***Supporting Information***

# Addition of Sialic Acid to Insulin Confers Superior Physical Properties and Bioequivalence

*Daniel E. K. Kabotso,[a,b] David Smiley,[b] John Mayer,[b] Vasily M. Gelfanov,[c] , Diego Perez-Tilve,[d] Richard D. DiMarchi,[b] Nicola L. B. Pohl*[b], Fa Liu*[e]*

*[a] School of Basic and Biomedical Sciences, University of Health and Allied Health Sciences, PMB 31 Ho, Volta Region, Ghana.*
*[b] Department of Chemistry, Indiana University, Bloomington, Indiana 47405, USA*
*[c] Novo Nordisk Research Center, 5225 Exploration Dr., Indianapolis, Indiana 46241, USA*
*[d] Department of Pharmacology and Systems Physiology, University of Cincinnati-College of Medicine, Cincinnati, Ohio 45267, USA.*
*[e] Novo Nordisk Research Center, 530 Fairview Avenue, North Seatle, Washington 98109, USA*

**Table of Content**

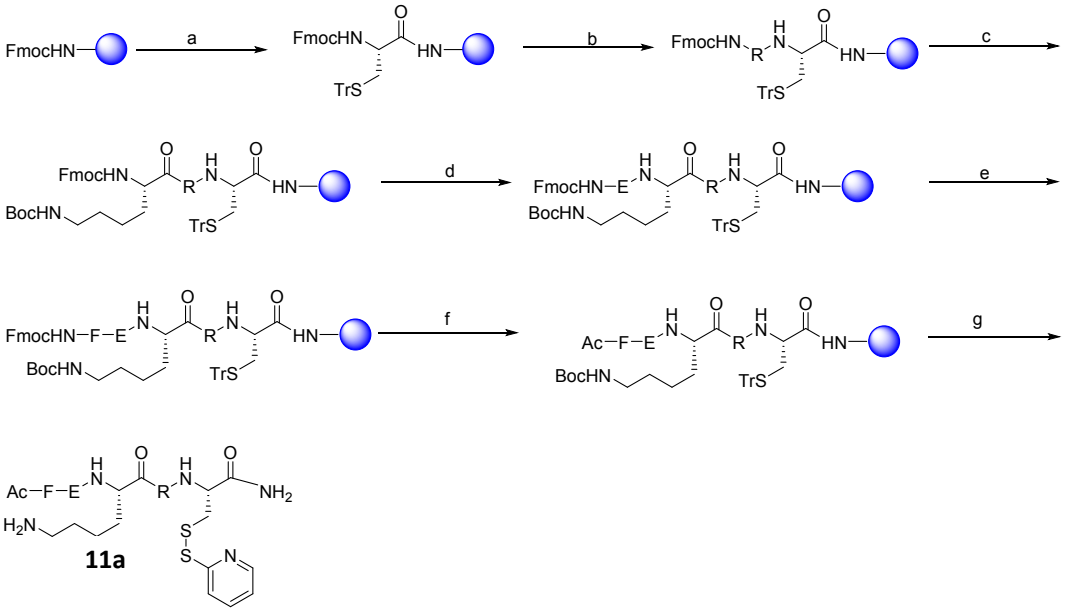
**List of Schemes**


| | | |
|---|---|---|
| **Scheme S1.** | Fmoc-based manual synthesis peptide **11a** | **S3** |
| **Scheme S2.** | Synthesis of pyridyl disulfide activated sialopeptides **11b-d** | **S5** |


**List of Figures**


| | | |
|---|---|---|
| **Figure S1.** | HPLC traces and MS of peptide **11a** | **S4** |
| **Figure S2.** | HPLC traces and MS of peptide **11b** | **S6** |
| **Figure S3.** | HPLC traces and MS of peptide **11c** | **S7** |
| **Figure S4.** | HPLC traces and MS of peptide **11d** | **S8** |
| **Figure S5.** | HPLC traces and MS of sialopeptide **12a** | **S9** |
| **Figure S6.** | HPLC traces and MS of sialopeptide **12b** | **S10** |
| **Figure S7.** | HPLC traces and MS of sialopeptide **12c** | **S11** |
| **Figure S8.** | HPLC traces and MS of sialopeptide **12d** | **S12** |
| **Figure S9.** | HPLC traces and MS of 3-(tritylthio)propyl insulin **17** | **S13** |
| **Figure S10.** | HPLC traces and MS of 3-thiopropyl insulin **18** | **S14** |
| **Figure S11** | HPLC traces and MS of protected Sialic-Ins **19a** | **S15** |
| **Figure S12.** | HPLC traces and MS of protected Sialic-Ins **19b** | **S16** |
| **Figure S13.** | HPLC traces and MS of protected Sialic-Ins **19c** | **S17** |
| **Figure S14.** | HPLC traces and MS of protected Sialic-Ins **19d** | **S18** |
| **Figure S15.** | HPLC traces and MS of Sialic-Ins **1** | **S19** |
| **Figure S16.** | HPLC traces and MS of Sialic-Ins **2** | **S20** |
| **Figure S17.** | HPLC traces and MS of Sialic-Ins **3** | **S21** |
| **Figure S18.** | HPLC traces and MS of Sialic-Ins **4** | **S22** |

***Fmoc-based Synthesis of Peptide 11a***




**Reagents and conditions**: (a) (i) 20% piperidine, (ii) Fmoc-L-Cys(Trt)-OH (5 equiv.), DEPBT (5 equiv.), DIPEA (10 equiv.), DMF (b) (i) 20% piperidine, (ii) Fmoc-Arg(Pbf)-OH (5 equiv.), DEPBT (5 equiv.), DIPEA (10 equiv.), (c) (i) 20% piperidine, (ii) Fmoc-Lys(Boc)-OH (5 equiv.), DEPBT (5 equiv.), DIPEA (10 equiv.), (d) (i) 20% piperidine, (ii) Fmoc-Glu(O*t*Bu)-OH (5 equiv.), DEPBT (5 equiv.), DIPEA (10 equiv.), (e) (i) 20% piperidine, (ii) Fmoc-Phe-OH (5 equiv.), DEPBT (5 equiv.), DIPEA (10 equiv.) (f) (i) 20% piperidine, (ii) $Ac_2O$ (10 equiv.), DMF, (g) TFA 95%, TIPS 2.5%, $H_2O$ 2.5%, DTDP (10 equiv.).

