SUPPORTING INFORMATION S1-S7

Ola Blixt *et al*

A High-throughput O-Glycopeptide Discovery Platform for Seromic Profiling

S1 Preparation of amine-reactive Sepharose 4B beads

EAH Sepharose 4B beads slurry (5 mL, 30 μmol) was placed in a frit-fitted plastic reaction barrel and drained by suction. The beads were washed with 50 mL of 50% ethanol in water whereupon a freshly prepared solution of 3,4-diethoxy-3-cyclobutene-1,2-dione (diethyl squarate, 0.4 mL, 2.7 mmol) in 1.5 mL of ethanol was added dropwise to 1.5 mL of NaHCO_3 0.1 M under stirring. The resulting solution was mixed with the beads and the resulting slurry was vigorously shaken for 1 h at rt, then the beads were drained and washed with 50 mL of 50% ethanol in water and 50 mL of water. A negative ninhydrin test (amino group content) was found at this stage. The drained beads were then mixed with cystamine dihydrochloride (600 mg, 2.7 mmol) in 3.5 mL of NaHCO_3 0.1 M and vigorously shaken for 1 h at rt. After extensive washings with water (>50 mL) and confirmation of a positive ninhydrin test from the beads and negative from the last washings, the Sepharose beads were considered properly functionalized with the cleavable linker. At this stage the beads could be stored in 20% aqueous ethanol for weeks without any noticeable loss in

performance. Immediately before undertaking the glycopeptide capture-release procedure, the Sepharose 4B beads equipped cleavable cystamine linker were activated in order to become amine-reactive. To achieve so, a second unit of diethyl squarate was introduced with the same procedure as described above for the first unit and, upon assessing a negative ninhydrin test, the bead slurry was quickly evenly aliquoted into the filter plate wells for subsequent glycopeptide capture.

S2 MS data bead capture (Table: Complete list of MUC1 glycopeptide library)

no.	Sequence	MS found	Glycan hapten	Type
1	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
2	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
3	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
4	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
5	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
6	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
7	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
8	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
9	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
10	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
11	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
12	VT S APDTRPAPG S TAPPAHG	x	(GalNAc α)-	Tn
13	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
14	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
15	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
16	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
17	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
18	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
19	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
20	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
21	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
22	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
23	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
24	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
25	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
26	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
27	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
28	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
29	VT S APDTRPAPG S TAPPAHG	x	(GalNAc α)-	Tn
30	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
31	VT S APDTRPAPG S TAPPAHG	✓	(GalNAc α)-	Tn
32	APG S TAPPAHGVTSAPDTRP	x	(GalNAc α)-	Tn
33	APG S TAPPAHGVTSAPDTRP	x	(GalNAc α)-	Tn
34	APGSTAPPAHGVTSAPD T RP	✓	(GalNAc α)-	Tn
35	APGSTAPPAHGV T SAPDTRP	✓	(GalNAc α)-	Tn
36	APGSTAPPAHGV T SAPDTRP	✓	(GalNAc α)-	Tn
37	APGSTAPPAHGV T SAPDTRP	✓	(GalNAc α)-	Tn
38	APGSTAPPAHGV T SAPD T RP	✓	(GalNAc α)-	Tn
39	APG S TAPPAHGV T SAPDTRP	✓	(GalNAc α)-	Tn

