Supporting Information

Irreversible Inactivation of Arylamine N-Acetyltransferases in the Presence of N-Hydroxy-4-Acetylaminobiphenyl: A Comparison of Human and Hamster Enzymes

Haiqing Wang, Carston R. Wagner and Patrick E. Hanna

Departments of Medicinal Chemistry and Pharmacology, University of Minnesota, Minneapolis, MN 55455

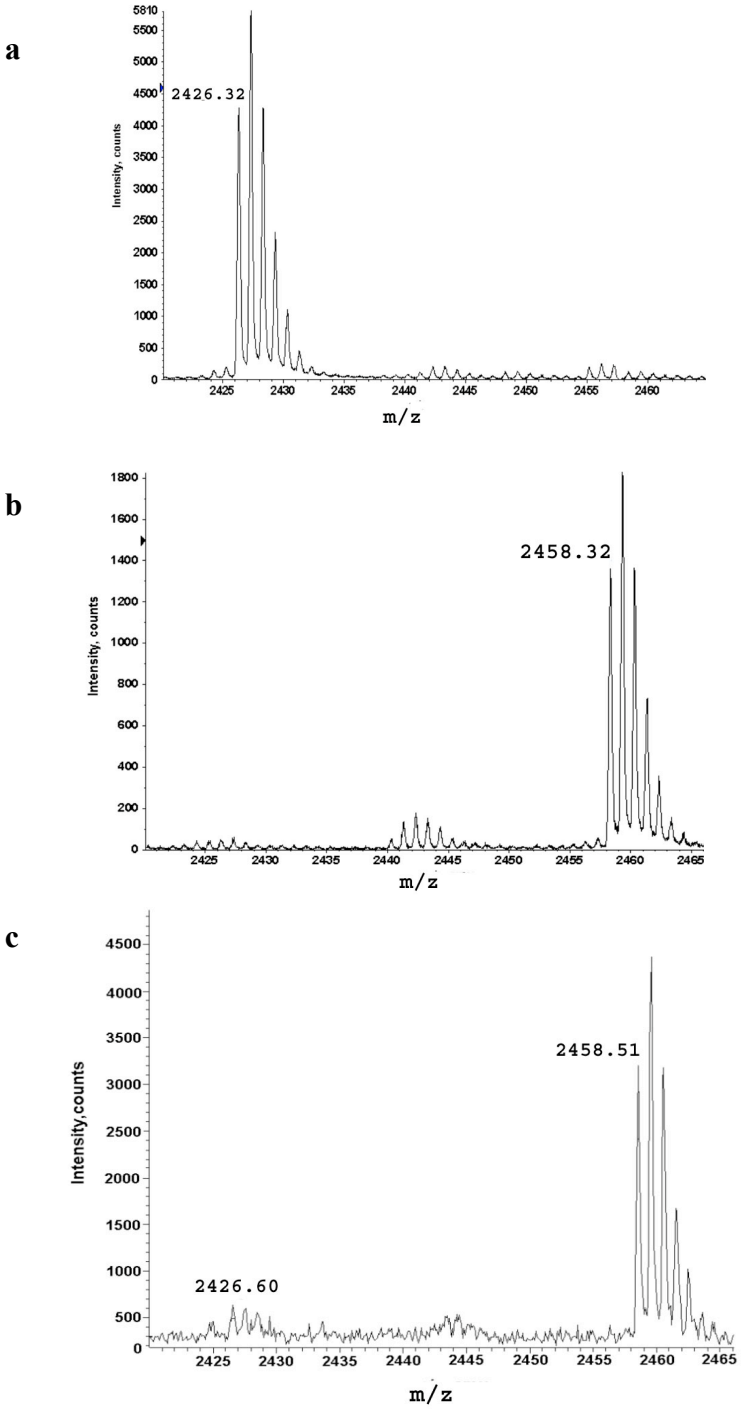

Figure S1. Segments of the MALDI Q-TOF mass spectra of pepsin digests of (a) native hamster NAT1; (b) N-OH-4-AABP-inactivated hamster NAT1; and (c) 4-nitrosobiphenyl-inacivated hamster NAT1.

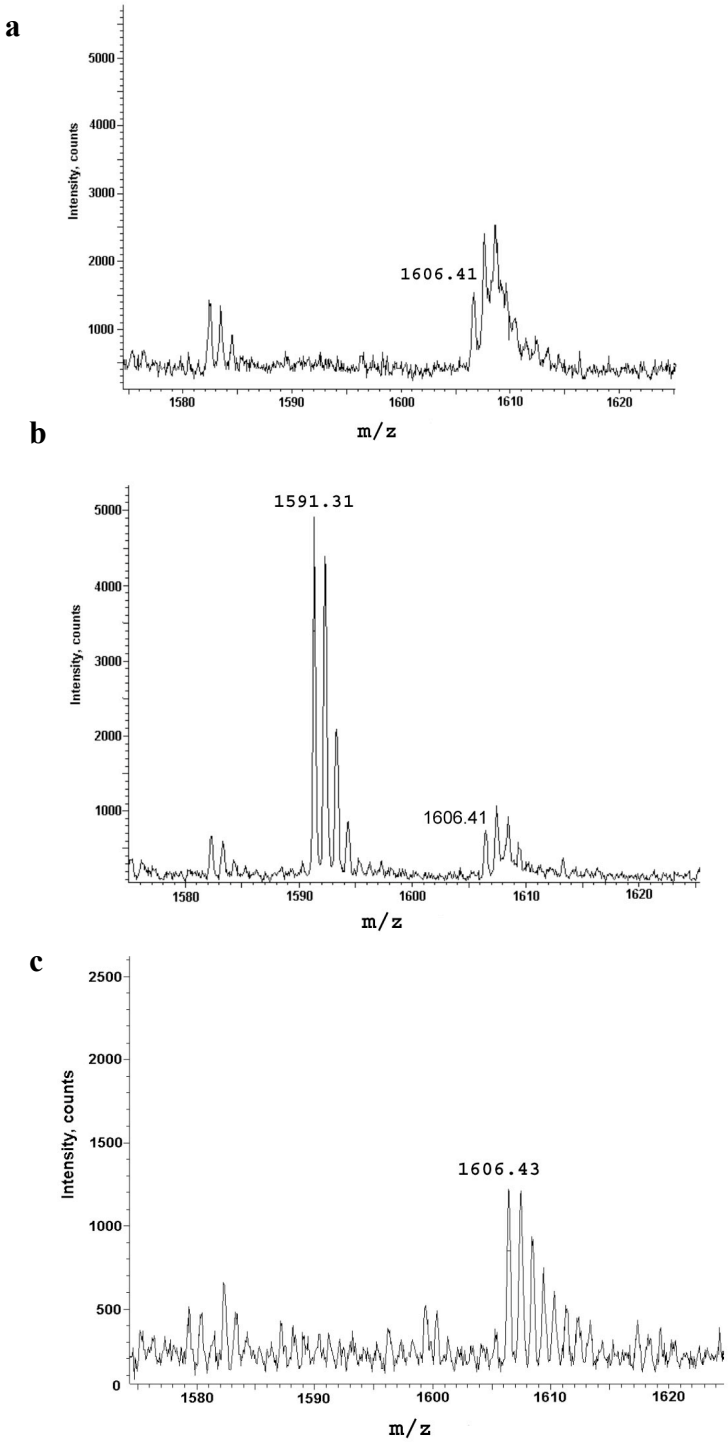

Figure S2. Segments of the MALDI Q-TOF mass spectra of endoproteinase Glu-C digests of (a) native hamster NAT1; (b) N-OH-4-AABP-inactivated hamster NAT1; (c) 4-nitrosobiphenyl-inactivated hamster NAT1.