**Scheme S1**. Fmoc-based manual synthesis of peptide **11a**

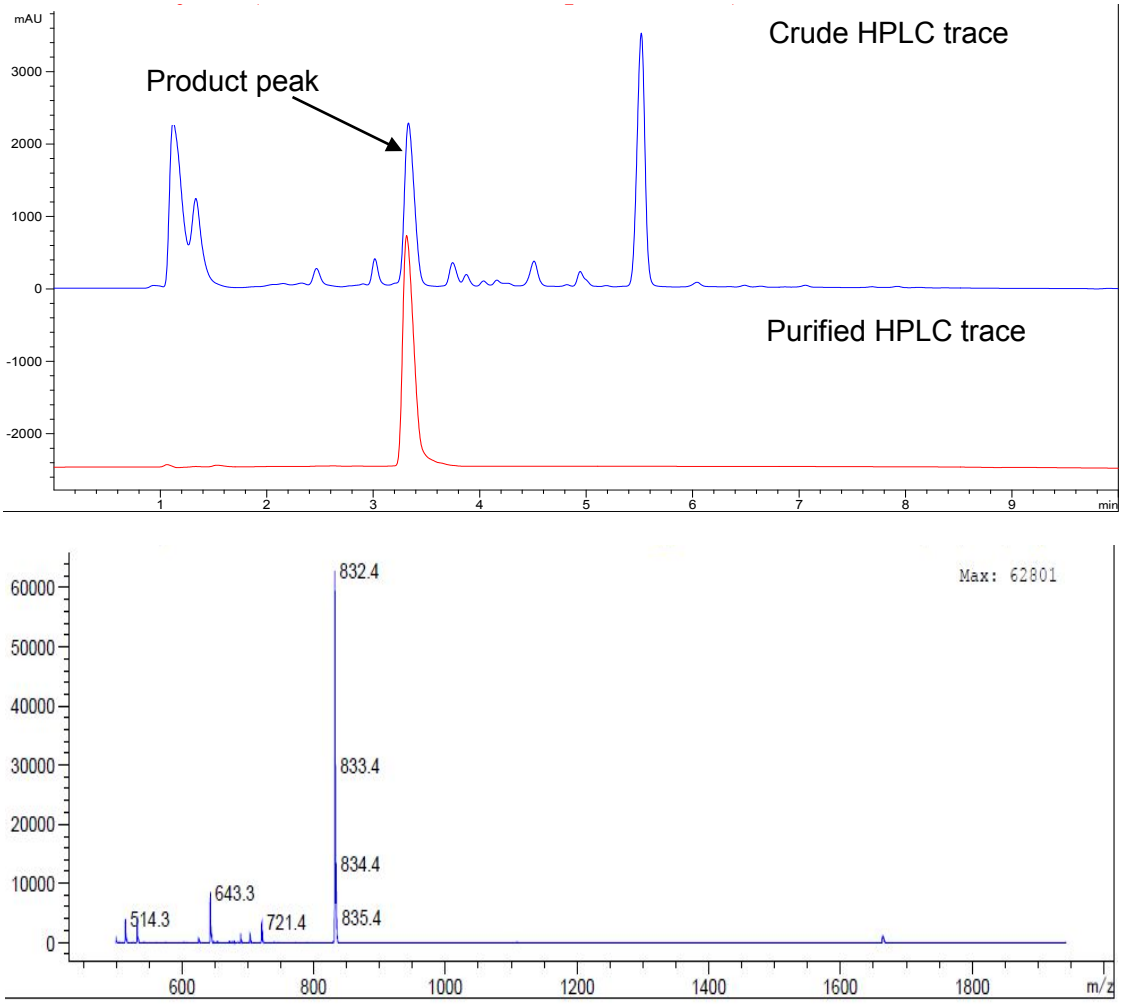
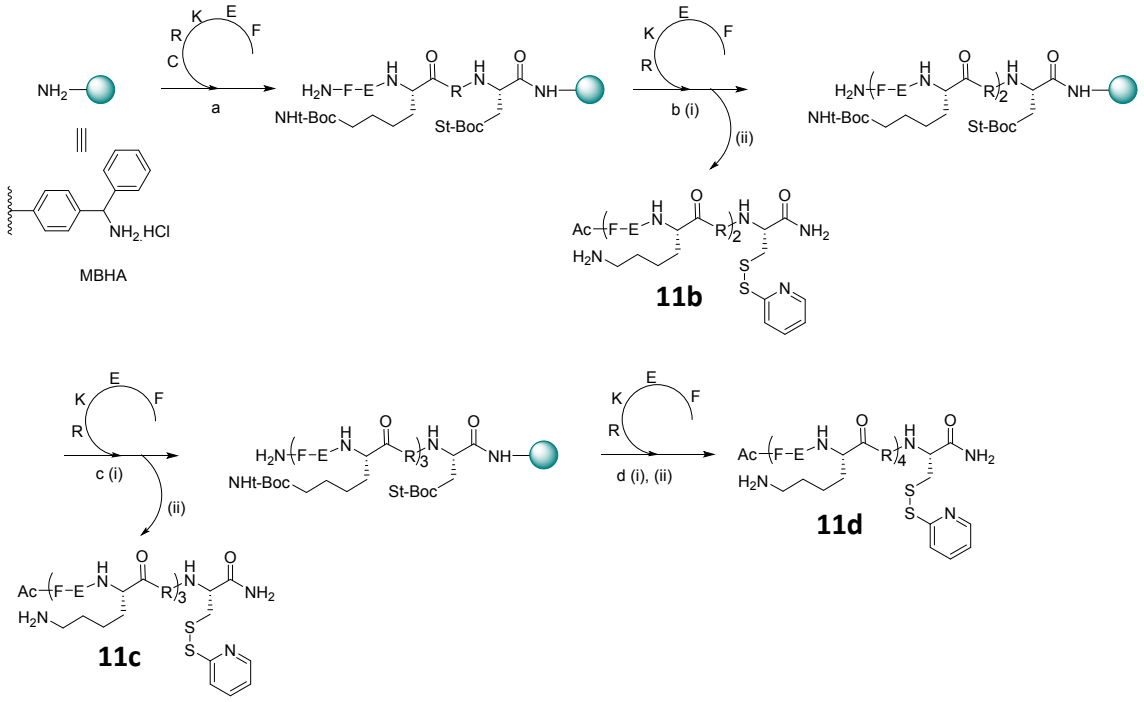



**Figure S1.** HPLC traces and MS of **peptide 11a**

***Boc-based Synthesis of Peptides 11b-d***




**Reagents and conditions:** (a) t-Boc-Cys-MeBzl, C; t-Boc-Arg-Tos, R; t-Boc-Lys-ClZ, K; t-Boc-Glu-OcHx, E; t-Boc-Phe, F; HOAc, 0.5M DEPBT in DMF, DIPEA, (b) (i) t-Boc-Arg-Tos, R; t-Boc-Lys-ClZ, K; t-Boc-Glu-OcHx, E; t-Boc-Phe, F; HOAc, 0.5M DEPBT in DMF, DIPEA, (ii) HF, 0 $^{o}$C; 2% HOAc/20%$CH_3CN$, DTDP; (c) (i) t-Boc-Arg-Tos, R; t-Boc-Lys-ClZ, K; t-Boc-Glu-OcHx, E; t-Boc-Phe, F; HOAc, 0.5M DEPBT in DMF, DIPEA, (ii) HF, 0 $^{o}$C; 2% HOAc/20%$CH_3CN$, DTDP; (d) (i) t-Boc-Arg-Tos, R; t-Boc-Lys-ClZ, K; t-Boc-Glu-OcHx, E; t-Boc-Phe, F; HOAc, 0.5M DEPBT in DMF, DIPEA, (ii) HF, 0 $^{o}$C; 2% HOAc/20%$CH_3CN$, DTDP.

