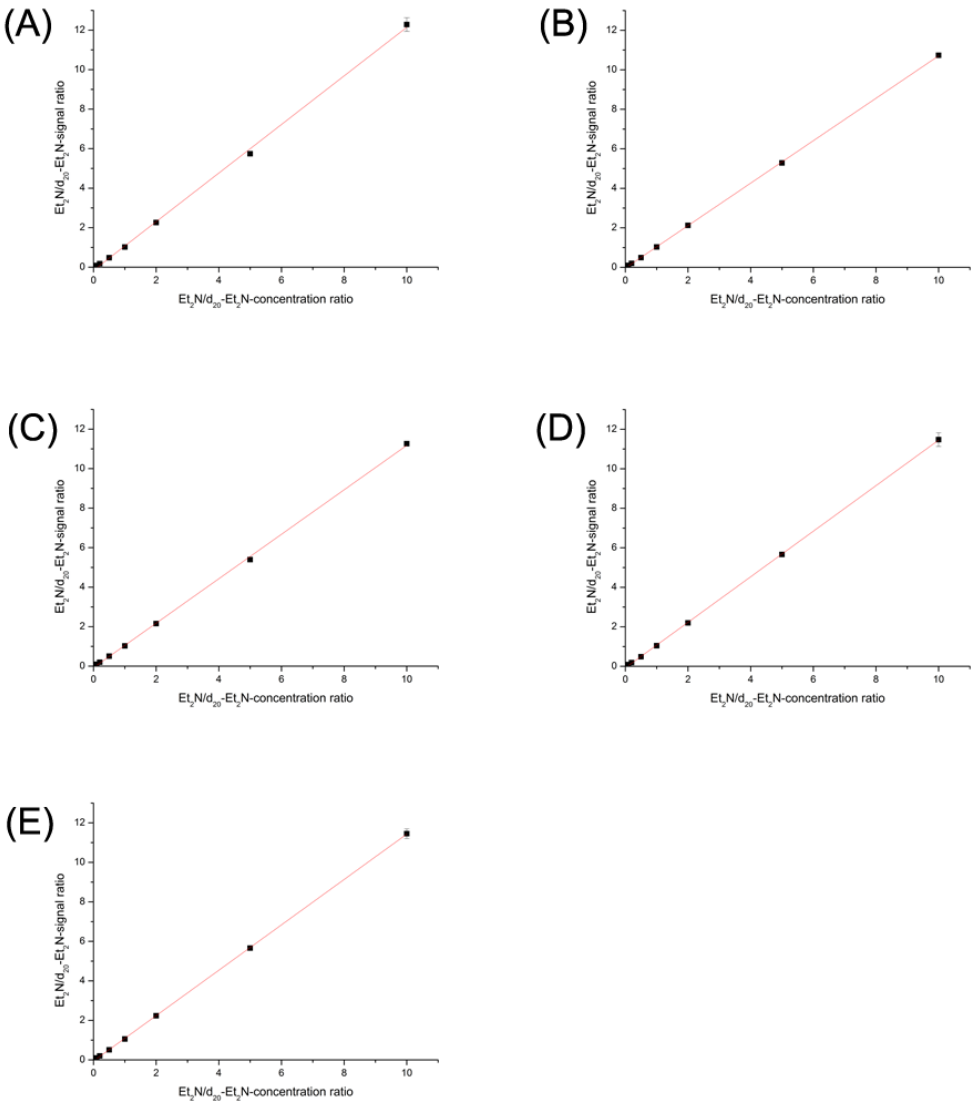


Fig. S3 Calibration curves of relative quantification analysis based on signal ratios of HDEAT and d_{20}-HDEAT labelled N-glycan from OVA in ESI-MS and corresponding concentration ratio: (A) $Hex_4HexNAc_2$, (B) $Hex_3HexNAc_3$, (C) $Hex_5HexNAc_2$, (D) $Hex_4HexNAc_3$, (E) $Hex_6HexNAc_2$.

References

1. M.-Z. Zhao, Y.-W. Zhang, F. Yuan, Y. Deng, J.-X. Liu, Y.-L. Zhou and X.-X. Zhang, *Talanta*, 2015, **144**, 992-997.