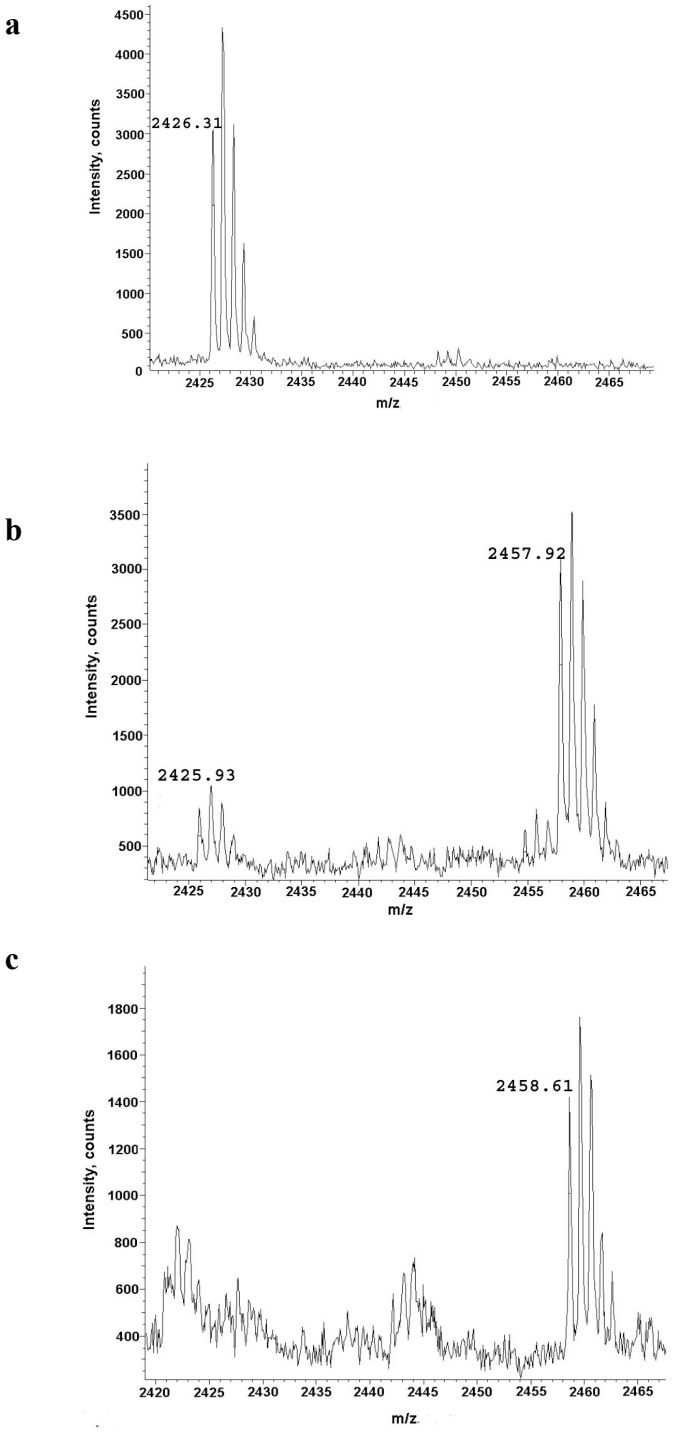

Figure S3. Segments of the MALDI Q-TOF mass spectra of pepsin digests of (a) native hamster NAT2; (b) N-OH-4-AABP-inactivated hamster NAT2; and (c) 4-nitrosobiphenyl-inactivated hamster NAT2.

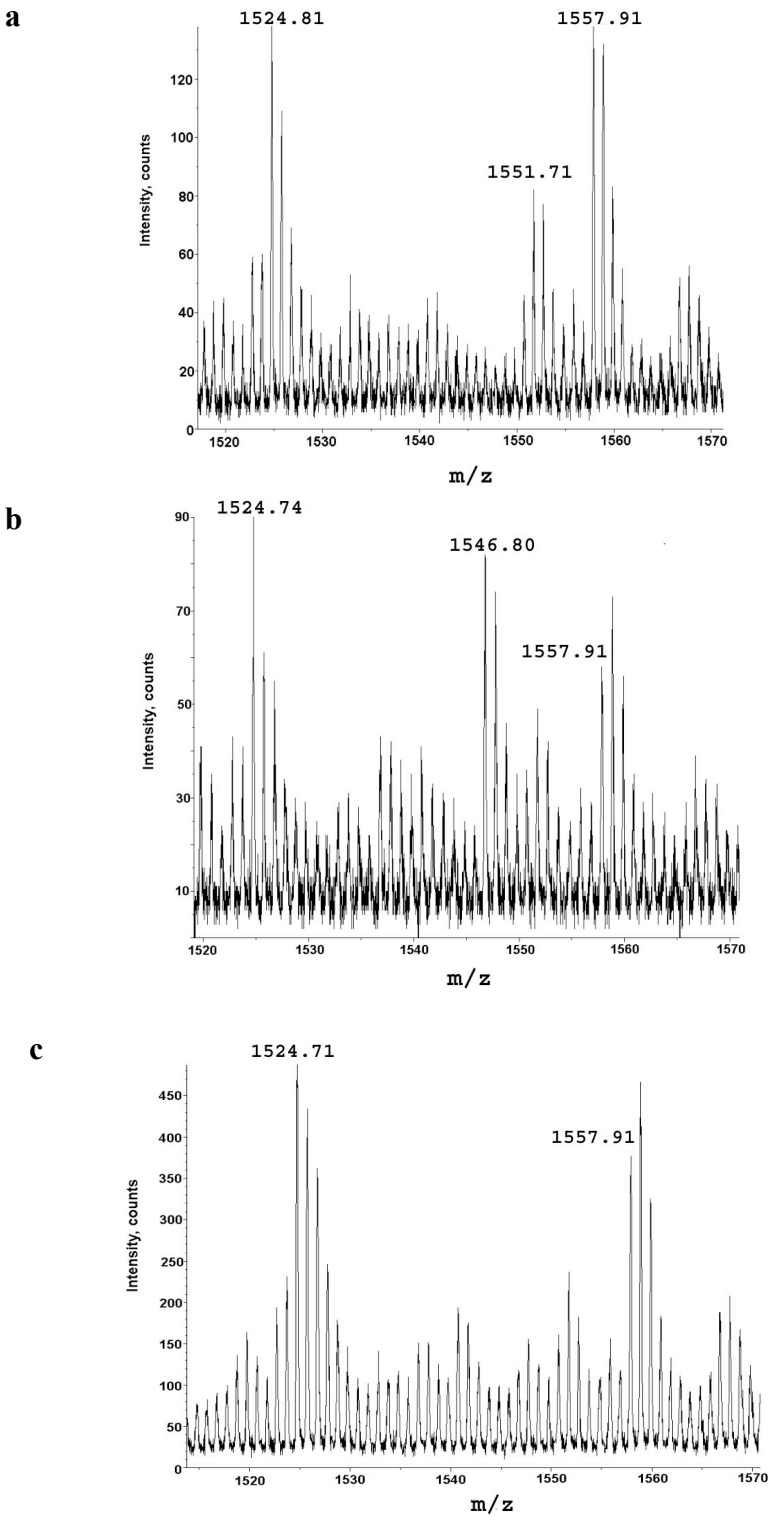

Figure S4. Segments of the MALDI Q-TOF mass spectra of endoproteinase Glu-C digests of (a) native hamster NAT2; (b) N-OH-4-AABP-inactivated hamster NAT2; and (c) 4-nitrosobiphenyl-inactivated hamster NAT2.