***Scheme S2. Boc-based synthesis of peptides 11b-d***

***Peptide 11b***

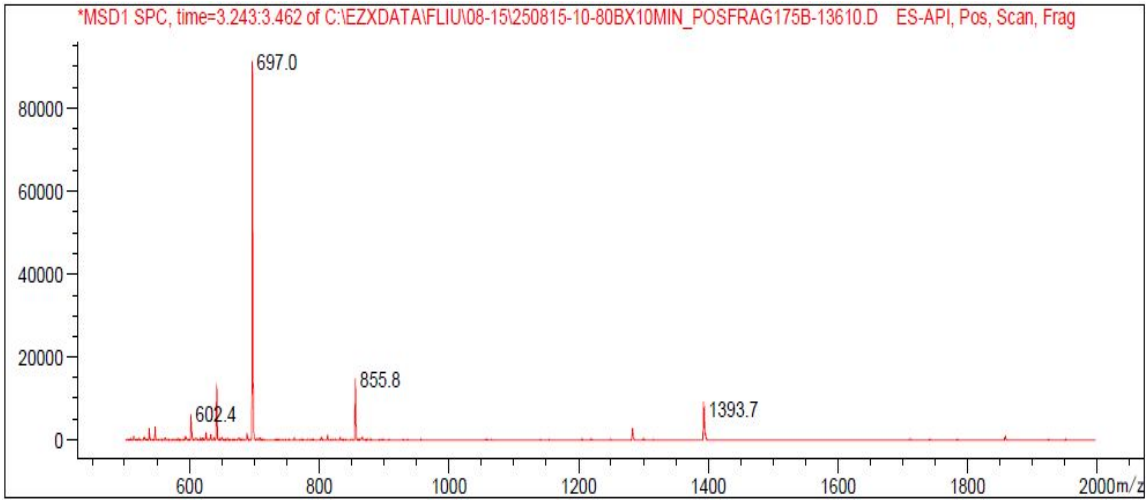



**Figure S2.** MS of peptide **11b**

***Peptide 11c***

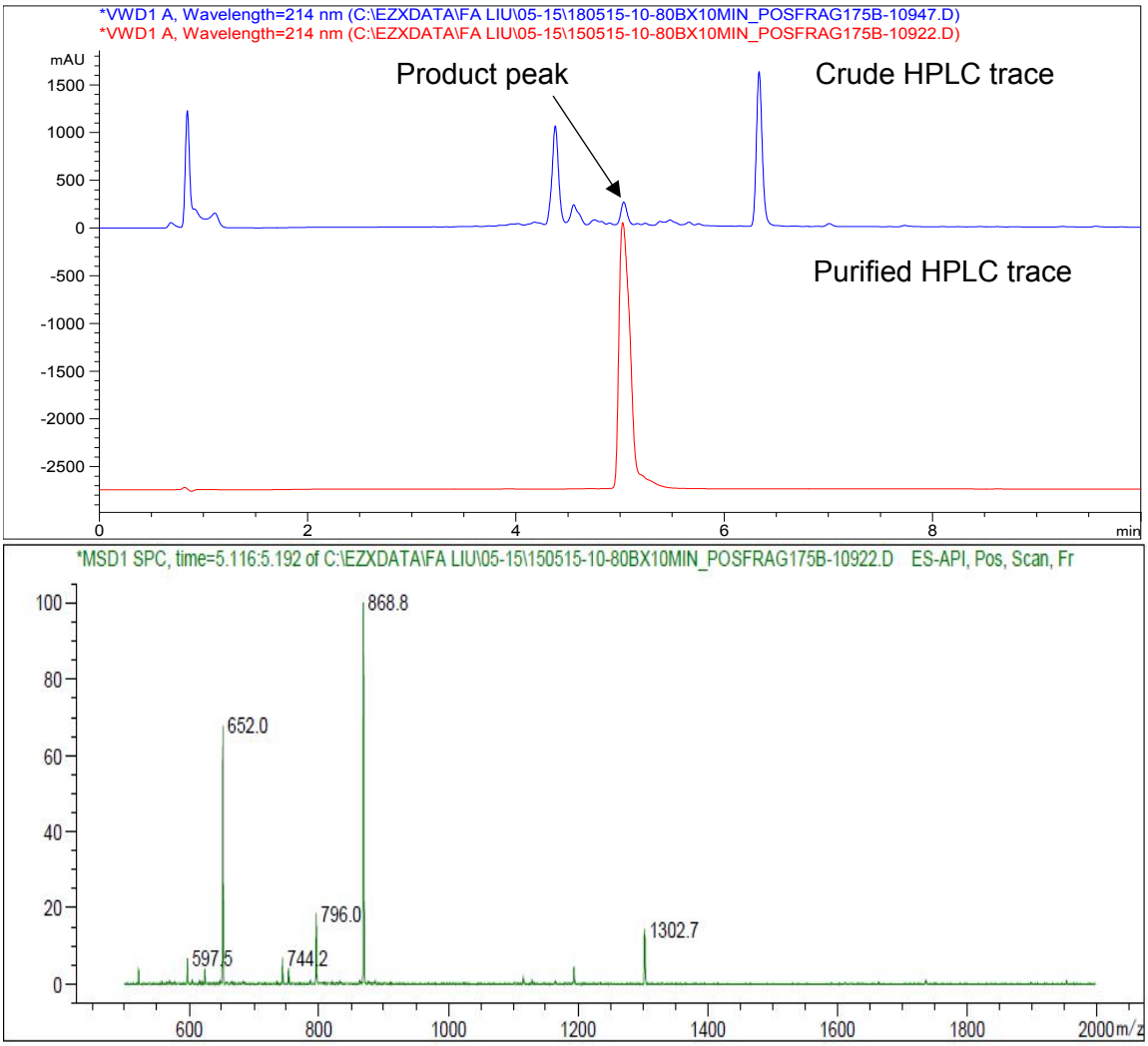



**Figure S3.** HPLC traces and MS of peptide **11c**

***Peptide 11d***

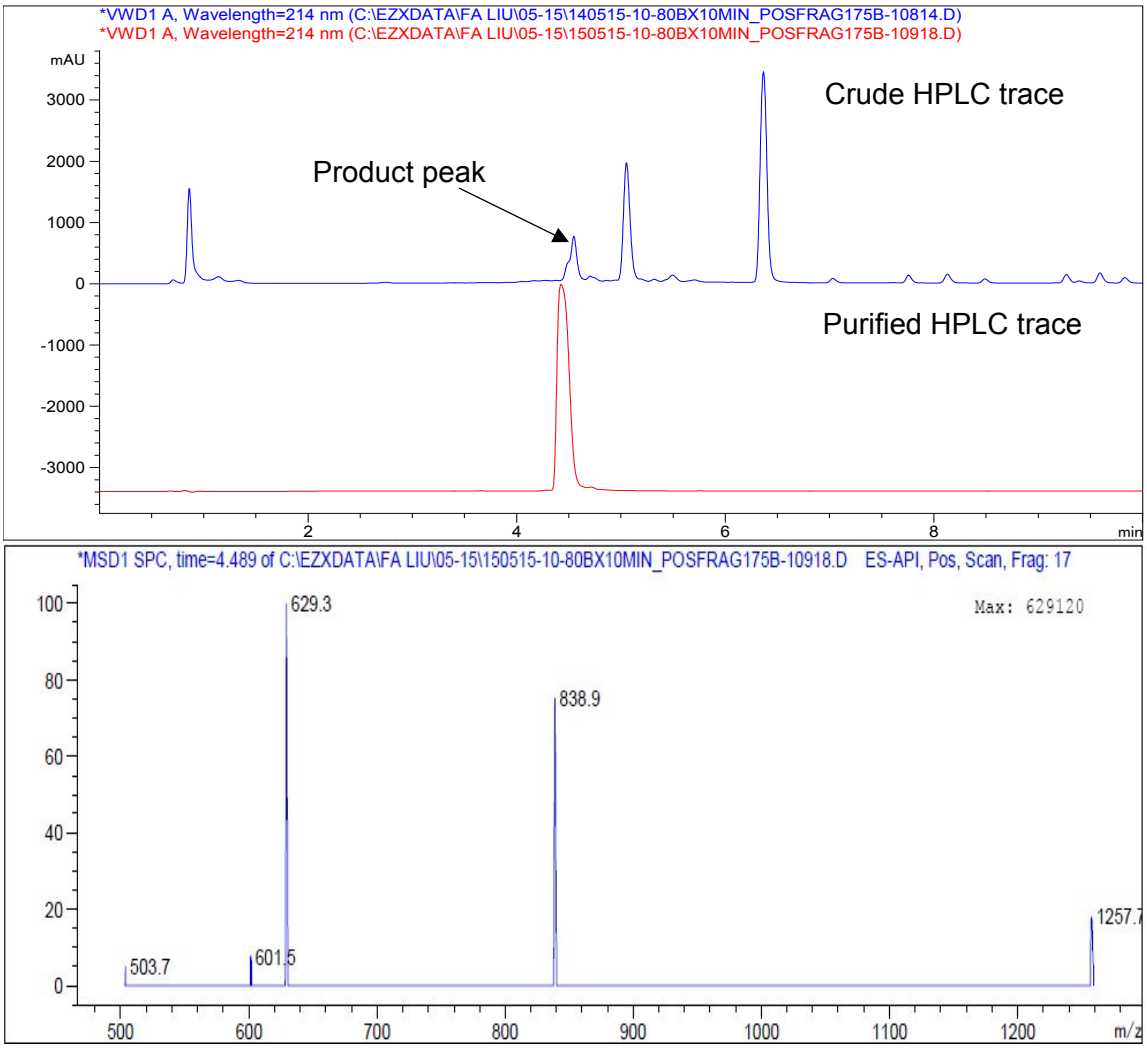