40	APGSTAPPAHGVTSAPDTRP	x	(GalNAc α)-	Tn
41	APGSTAPPAHGVTSAPDTRP	√	(GalNAc α)-	Tn
42	APGSTAPPAHGVTSAPDTRP	√	(GalNAc α)-	Tn
43	APGSTAPPAHGVTSAPDTRP	√	(GalNAc α)-	Tn
44	APGSTAPPAHGVTSAPDTRP	√	(GalNAc α)-	Tn
45	APGSTAPPAHGVTSAPDTRP	√	(GalNAc α)-	Tn
46	APGSTAPPAHGVTSAPDTRP	√	(GalNAc α)-	Tn
47	APGSTAPPAHGVTSAPDTRP	√	(GalNAc α)-	Tn
48	APGSTAPPAHGVTSAPDTRP	√	(GalNAc α)-	Tn
49	APGSTAPPAHGVTSAPDTRP	√	(GalNAc α)-	Tn
50	APGSTAPPAHGVTSAPDTRP	√	(GalNAc α)-	Tn
51	APGSTAPPAHGVTSAPDTRP	√	(GalNAc α)-	Tn
52	APGSTAPPAHGVTSAPDTRP	√	(GalNAc α)-	Tn
53	APGSTAPPAHGVTSAPDTRP	√	(GalNAc α)-	Tn
54	APGSTAPPAHGVTSAPDTRP	√	(GalNAc α)-	Tn
55	APGSTAPPAHGVTSAPDTRP	√	(GalNAc α)-	Tn
56	APGSTAPPAHGVTSAPDTRP	√	(GalNAc α)-	Tn
57	APGSTAPPAHGVTSAPDTRP	x	(GalNAc α)-	Tn
58	APGSTAPPAHGVTSAPDTRP	√	(GalNAc α)-	Tn
59	APGSTAPPAHGVTSAPDTRP	√	(GalNAc α)-	Tn
60	APGSTAPPAHGVTSAPDTRP	√	(GalNAc α)-	Tn
61	APGSTAPPAHGVTSAPDTRP	x	(GalNAc α)-	Tn
62	APGSTAPPAHGVTSAPDTRP	√	(GalNAc α)-	Tn
63	VSAPDTRPAPGSTAPPAHG	√	(GalNAc α)-	Tn
64	TSAPDTRPAPGSTAPPAHGV	√	(GalNAc α)-	Tn
65	SAPDTRPAPGSTAPPAHGV	√	(GalNAc α)-	Tn
66	APDTRPAPGSTAPPAHGV	√	(GalNAc α)-	Tn
67	PDTRPAPGSTAPPAHGV	√	(GalNAc α)-	Tn
68	DTRPAPGSTAPPAHGV	√	(GalNAc α)-	Tn
69	TRPAPGSTAPPAHGV	√	(GalNAc α)-	Tn
70	RPAPGSTAPPAHGV	√	(GalNAc α)-	Tn
71	PAPGSTAPPAHGV	√	(GalNAc α)-	Tn
72	APGSTAPPAHGV	√	(GalNAc α)-	Tn
73	PGSTAPPAHGV	√	(GalNAc α)-	Tn
74	GSTAPPAHGV	√	(GalNAc α)-	Tn
75	STAPPAHGV	√	(GalNAc α)-	Tn
76	TAPPAHGV	√	(GalNAc α)-	Tn
77	APPAHGV	√	(GalNAc α)-	Tn
78	PPAHGV	√	(GalNAc α)-	Tn
79	PAHGV	√	(GalNAc α)-	Tn
80	AHGV	√	(GalNAc α)-	Tn
81	HGV	√	(GalNAc α)-	Tn
82	GVT	√	(GalNAc α)-	Tn
83	APGSTAPPAHGLLLTVLTV	x	(GalNAc α)-	Tn
84	VSAPDTRPAPGSTAPPAHG	√	(GalNAc α)-	Tn