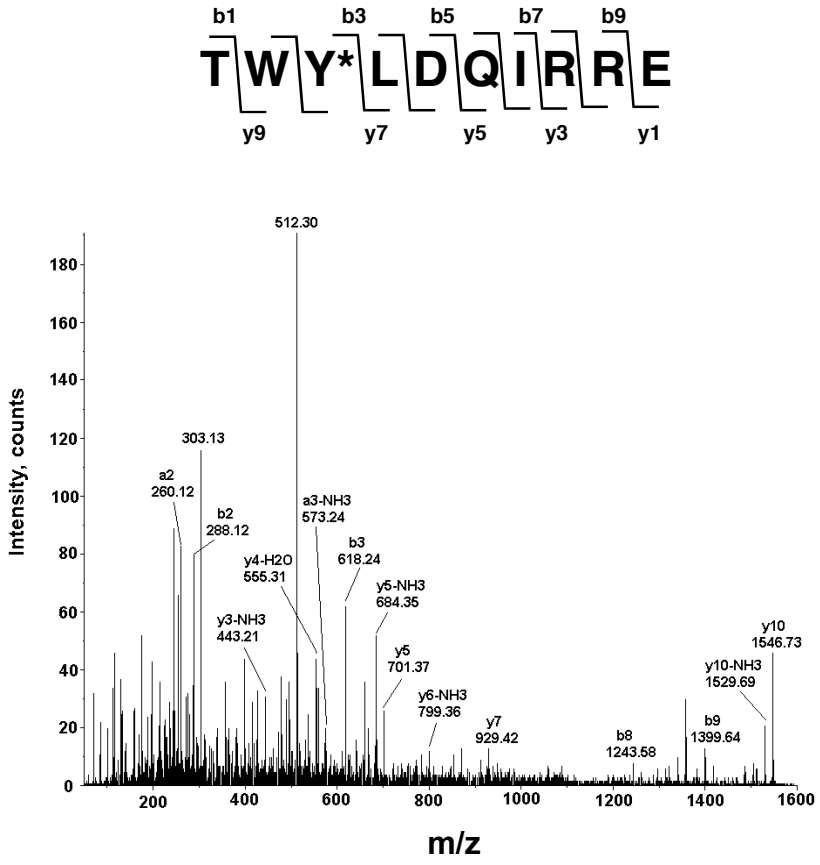

Figure S5. MALDI Q-TOF tandem mass spectrum of the 1546.8 Da peptide obtained by endoproteinase Glu-C-catalyzed digestion of N-OH-4-AABP-inactivated hamster NAT2. See also, Table S4.

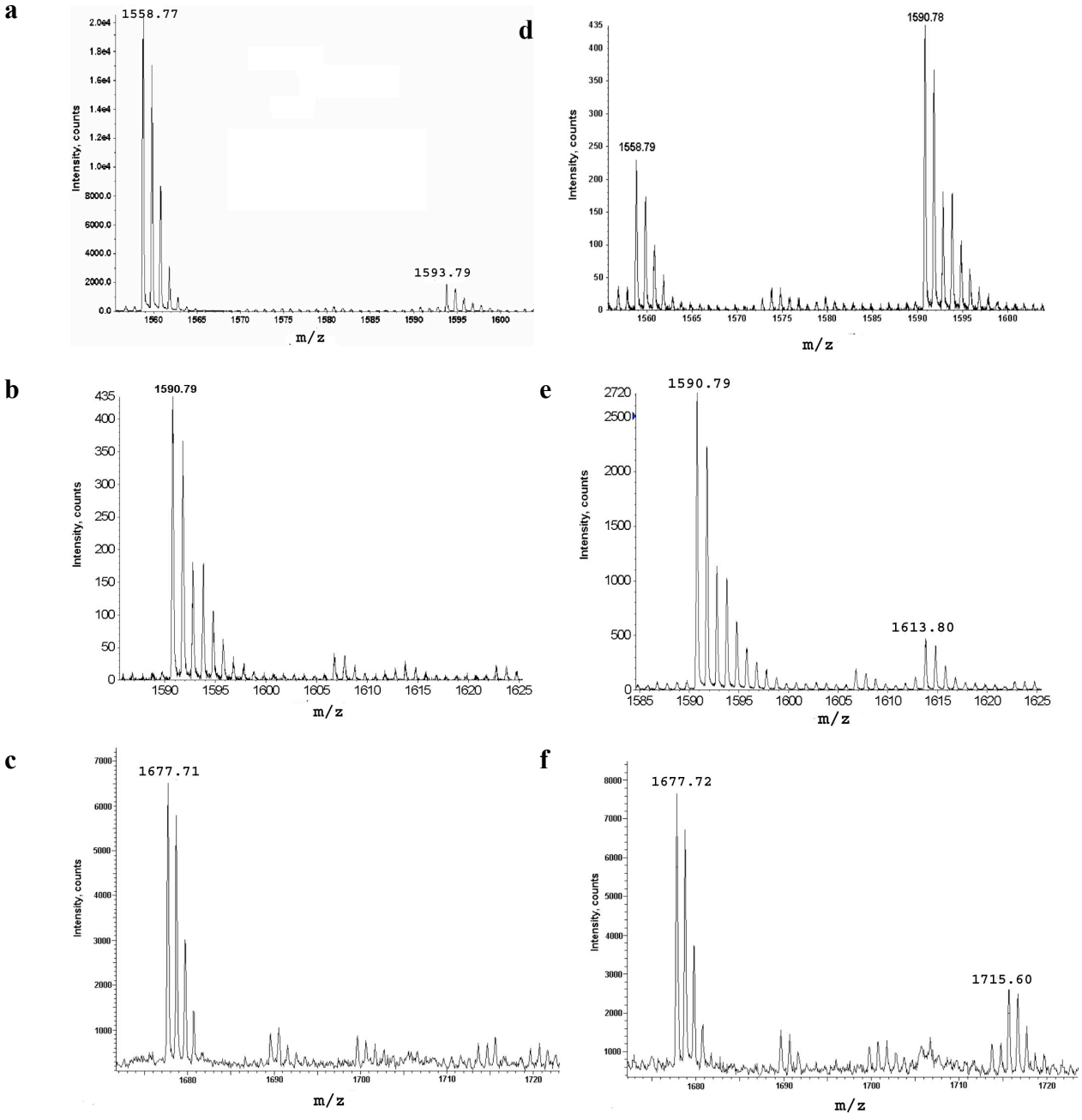

Figure S6. Segments of the MALDI-Q-TOF mass spectra of pepsin digests of (a, b, and c) native human NAT1; and (d, e, and f) N-OH-4-AABP-inactivated human NAT1