**Figure S4.** HPLC traces and MS of peptide **11d**

***Sialopeptide 12a***

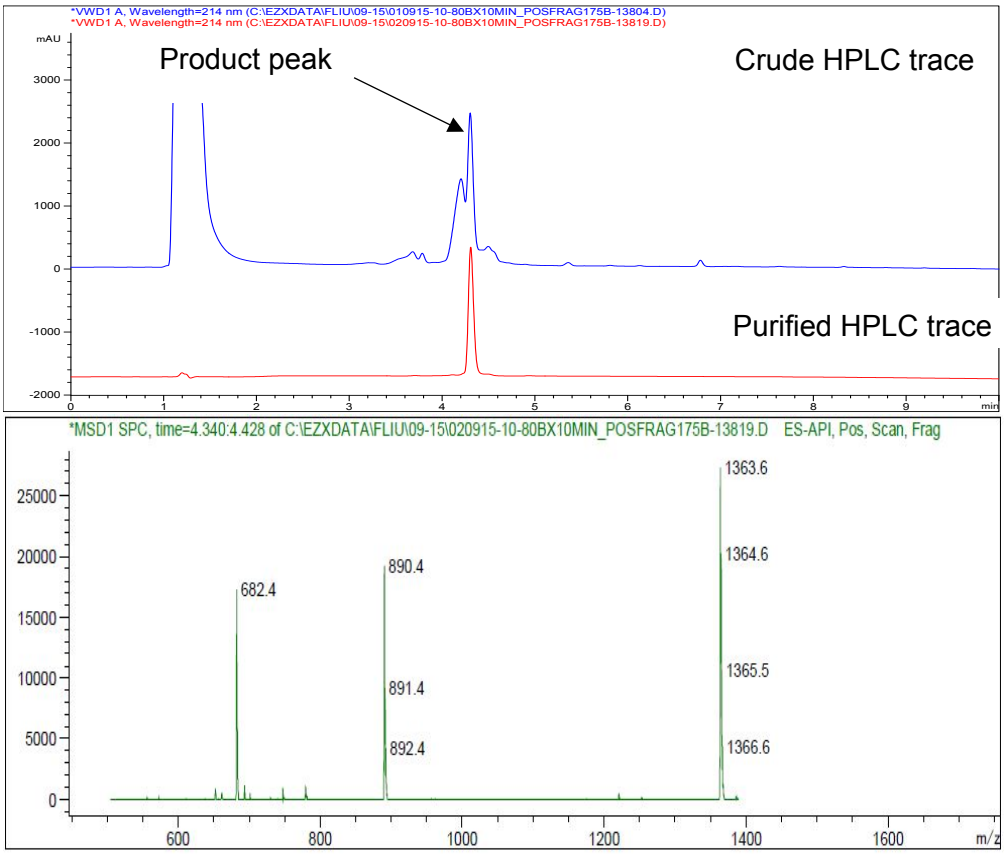



**Figure S5.** HPLC traces and MS of sialopeptide **12a**

***Sialopeptide 12b***

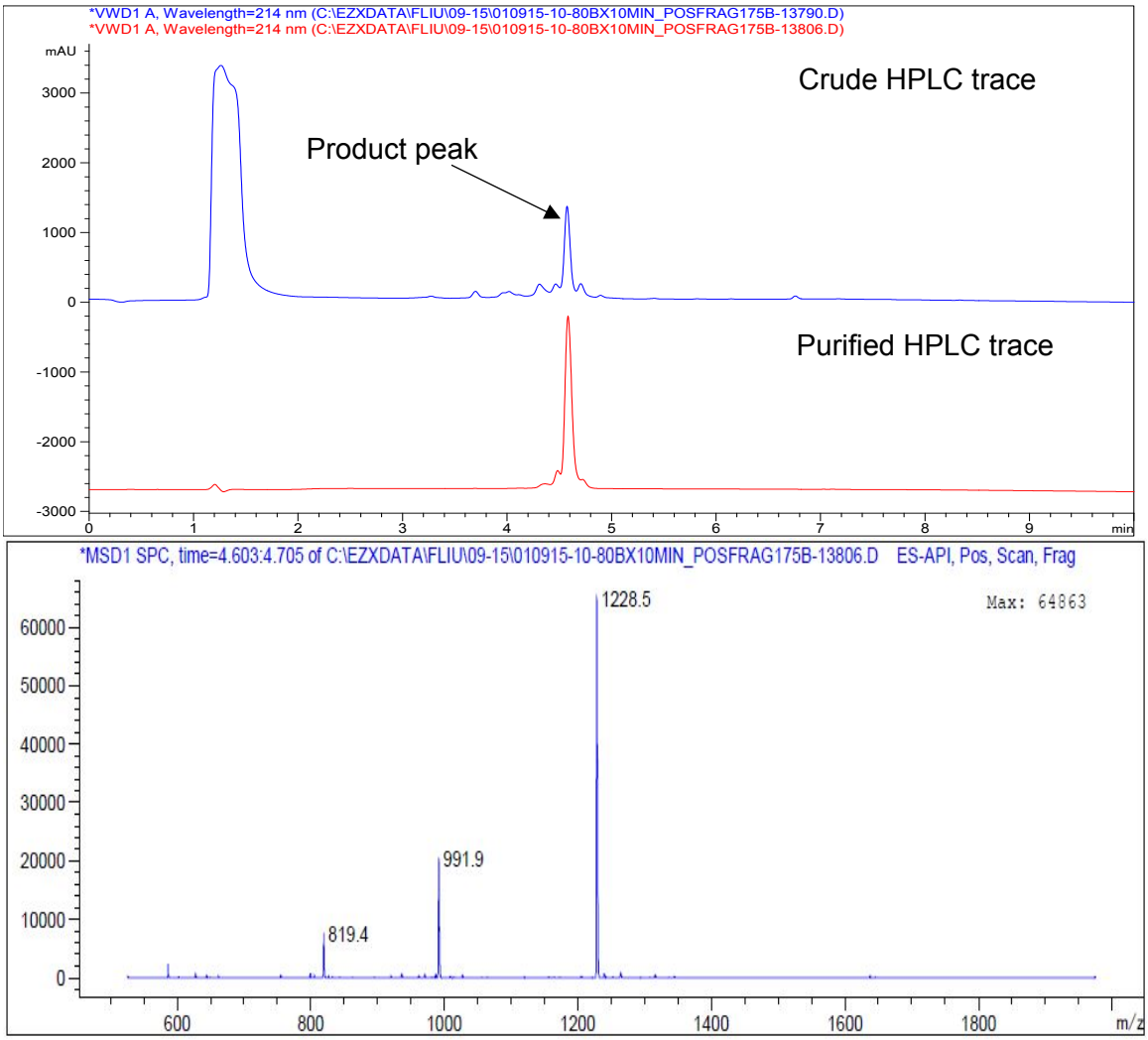



**Figure S6.** HPLC traces and MS of sialopeptide **12b**

***Sialopeptide 12c***

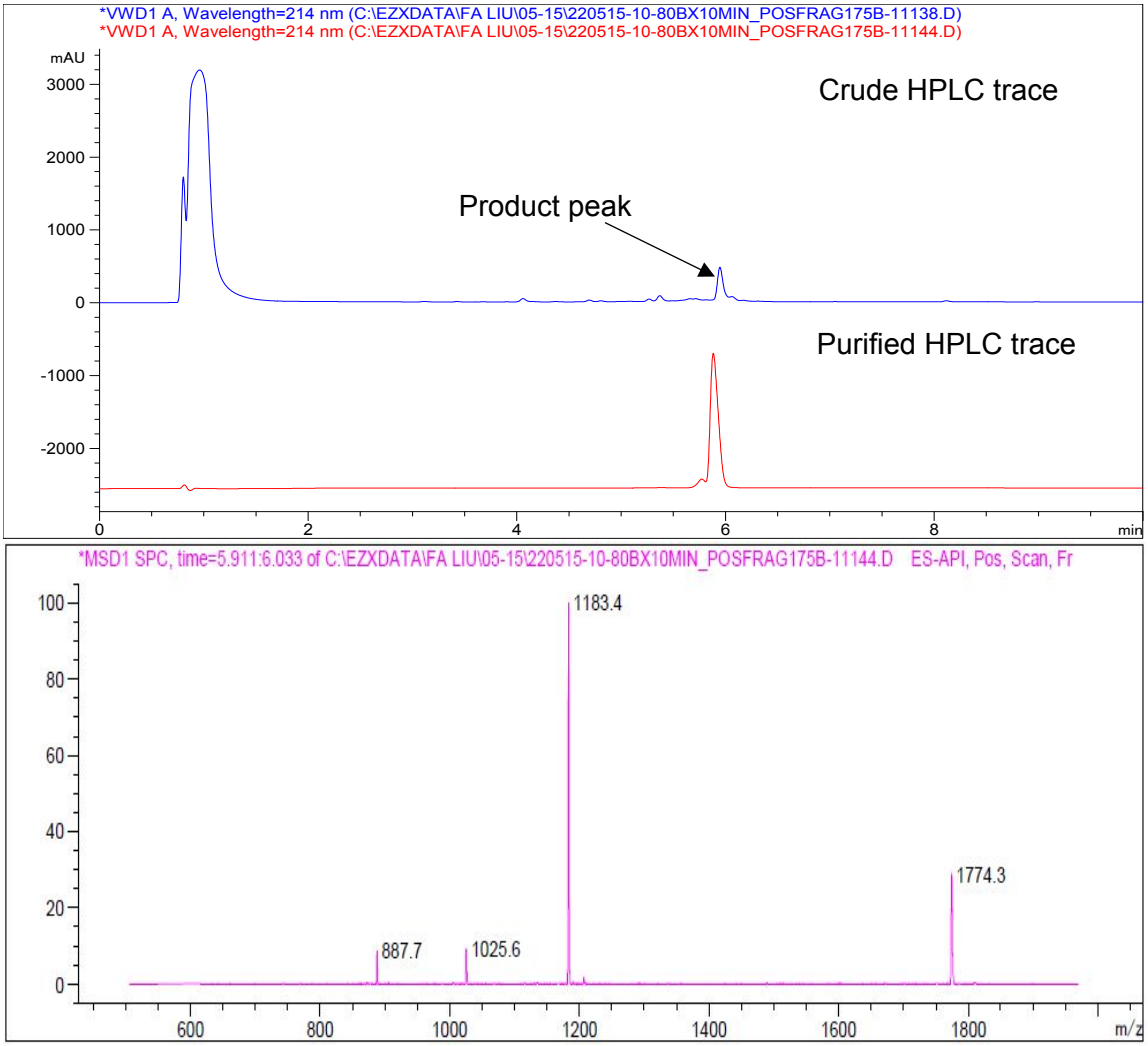



**Figure S7.** HPLC traces and MS of sialopeptide **12c**

***Sialopeptide 12d***

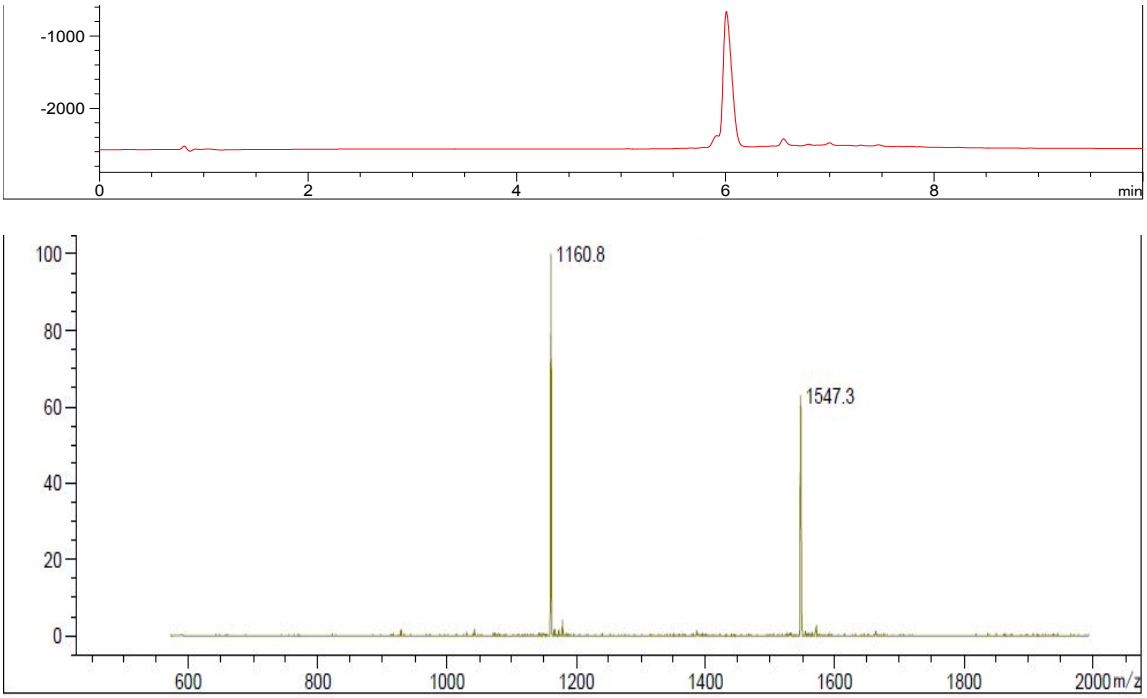