85	VTSAPDTRPAPGSTNPPAHG	✓	(GalNAcα)-	Tn
86	VTSAPDTRPAPGSTDPPAHG	✓	(GalNAcα)-	Tn
87	VTSAPDTRPAPGSTQPPAHG	✓	(GalNAcα)-	Tn
88	VTSAPDTRPAPGSTTEPPAHG	✓	(GalNAcα)-	Tn
89	VTSAPDTRPAPGSTHPPAHG	✓	(GalNAcα)-	Tn
90	VTSAPDTRPAPGSTIPPAHG	✓	(GalNAcα)-	Tn
91	VTSAPDTRPAPGSTLPPAHG	✓	(GalNAcα)-	Tn
92	VTSAPDTRPAPGSTMPPAHG	✓	(GalNAcα)-	Tn
93	VTSAPDTRPAPGSTFPPAHG	✓	(GalNAcα)-	Tn
94	VTSAPDTRPAPGSTPPPAHG	✓	(GalNAcα)-	Tn
95	VTSAPDTRPAPGSTSPPAHG	✓	(GalNAcα)-	Tn
96	VTSAPDTRPAPGSTTPPAHG	✓	(GalNAcα)-	Tn
97	VTSAPDTRPAPGSTWPPAHG	✓	(GalNAcα)-	Tn
98	VTSAPDTRPAPGSTYPPAHG	✓	(GalNAcα)-	Tn
99	VTSAPDTRPAPGSTVPPAHG	✓	(GalNAcα)-	Tn
100	VTSAPDTRPAPGSTCPPAHG	x	(GalNAcα)-	Tn
101	VTSAPDTRPAPGSTAPPAHG	✓	(GalNAcα)-	Tn
102	ATSAPDTRPAPGSTAPPAHG	✓	(GalNAcα)-	Tn
103	VASAPDTRPAPGSTAPPAHG	✓	(GalNAcα)-	Tn
104	VTAAPDTRPAPGSTAPPAHG	✓	(GalNAcα)-	Tn
105	VTSAADTRPAPGSTAPPAHG	✓	(GalNAcα)-	Tn
106	VTSAPATRPAPGSTAPPAHG	✓	(GalNAcα)-	Tn
107	VTSAPDARPAPGSTAPPAHG	✓	(GalNAcα)-	Tn
108	VTSAPDTAPAPGSTAPPAHG	✓	(GalNAcα)-	Tn
109	VTSAPDTRAAPGSTAPPAHG	✓	(GalNAcα)-	Tn
110	VTSAPDTRPAAGSTAPPAHG	✓	(GalNAcα)-	Tn
111	VTSAPDTRPAPASTAPPAHG	✓	(GalNAcα)-	Tn
112	VTSAPDTRPAPGATAPPAHG	✓	(GalNAcα)-	Tn
113	VTSAPDTRPAPGSAAPPAHG	✓	(GalNAcα)-	Tn
114	VTSAPDTRPAPGSTAAPAHG	x	(GalNAcα)-	Tn
115	VTSAPDTRPAPGSTAPAAHG	✓	(GalNAcα)-	Tn
116	VTSAPDTRPAPGSTAPPAAG	✓	(GalNAcα)-	Tn
117	VTSAPDTRPAPGSTAPPAHA	✓	(GalNAcα)-	Tn
118	VTSAPDTRPAPGSTAPPAHG	✓	(GalNAcα)-	Tn
119	VTSAPDTRPAPGSTAPPAHG	✓	(GalNAcα)-	Tn
120	VTSAPDTRPAPLSTAPPAHG	✓	(GalNAcα)-	Tn
121	VTSAPDTRPAPGLTAPPAHG	✓	(GalNAcα)-	Tn
122	VTSAPDTRPAPGSTLPPAHG	✓	(GalNAcα)-	Tn
123	VTSAPDTRPAPGSTALPAHG	✓	(GalNAcα)-	Tn
124	VTSAPDTRPAPGSTAPLAHG	✓	(GalNAcα)-	Tn
125	VTSAPDTRPAPGSTAPPLHG	✓	(GalNAcα)-	Tn
126	VTSAPDTRPALLSTAPPAHG	✓	(GalNAcα)-	Tn
127	VTSAPDTRPAPGSTAPPLLG	✓	(GalNAcα)-	Tn
128	VTSAPDTRPALLSTAPPLLG	✓	(GalNAcα)-	Tn
129	VTSAPDTRPALLLTVPPAHG	✓	(GalNAcα)-	Tn