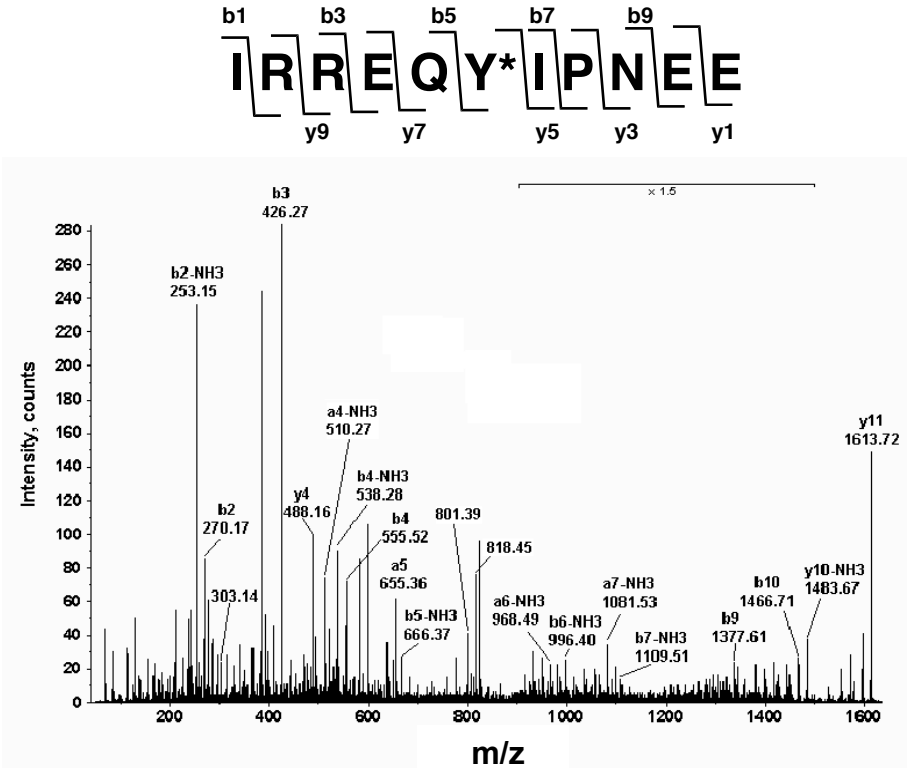

Figure S7. MALDI Q-TOF tandem mass spectrum of the 1613.80 Da peptide obtained by pepsin-catalyzed digestion of N-OH-4-AABP-inactivted human NAT1. See also, Table S6.

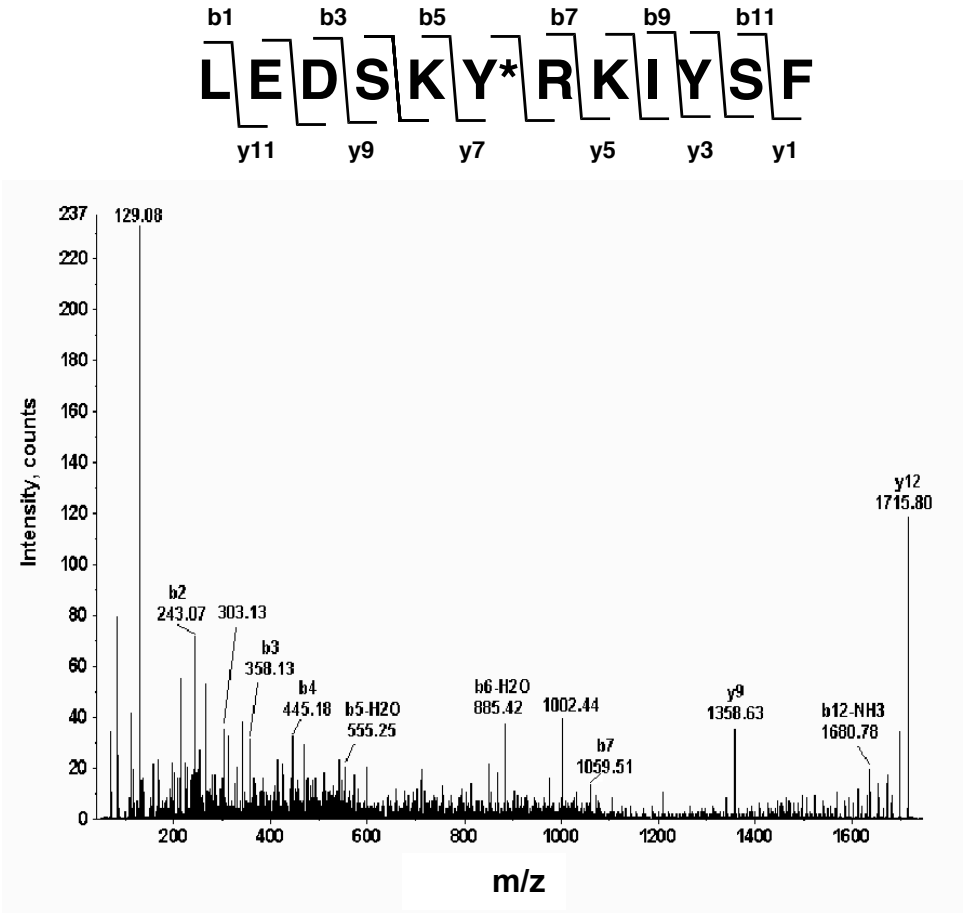

Figure S8. MALDI Q-TOF tandem mass spectrum of the 1715.60 Da peptide obtained by pepsin digestion of N-OH-4-AABP-inactivated human NAT1. See also, Table S7.

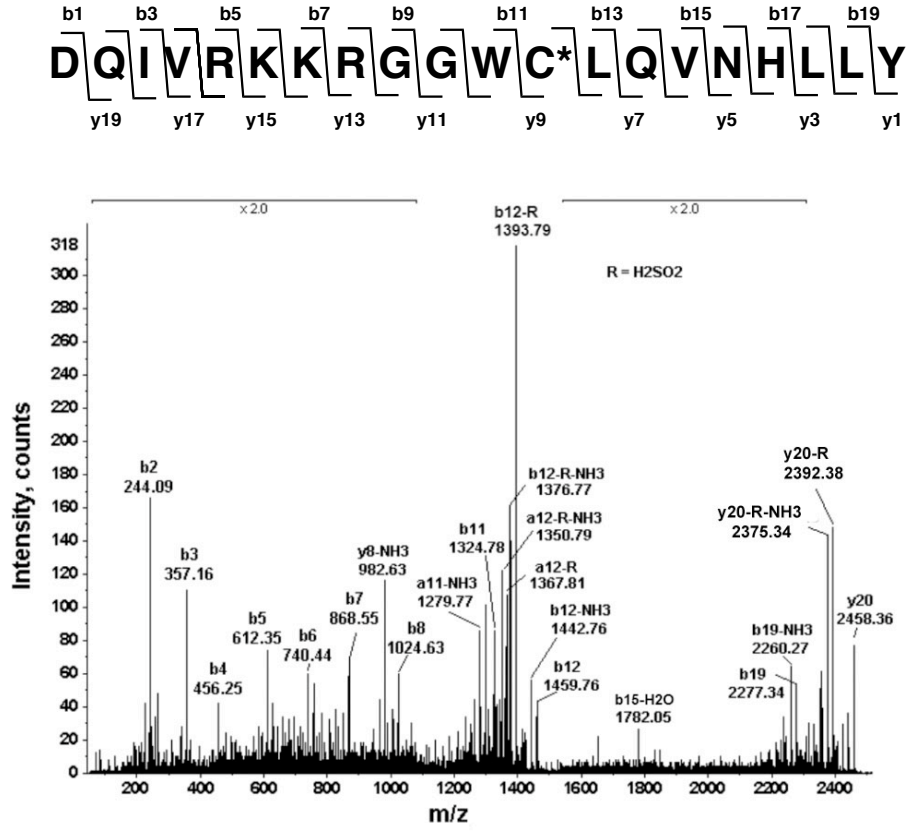

Figure S9. MALDI Q-TOF tandem mass spectrum of the 2458.51 Da peptide obtained by pepsin digestion of 4-nitrosobiphenyl-inactivated hamster NAT1. See also, Table S8.