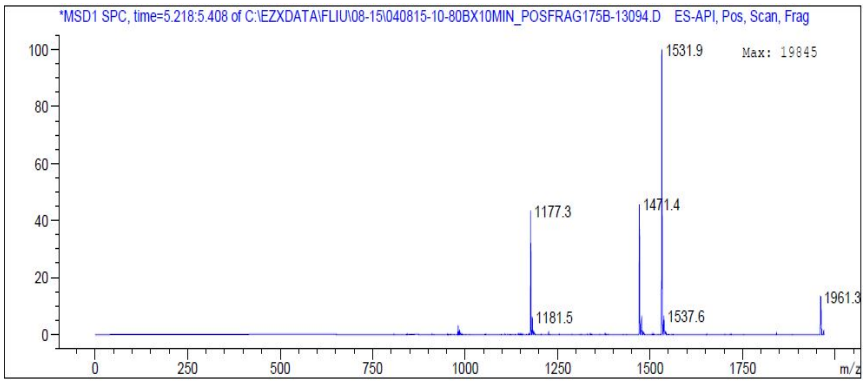



**Figure S8.** HPLC traces and MS of sialopeptide **12d**

*3-Thiopropyl Insulin 17*



| Fragment | Retention Time | Observed Mass (Da) | Calculated Mass (Da) |
|---|---|---|---|
| A | - | - | 416.5 |
| B | 5.827 | 3285.0 | 3285.8 |
| C | 4.467 | 1377.3 | 1377.5 |
| D | 4.276 | 1116.6 | 1116.3 |