130	VTSA PDTRPALLLTVLLAHG	✓	(GalNAc α)-	Tn
131	VTSA PDTRPALLLTVLLAHG	✓	(GalNAc α)-	Tn
132	VTSA PDTRPALLLTLPPAHG	✓	(GalNAc α)-	Tn
133	VTSA PDTRPAPGSTAPPAHG	✓	(GlcNAc β 1-3GalNAc α)-	Core 3
134	VTSA PDTRPAPLSAPPAHG	x	(GlcNAc β 1-3GalNAc α)-	Core 3
135	VTSA PDTRPAPGLTAPPAHG	✓	(GlcNAc β 1-3GalNAc α)-	Core 3
136	VTSA PDTRPAPGSTLPPAHG	✓	(GlcNAc β 1-3GalNAc α)-	Core 3
137	VTSA PDTRPAPGSTALPAHG	✓	(GlcNAc β 1-3GalNAc α)-	Core 3
138	VTSA PDTRPAPGSTAPLAHG	✓	(GlcNAc β 1-3GalNAc α)-	Core 3
139	VTSA PDTRPAPGSTAPPLHG	✓	(GlcNAc β 1-3GalNAc α)-	Core 3
140	VTSA PDTRPALLSTAPPAHG	x	(GlcNAc β 1-3GalNAc α)-	Core 3
141	VTSA PDTRPAPGSTAPPLLG	✓	(GlcNAc β 1-3GalNAc α)-	Core 3
142	VTSA PDTRPALLSTAPPLLG	✓	(GlcNAc β 1-3GalNAc α)-	Core 3
143	VTSA PDTRPALLLTVPPAHG	✓	(GlcNAc β 1-3GalNAc α)-	Core 3
144	VTSA PDTRPALLLTVLLAHG	✓	(GlcNAc β 1-3GalNAc α)-	Core 3
145	APGSTAPPAHGVTSAPDTRP	✓	(GalNAc α)-	Tn
146	APGSTAPPAHGVTSAPDTRP	✓	(GlcNAc β 1-3GalNAc α)-	Core 3
147	APGSTAPPAHGVTSAPDTRP	✓	(GlcNAc β 1-6[Gal β 1-3]GalNAc α)-	Core 2
148	APGSTAPPAHGVTSAPDTRP	✓	(GlcNAc β 1-6[GlcNAc β 1-3]GalNAc α)-	Core 4
149	APGSTAPAAHGVTSAPDTRP	✓	(GalNAc α)-	Tn
150	APGSTAPAAHGVTSAPDTRP	✓	(GlcNAc β 1-3GalNAc α)-	Core 3
151	APGSTAPAAHGVTSAPDTRP	✓	(GlcNAc β 1-6[Gal β 1-3]GalNAc α)-	Core 2
152	APGSTAPAAHGVTSAPDTRP	✓	(GlcNAc β 1-6[GlcNAc β 1-3]GalNAc α)-	Core 4
153	APGSTAPQAHGVTSAPDTRP	✓	(GalNAc α)-	Tn
154	APGSTAPQAHGVTSAPDTRP	✓	(GlcNAc β 1-3GalNAc α)-	Core 3
155	APGSTAPQAHGVTSAPDTRP	✓	(GlcNAc β 1-6[Gal β 1-3]GalNAc α)-	Core 2
156	APGSTAPQAHGVTSAPDTRP	✓	(GlcNAc β 1-6[GlcNAc β 1-3]GalNAc α)-	Core 4
157	APGSTAPPAHGVESAPDTRP	✓	(GalNAc α)-	Tn
158	APGSTAPPAHGVESAPDTRP	✓	(GlcNAc β 1-3GalNAc α)-	Core 3
159	APGSTAPPAHGVESAPDTRP	✓	(GlcNAc β 1-6[Gal β 1-3]GalNAc α)-	Core 2
160	APGSTAPPAHGVESAPDTRP	x	(GlcNAc β 1-6[GlcNAc β 1-3]GalNAc α)-	Core 4

S3 Alanine Tn MUC1a mutation walk and detection with 5E5 and 2D9 mAbs

Binding of mAbs 5E5 and 2D9 on walking single site alanine-mutation of Bis-Tn-MUC1a glycopeptides (VTSAPDTRPAPGSTAPPAHG). Each bar represent an alanine mutation of native amino acid displayed on the x-axis.

S4 Core-3 mAb 1E10 Tn-MUC1a specificity (A). Immunogen (B).

Generation of Core-3 mAb 1E10 was performed as described previously (Reis, C.A., et al. 1998, *Glycoconj. J.*, **15**, 51–62). Briefly, female Balb/c mice were immunized with Core3-MUC1 20-mer glycopeptides (**B**), conjugated to KLH in Freund's complete adjuvant followed by two injections with Freund's incomplete adjuvant and finally an intravenous boost without adjuvant. Eye bleeds were collected 7 days after the third immunization and sera tested by array. Three days after the fourth immunization, spleen cells from one mouse were fused with NS1 myeloma cells (Kohler and Milstein 1975). Hybridomas specific to the antigens of interest were cloned by limiting dilution.

S5 On-bead enzymatic reactions (Core3 and STn) with MALDI-TOF spectra

S6 *Competitive glycopeptide inhibition reactions of core3-specific serum autoantibody. Serum autoantibody binding to Core3-MUC1a glycopeptides (25) was inhibited with a pre-incubated serum mixture at different inhibitor concentrations of Core3-MUC1a 20mer (A) or Tn-MUC1a 20mer (B) and their corresponding 60mer glycoforms.*

S7 *Tn*-specific VVA lectin microarray staining on glycopeptides array. Structures for compounds 1-160 are presented in S2.