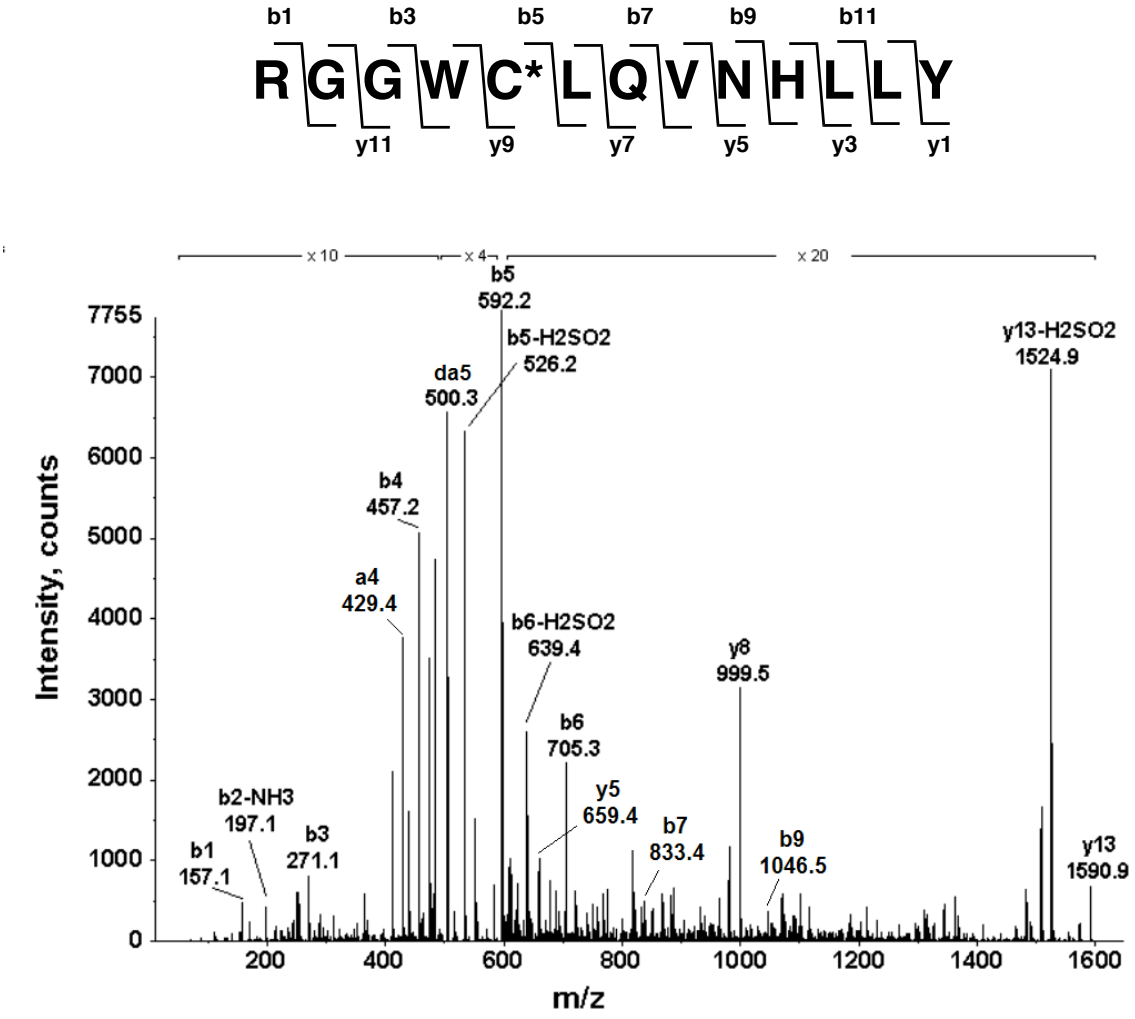

Figure S10. MALDI Q-TOF tandem mass spectrum of the 1590.90 Da peptide obtained by sequential pepsin and endopeptidase Lys-C digestion of 4-nitrosobiphenyl-treated hamster NAT2. See also, Table S9.

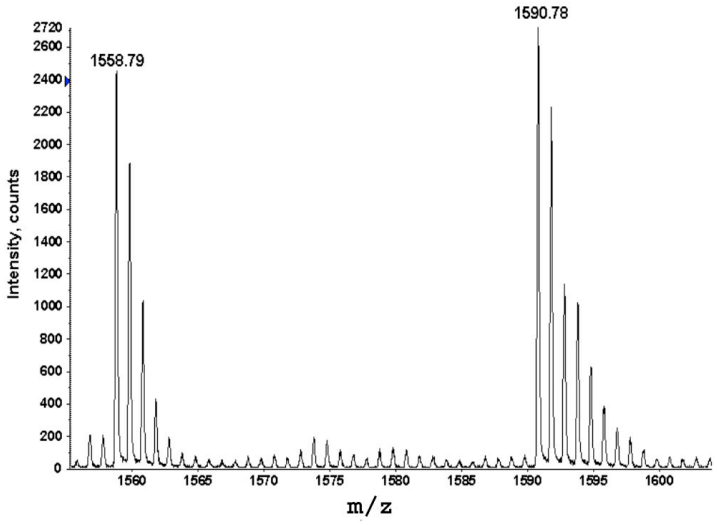

Figure S11. Segment of the MALDI Q-TOF mass spectrum of the pepsin digest of 4-nitrosobiphenyl-inactivated human NAT1. The corresponding segment of the MALDI Q-TOF mass spectrum of the pepsin digest of native human NAT1is shown Figure S6a.