**Figure S9.** MS of 3-(tritylthio)propyl insulin **17** and the assigment of the Glu-C digestive fragments

*3-Thiopropyl Insulin 18*

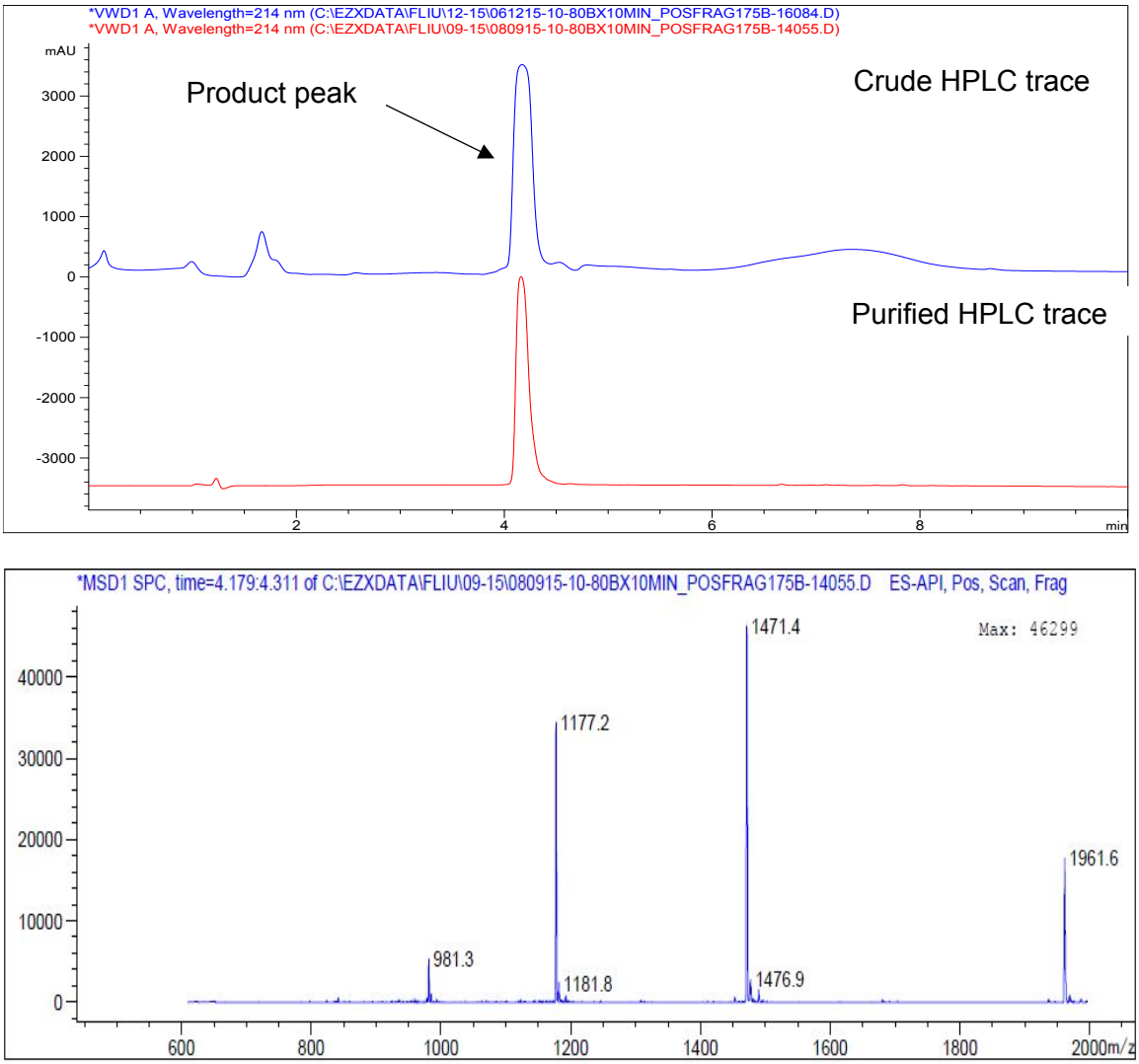



**Figure S10.** HPLC traces and MS of 3-thiopropyl insulin **18**

***Protected Sialic-Ins 19a***

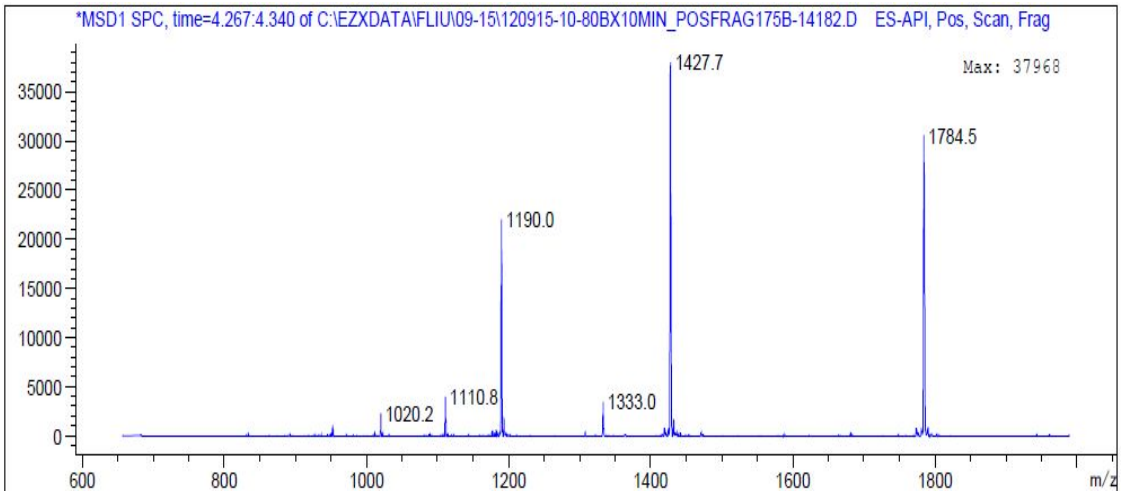



**Figure S11** MS of protected Sialic-ins **19a**

***Protected Sialic-Ins 19b***

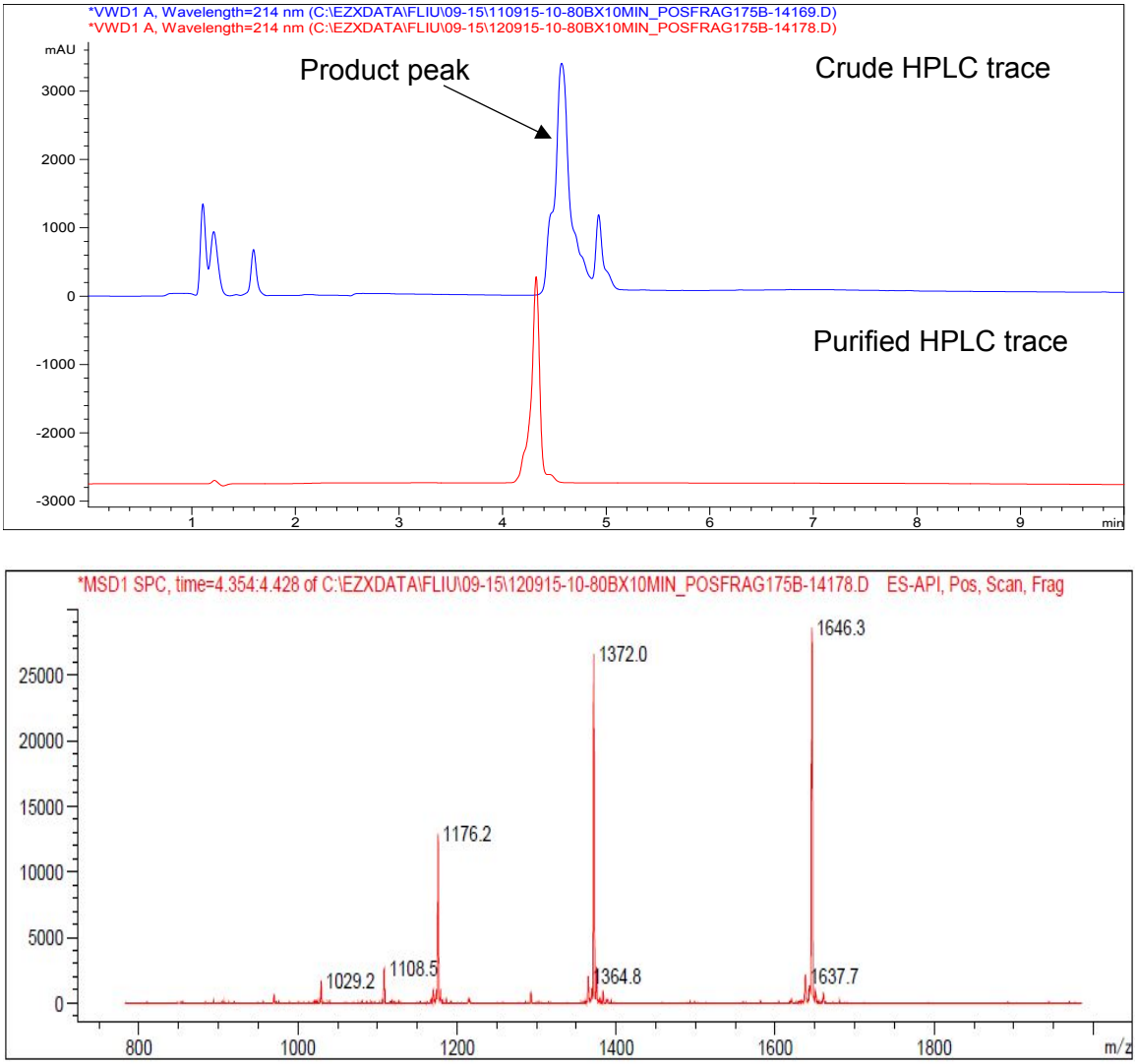



**Figure S12.** HPLC traces and MS of protected Sialic-Ins **19b**

***Protected Sialic-Ins 19c***

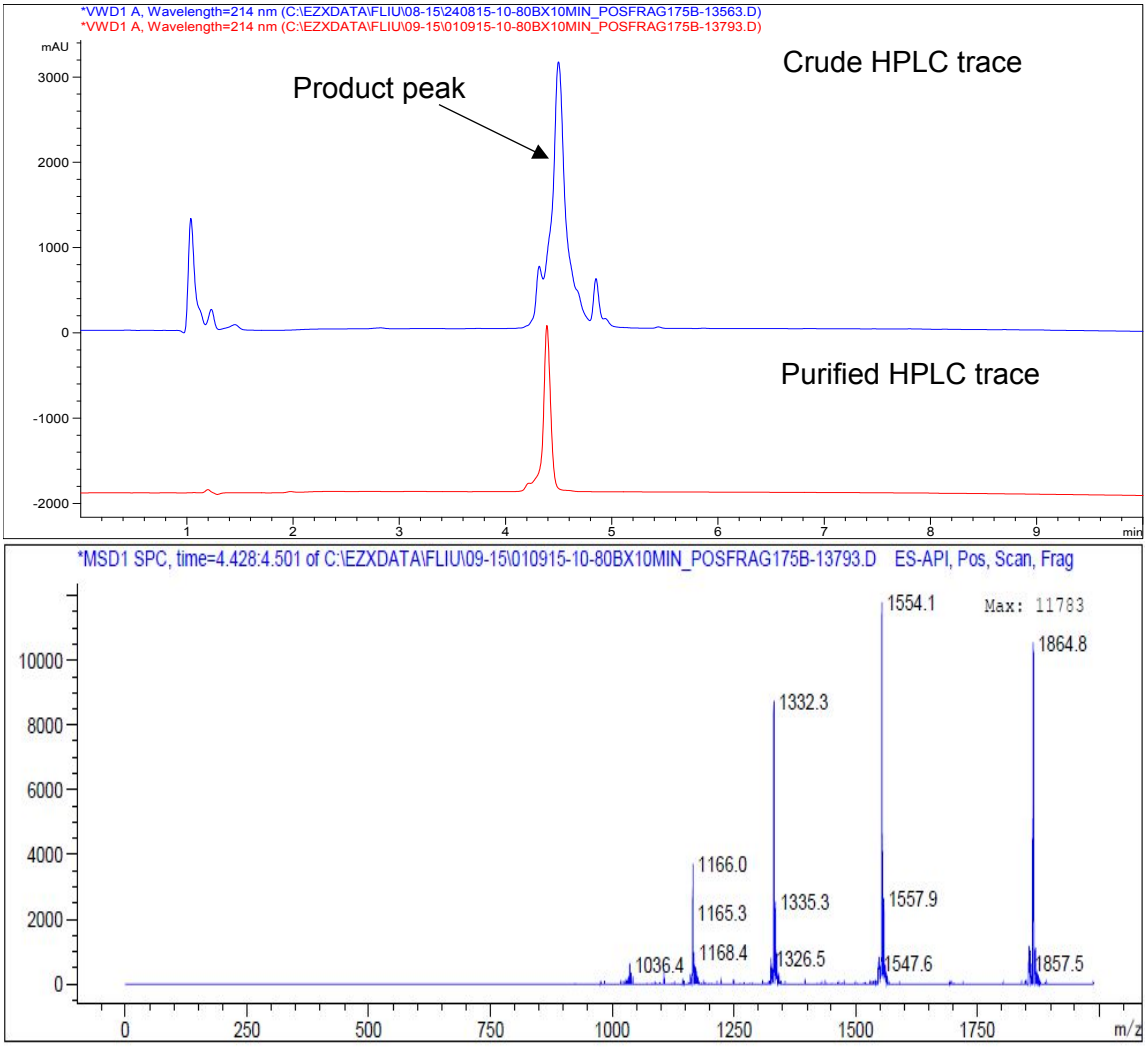



**Figure S13.** HPLC traces and MS of protected Sialic-Ins **19c**

***Protected Sialic-Ins 19d***

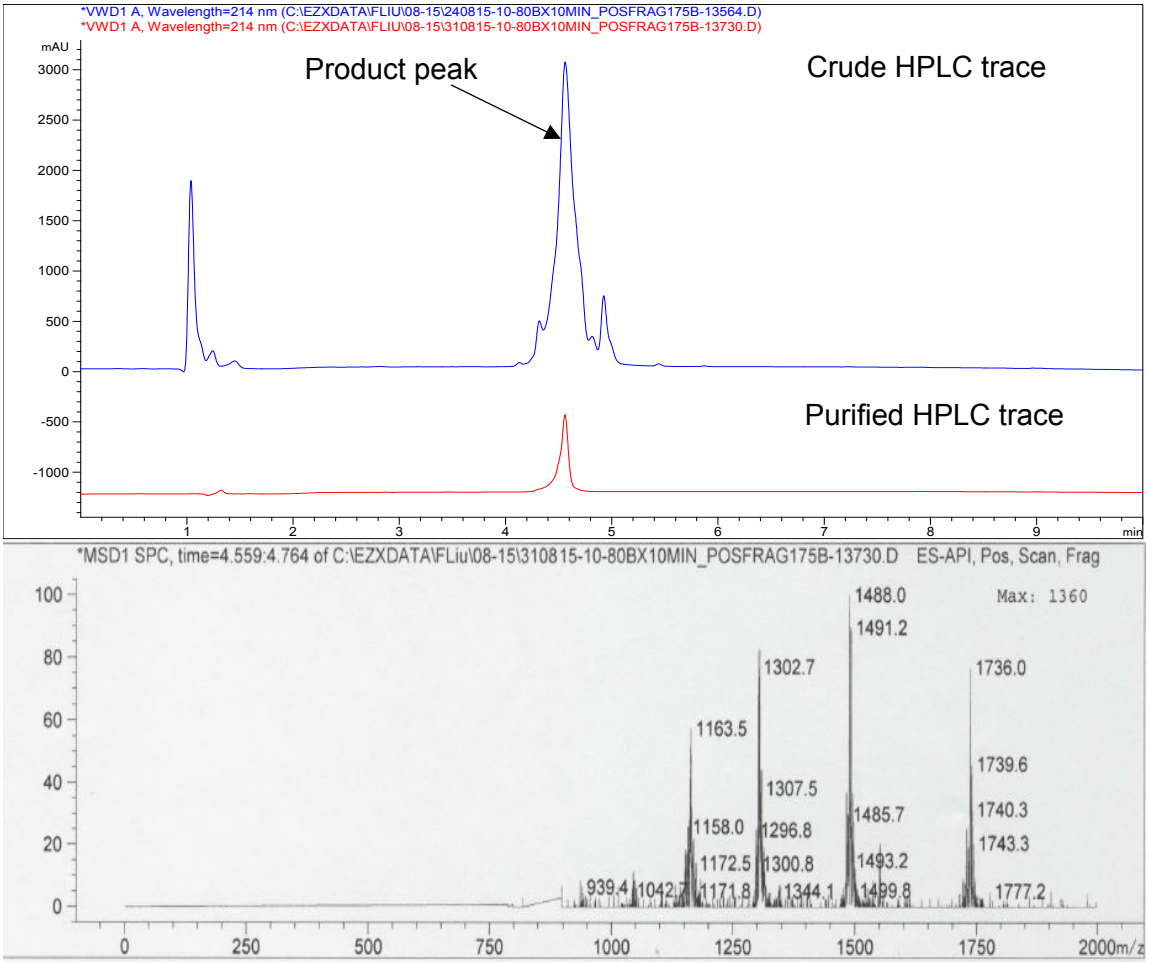



**Figure S14.** HPLC traces and MS of protected Sialic-Ins **19d**

***Sialic-Ins 1***

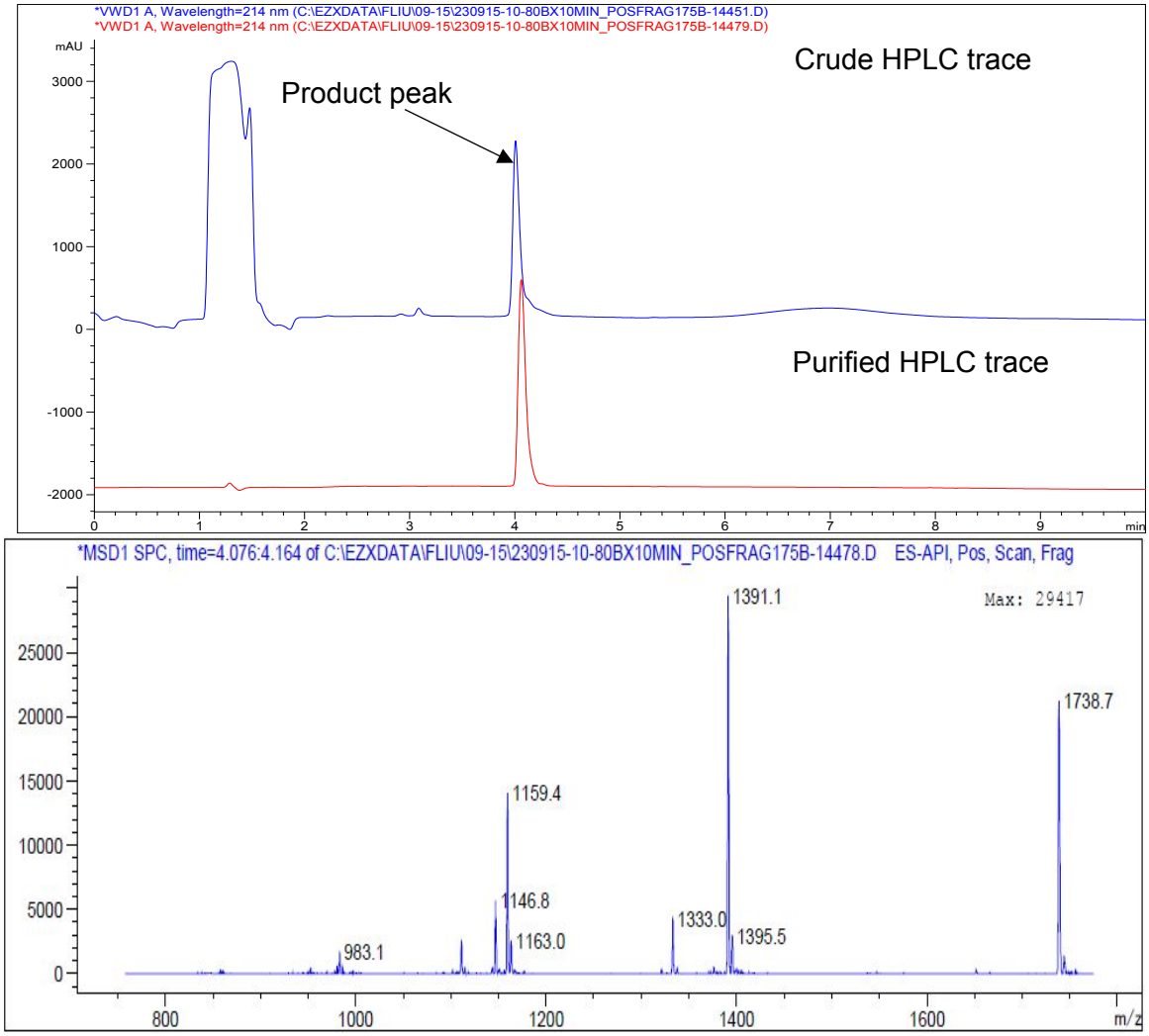