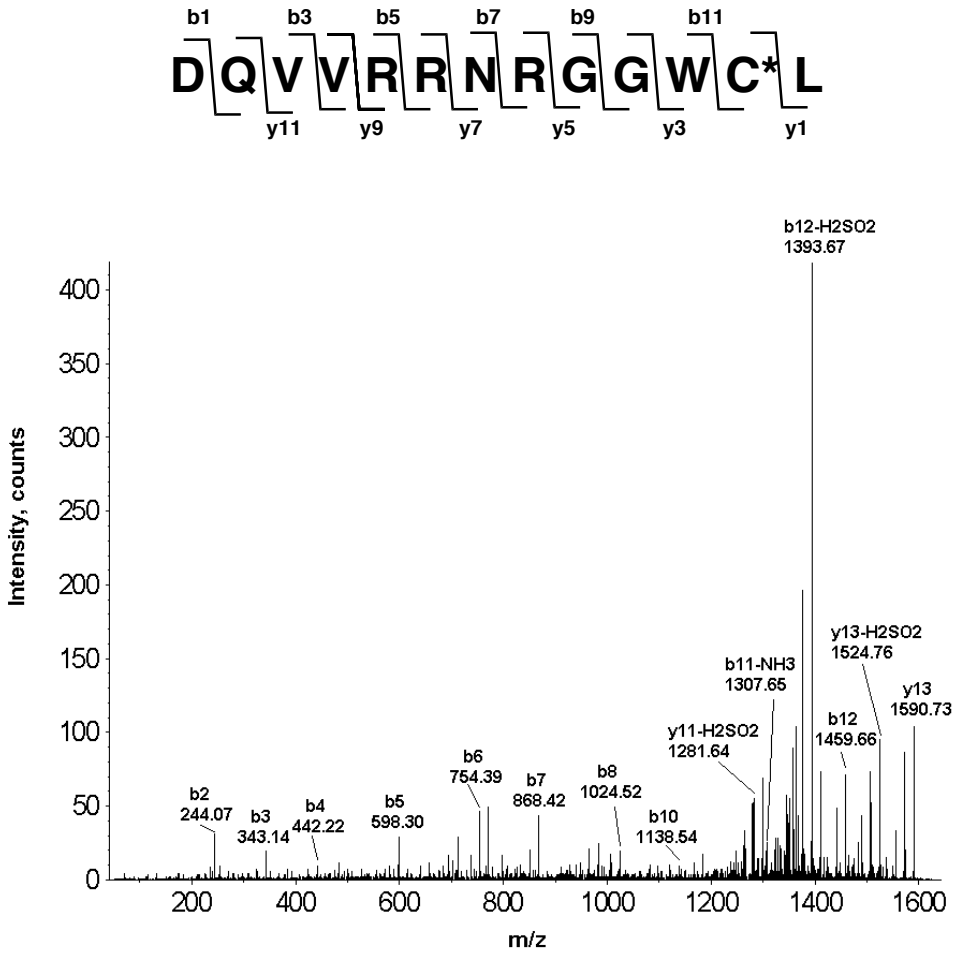

Figure S12. MALDI Q-TOF tandem mass spectrum of the 1590.78 Da peptide obtained by pepsin digestion of 4-nitrosobiphenyl-treated human NAT1. See also, Table S10.

Table S1. Theoretical and Experimental m/z for b and y Ions for the Peptide (m/z 2458.32) Obtained by Pepsin-Catalyzed Digestion of N-OH-4-AABP-Treated Hamster NAT1[a]

			DQIVRKKRGGWC*LQVNHLLY			
b ions	Theoretical m/z	Experimental m/z	Sequence	Theoretical m/z	Experimental m/z	y ions
			DQIVRKKRGGWC*LQVNHLLY	2458.31	2458.36	y20
b1	116.03	~~~~	D QIVRKKRGGWC*LQVNHLLY	2343.29	~~~~	y19
b2	244.09	244.09	DQ IVRKKRGGWC*LQVNHLLY	2215.23	~~~~	y18
b3	357.17	357.16	DQI VRKKRGGWC*LQVNHLLY	2102.14	~~~~	y17
b4	456.25	456.25	DQIV RKKRGGWC*LQVNHLLY	2003.08	~~~~	y16
b5	612.35	612.35	DQIVR KKRGGWC*LQVNHLLY	1846.97	~~~~	y15
b6	740.44	740.44	DQIVRK KRGGWC*LQVNHLLY	1718.88	~~~~	y14
b7	868.53	868.55	DQIVRKK RGGWC*LQVNHLLY	1590.78	~~~~	y13
b8	1024.64	1024.63	DQIVRKKR GGWC*LQVNHLLY	1434.68	~~~~	y12
b9	1081.66	~~~~	DQIVRKKRG GWC*LQVNHLLY	1359.65	1359.76	y11-H2O
b10	1138.68	~~~~	DQIVRKKRGG WC*LQVNHLLY	1320.64	~~~~	y10
b11	1324.76	1324.78	DQIVRKKRGGW C*LQVNHLLY	1134.56	~~~~	y9
b12	1459.76	1459.76	DQIVRKKRGGWC* LQVNHLLY	982.52	982.63	y8-NH3
b13	1572.84	~~~~	DQIVRKKRGGWC*L QVNHLLY	886.48	~~~~	y7
b14	1700.90	~~~~	DQIVRKKRGGWC*LQ VNHLLY	758.42	~~~~	y6
b15-H2O	1781.96	1782.05	DQIVRKKRGGWC*LQV NHLLY	659.35	~~~~	y5
b16	1914.01	~~~~	DQIVRKKRGGWC*LQVN HLLY	545.31	~~~~	y4
b17	2051.07	~~~~	DQIVRKKRGGWC*LQVNH LLY	408.25	~~~~	y3
b18	2164.16	~~~~	DQIVRKKRGGWC*LQVNHL LY	295.16	~~~~	y2
b19	2277.24	2277.33	DQIVRKKRGGWC*LQVNHLL Y	182.08	~~~~	y1
b20	2440.30	~~~~	DQIVRKKRGGWC*LQVNHLLY			

[a]The modified residue (Cys68) is designated C*

Table S2. Theoretical and Experimental m/z for a and y Ions for the Peptide (m/z 1591.31) Obtained by Endoproteinase-Glu-C-Catalyzed Digestion of N-OH-4-AABP-Treated Hamster NAT1[a]

			RIGYNNPVY*TLD			
a ions	Theoretical m/z	Experimental m/z	Sequence	Theoretical m/z	Experimental m/z	y ions
			RIGYNNPVY*TLD	1591.79	1591.79	y12
a1-NH3	112.11	112.08	R IGYNNPVY*TLD	1435.69	~~~~	y11
a2	242.20	242.19	RI GYNNPVY*TLD	1322.61	~~~~	y10
a3	299.22	~~~~	RIG YNNPVY*TLD	1265.58	~~~~	y9
a4	462.28	462.26	RIGY NNPVY*TLD	1102.52	~~~~	y8
a5	576.33	~~~~	RIGYN NPVY*TLD	988.48	~~~~	y7
a6	690.37	690.35	RIGYNN PVY*TLD	874.44	~~~~	y6
a7	787.42	787.40	RIGYNNP VY*TLD	777.38	~~~~	y5
a8	886.49	886.45	RIGYNNPV Y*TLD	678.31	~~~~	y4
a9-NH3	1199.63	1199.60	RIGYNNPVY* TLD	348.18	~~~~	y3
a10	1317.67	1317.65	RIGYNNPVY*T LD	247.13	~~~~	y2
a11	1430.76	1430.73	RIGYNNPVY*TL D	134.05	~~~~	y1
a12	1545.78	~~~~	RIGYNNPVY*TLD			

[a]The modified tyrosine residue (Tyr17) is designated Y*

Table S3. Theoretical and Experimental m/z for b and y Ions for the Peptide (m/z 2457.92) Obtained by Pepsin-Catalyzed Digestion of N-OH-4-AABP-Treated Hamster NAT2[a]