**Figure S15.** HPLC traces and MS of Sialic-Ins **1**

***Sialic-Ins 2***

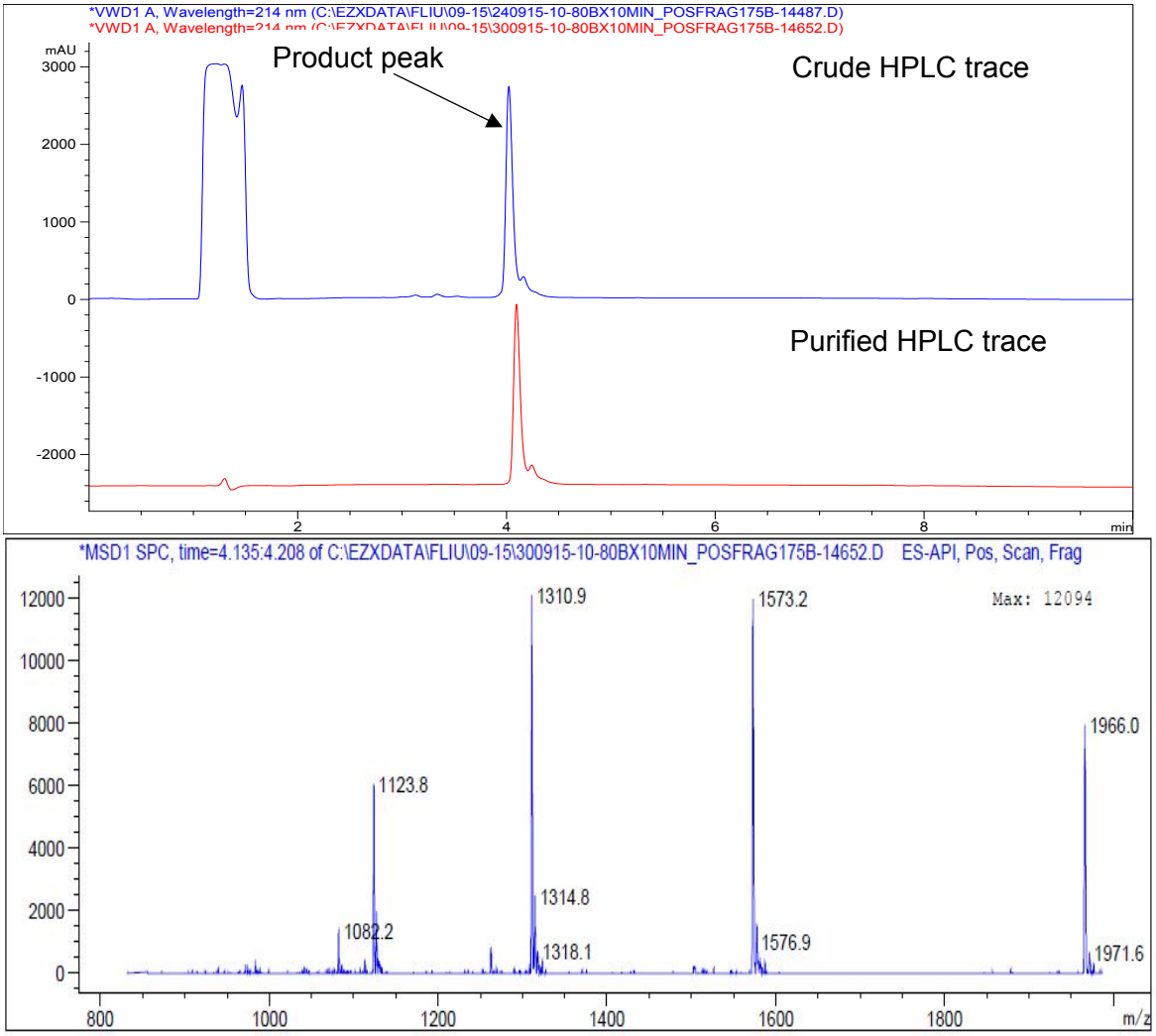



**Figure S16.** HPLC traces and MS of Sialic-Ins **2**

***Sialic-Ins 3***

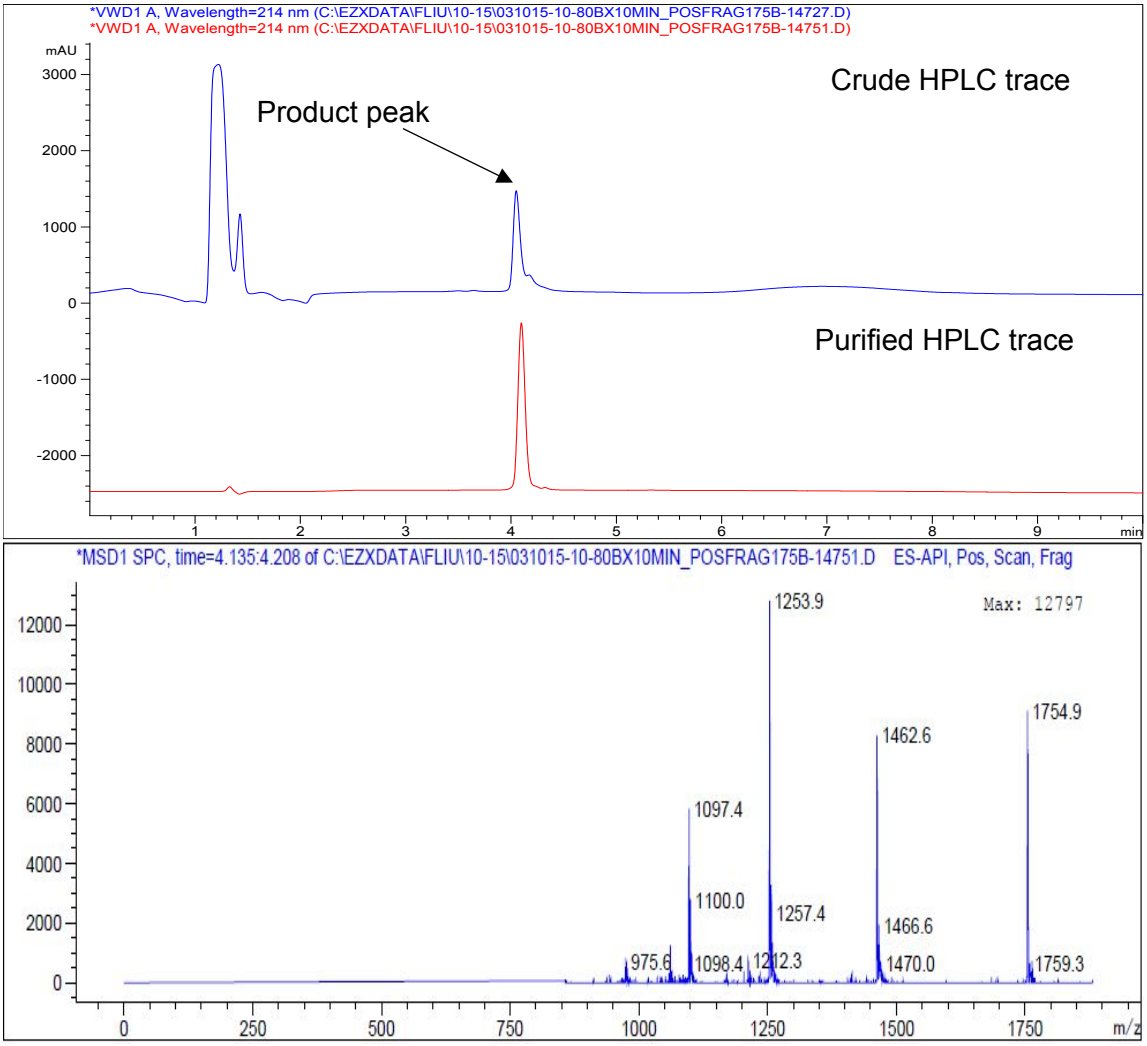



**Figure S17.** HPLC traces and MS of Sialic-Ins **3**

***Sialic-Ins 4***

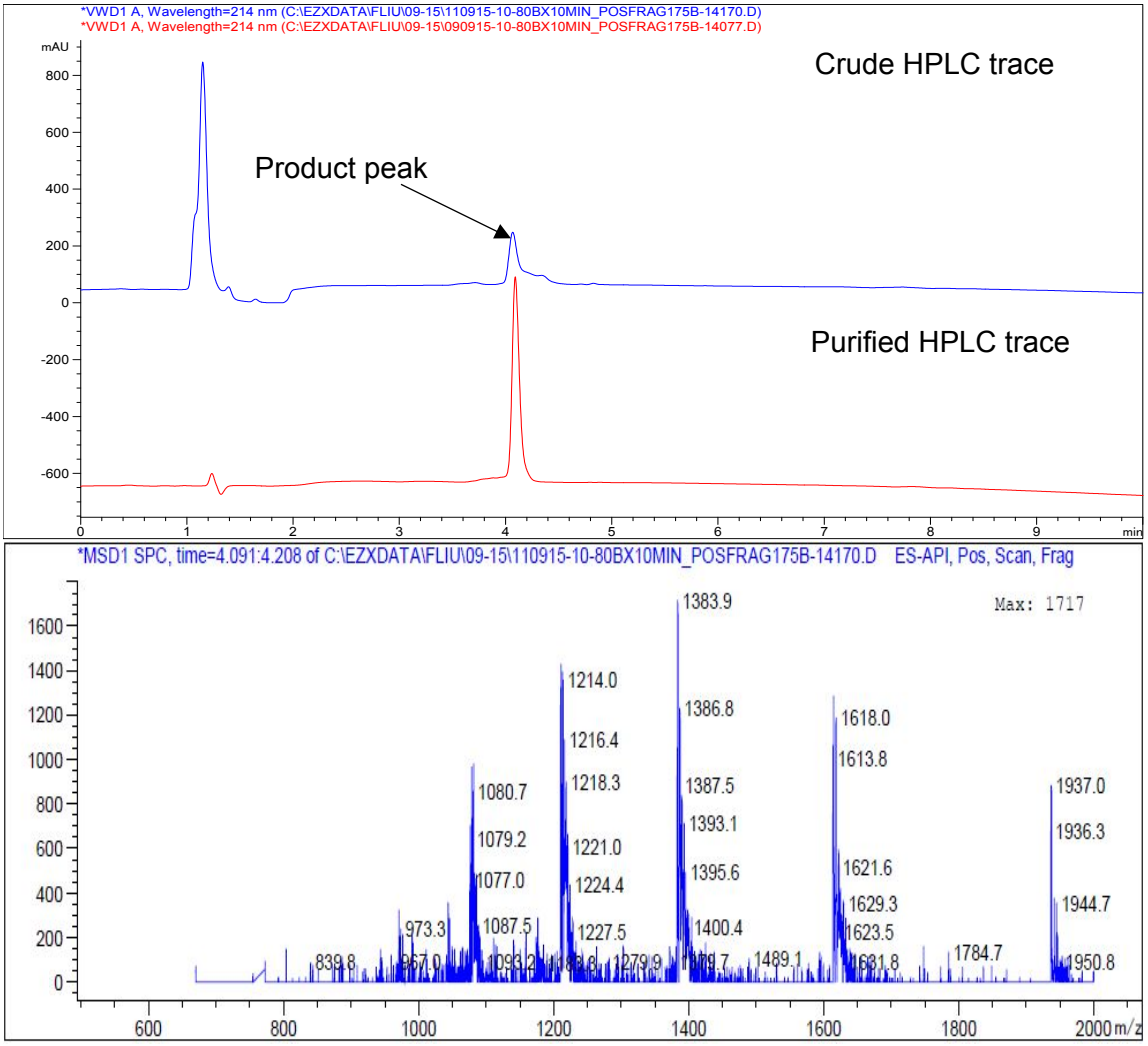



**Figure S18.** HPLC traces and MS of Sialic-Ins **4**