			DQIVRKKRGGWC*LQVNHLLY			
b ions	Theoretical m/z	Experimental m/z	Sequence	Theoretical m/z	Experimental m/z	y ions
			DQIVRKKRGGWC*LQVNHLLY	2458.31	2458.29	y20
b1	116.03	~~~~	D QIVRKKRGGWC*LQVNHLLY	2343.29	~~~~	y19
b2	244.09	244.09	DQ IVRKKRGGWC*LQVNHLLY	2215.23	~~~~	y18
b3	357.17	357.16	DQI VRKKRGGWC*LQVNHLLY	2102.14	~~~~	y17
b4	456.25	456.23	DQIV RKKRGGWC*LQVNHLLY	2003.08	~~~~	y16
b5	612.35	612.33	DQIVR KKRGGWC*LQVNHLLY	1846.97	~~~~	y15
b6	740.44	740.42	DQIVRK KRGGWC*LQVNHLLY	1718.88	~~~~	y14
b7	868.53	868.52	DQIVRKK RGGWC*LQVNHLLY	1590.78	~~~~	y13
b8	1024.64	1024.62	DQIVRKKR GGWC*LQVNHLLY	1434.68	~~~~	y12
b9	1081.66	~~~~	DQIVRKKRG GWC*LQVNHLLY	1359.65	1359.76	y11-H2O
b10	1138.68	~~~~	DQIVRKKRGG WC*LQVNHLLY	1320.64	~~~~	y10
b11	1324.76	1324.74	DQIVRKKRGGW C*LQVNHLLY	1134.56	~~~~	y9
b12	1459.76	1459.76	DQIVRKKRGGWC* LQVNHLLY	982.52	982.62	y8-NH3
b13	1572.84	~~~~	DQIVRKKRGGWC*L QVNHLLY	886.48	~~~~	y7
b14	1700.9	~~~~	DQIVRKKRGGWC*LQ VNHLLY	758.42	~~~~	y6
b15-H2O	1781.96	1781.90	DQIVRKKRGGWC*LQV NHLLY	659.35	~~~~	y5
b16	1914.01	~~~~	DQIVRKKRGGWC*LQVN HLLY	545.31	~~~~	y4
b17	2051.07	~~~~	DQIVRKKRGGWC*LQVNH LLY	408.25	~~~~	y3
b18	2164.16	~~~~	DQIVRKKRGGWC*LQVNHL LY	295.16	~~~~	y2
b19	2277.24	2277.33	DQIVRKKRGGWC*LQVNHLL Y	182.08	~~~~	y1
b20	2440.3	~~~~	DQIVRKKRGGWC*LQVNHLLY			

[a]The modified residue (Cys68) is designated C*

Table S4. Theoretical and Experimental m/z for b and y Ions for the Peptide (m/z 1546.80) Obtained by Endoproteinase-Glu-C-Catalyzed Digestion of N-OH-4-AABP-Treated Hamster NAT2[a]

			TWY*LDQIRRE			
b ions	Theoretical m/z	Experimental m/z	Sequence	Theoretical m/z	Experimental m/z	y ions
			TWY*LDQIRRE	1546.78	1546.73	y10
b1	102.06	~~~~	T WY*LDQIRRE	1445.733	~~~~	y9
b2	288.14	288.12	TW Y*LDQIRRE	1259.654	~~~~	y8
b3	618.27	618.24	TWY* LDQIRRE	929.517	929.42	y7
b4	731.36	~~~~	TWY*L DQIRRE	799.43	799.36	y6-NH3
b5	846.38	~~~~	TWY*LD QIRRE	701.406	701.37	y5
b6	974.44	~~~~	TWY*LDQ IRRE	555.35	555.31	y4-H2O
b7	1087.53	~~~~	TWY*LDQI RRE	443.26	443.21	y3-NH3
b8	1243.63	1243.58	TWY*LDQIR RE	304.162	~~~~	y2
b9	1399.73	1399.64	TWY*LDQIRR E	148.061	~~~~	y1
b10	1528.68	~~~~	TWY*LDQIRRE			

[a]The modified residue (Tyr160) is designated Y*

Table S5. Theoretical and Experimental m/z for b and y Ions for the Peptide (m/z 1590.78) Obtained by Pepsin-Catalyzed Digestion of N-OH-4-AABP-Treated Human NAT1[a]

			DQVVRRNRGGWC*L			
b ions	Theoretical	Experimental	Sequence	Theoretical	Experimental	y ions
	m/z	m/z		m/z	m/z	
			DQVVRRNRGGWC*L	1590.79	1590.73	y13
b1	116.03	~~~~	D QVVRRNRGGWC*L	1475.76	1475.71	y12
b2	244.09	244.07	DQ VVRRNRGGWC*L	1347.71	~~~~	y11
b3	343.16	343.14	DQV VRRNRGGWC*L	1248.64	1248.60	y10
b4	442.23	442.22	DQVV RRNRGGWC*L	1149.57	~~~~	y9
b5	598.33	598.30	DQVVR RNRGGWC*L	993.47	~~~~	y8
b6	754.43	754.39	DQVVRR NRGGWC*L	837.37	~~~~	y7
b7	868.47	868.42	DQVVRRN RGGWC*L	723.32	~~~~	y6
b8	1024.58	1024.52	DQVVRRNR GGWC*L	567.22	~~~~	y5
b9	1081.60	~~~~	DQVVRRNRG GWC*L	510.21	~~~~	y4
b10	1138.62	1138.54	DQVVRRNRGG WC*L	453.18	~~~~	y3
b11-NH3	1307.67	1307.65	DQVVRRNRGGW C*L	267.10	~~~~	y2
b12	1459.7	1459.66	DQVVRRNRGGWC* L	132.10	~~~~	y1
b13	1572.78	~~~~	DQVVRRNRGGWC*L			

[a]The modified residue (Cys68) is designated C*

Table S6. Theoretical and Experimental m/z for b and y Ions for the Peptide (m/z 1613.80) Obtained by Pepsin-Catalyzed Digestion of N-OH-4-AABP-Treated Human NAT1[a]

			IRREQY*IPNEE			
b ions	Theoretical	Experimental	Sequence	Theoretical	Experimental	y ions
	m/z	m/z		m/z	m/z	
			IRREQY*IPNEE	1613.80	1613.72	y11
b1	114.09	~~~~	I RREQY*IPNEE	1483.70	1483.67	y10-NH3
b2	270.19	270.17	IR REQY*IPNEE	1344.62	~~~~	y9
b3	426.29	426.27	IRR EQY*IPNEE	1188.52	~~~~	y8
b4	555.34	555.52	IRRE QY*IPNEE	1059.48	~~~~	y7
b5-NH3	666.37	666.37	IRREQ Y*IPNEE	931.42	~~~~	y6
b6-NH3	996.53	996.40	IRREQY* IPNEE	601.28	~~~~	y5
b7-NH3	1109.62	1109.51	IRREQY*I PNEE	488.20	488.16	y4
b8	1223.67	~~~~	IRREQY*IP NEE	391.14	~~~~	y3
b9	1337.71	1337.61	IRREQY*IPN EE	277.10	~~~~	y2
b10	1466.75	1466.71	IRREQY*IPNE E	148.06	~~~~	y1
b11	1596.8		IRREQY*IPNEE		~~~~	

[a]The modified residue (Tyr169) is designated Y*

Table S7. Theoretical and Experimental m/z for b and y Ions for the Peptide (m/z 1715.60) Obtained by Pepsin-Catalyzed Digestion of N-OH-4-AABP-Treated Human NAT1[a]

			LEDSKY*RKIYSF			
b ions	Theoretical	Experimental	Sequence	Theoretical	Experimental	y ions
	m/z	m/z		m/z	m/z	
			LEDSKY*RKIYSF	1715.88	1715.80	y12
b1	114.09	~~~~	L EDSKY*RKIYSF	1602.80	~~~~	y11
b2	243.13	243.07	LE DSKY*RKIYSF	1473.75	~~~~	y10
b3	358.16	358.13	LED SKY*RKIYSF	1358.72	1358.63	y9
b4	445.19	445.18	LEDS KY*RKIYSF	1271.69	~~~~	y8
b5-H2O	555.28	555.25	LEDSK Y*RKIYSF	1143.60	~~~~	y7
b6-H2O	885.42	885.42	LEDSKY* RKIYSF	813.46	~~~~	y6
b7	1059.53	1059.51	LEDSKY*R KIYSF	657.36	~~~~	y5
b8	1187.62	~~~~	LEDSKY*RK IYSF	529.27	~~~~	y4
b9	1300.70	~~~~	LEDSKY*RKI YSF	416.18	~~~~	y3
b10	1463.77	~~~~	LEDSKY*RKIY SF	253.12	~~~~	y2
b11	1550.80	~~~~	LEDSKY*RKIYS F	166.08	~~~~	y1
b12-NH3	1680.84	1680.78	LEDSKY*RKIYSF			

[a]The modified residue (Tyr186) is designated Y*

Table S8. Theoretical and Experimental m/z for b and y Ions for the Peptide (m/z 2458.51) Obtained by Pepsin-Catalyzed Digestion of 4-Nitrosobiphenyl-Treated Hamster NAT1[a]

			DQIVRKKRGGWC*LQVNHLLY			
b ions	Theoretical	Experimental	Sequence	Theoretical	Experimental	y ions
	m/z	m/z		m/z	m/z	
			DQIVRKKRGGWC*LQVNHLLY	2458.31	2458.36	y20
b1	116.03	~~~~	D QIVRKKRGGWC*LQVNHLLY	2343.29	~~~~	y19
b2	244.09	244.09	DQ IVRKKRGGWC*LQVNHLLY	2215.23	~~~~	y18
b3	357.17	357.16	DQI VRKKRGGWC*LQVNHLLY	2102.14	~~~~	y17
b4	456.25	456.25	DQIV RKKRGGWC*LQVNHLLY	2003.08	~~~~	y16
b5	612.35	612.35	DQIVR KKRGGWC*LQVNHLLY	1846.97	~~~~	y15
b6	740.44	740.44	DQIVRK KRGGWC*LQVNHLLY	1718.88	~~~~	y14
b7	868.53	868.55	DQIVRKK RGGWC*LQVNHLLY	1590.78	~~~~	y13
b8	1024.64	1024.63	DQIVRKKR GGWC*LQVNHLLY	1434.68	~~~~	y12
b9	1081.66	~~~~	DQIVRKKRG GWC*LQVNHLLY	1359.65	1359.76	y11-H2O
b10	1138.68	~~~~	DQIVRKKRGG WC*LQVNHLLY	1320.64	~~~~	y10
b11	1324.76	1324.78	DQIVRKKRGGW C*LQVNHLLY	1134.56	~~~~	y9
b12	1459.76	1459.76	DQIVRKKRGGWC* LQVNHLLY	982.52	982.63	y8-NH3
b13	1572.84	~~~~	DQIVRKKRGGWC*L QVNHLLY	886.48	~~~~	y7
b14	1700.90	~~~~	DQIVRKKRGGWC*LQ VNHLLY	758.42	~~~~	y6
b15-H2O	1781.96	1782.05	DQIVRKKRGGWC*LQV NHLLY	659.35	~~~~	y5
b16	1914.01	~~~~	DQIVRKKRGGWC*LQVN HLLY	545.31	~~~~	y4
b17	2051.07	~~~~	DQIVRKKRGGWC*LQVNH LLY	408.25	~~~~	y3
b18	2164.16	~~~~	DQIVRKKRGGWC*LQVNHL LY	295.16	~~~~	y2
b19	2277.24	2277.33	DQIVRKKRGGWC*LQVNHLL Y	182.08	~~~~	y1
b20	2440.30	~~~~	DQIVRKKRGGWC*LQVNHLLY			

[a]The modified residue (Cys68) is designated C*

Table S9. Theoretical and Experimental m/z for b and y Ions for the Peptide (m/z 1590.90) Obtained by Sequential Pepsin and Endopeptidase Lys-C-Catalyzed Digestion of 4-Nitrosobiphenyl-Treated Hamster NAT2[a]

			RGGWC*LQVNHLLY			
b ions	Theoretical	Experimental	Sequence	Theoretical	Experimental	y ions
	m/z	m/z		m/z	m/z	
			RGGWC*LQVNHLLY	1590.78	1590.89	y13
b1	157.11	157.11	R GGWC*LQVNHLLY	1434.68	~~~~	y12
b2-NH3	197.10	197.10	RG GWC*LQVNHLLY	1377.66	~~~~	y11
b3	271.15	271.15	RGG WC*LQVNHLLY	1320.64	~~~~	y10
b4	457.23	457.23	RGGW C*LQVNHLLY	1134.56	~~~~	y9
b5	592.23	592.24	RGGWC* LQVNHLLY	999.56	999.53	y8
b6	705.31	705.30	RGGWC*L QVNHLLY	886.48	886.48	y7
b7	833.37	833.39	RGGWC*LQ VNHLLY	758.42	758.43	y6
b8	932.44	932.48	RGGWC*LQV NHLLY	659.35	659.36	y5
b9	1046.48	1046.52	RGGWC*LQVN HLLY	545.31	~~~~	y4
b10	1183.54	~~~~	RGGWC*LQVNH LLY	408.25	~~~~	y3
b11	1296.63	~~~~	RGGWC*LQVNHL LY	295.16	~~~~	y2
b12	1409.71	~~~~	RGGWC*LQVNHLL Y	182.08	~~~~	y1
b13	1572.77	~~~~	RGGWC*LQVNHLLY			

[a]The modified residue (Cys68) is designated C*

Table S10. Theoretical and Experimental m/z for b and y Ions for the Peptide (m/z 1590.78) Obtained by Sequential Pepsin-Catalyzed Digestion of 4-Nitrosobiphenyl-Treated Human NAT1[a]

			DQVVRRNRGGWC*L			
b ions	Theoretical	Experimental	Sequence	Theoretical	Experimental	y ions
	m/z	m/z		m/z	m/z	
			DQVVRRNRGGWC*L	1590.79	1590.73	y13
b1	116.03	~~~~	D QVVRRNRGGWC*L	1475.76	1475.72	y12
b2	244.09	244.07	DQ VVRRNRGGWC*L	1347.71	~~~~	y11
b3	343.16	343.16	DQV VRRNRGGWC*L	1248.64	1248.61	y10
b4	442.23	422.21	DQVV RRNRGGWC*L	1149.57	~~~~	y9
b5	598.33	598.30	DQVVR RNRGGWC*L	993.47	~~~~	y8
b6	754.43	754.39	DQVVRR NRGGWC*L	837.37	~~~~	y7
b7	868.47	868.42	DQVVRRN RGGWC*L	723.32	~~~~	y6
b8	1024.58	1024.51	DQVVRRNR GGWC*L	567.22	~~~~	y5
b9	1081.60	~~~~	DQVVRRNRG GWC*L	510.21	~~~~	y4
b10	1138.62	1138.52	DQVVRRNRGG WC*L	453.18	~~~~	y3
b11-NH3	1307.67	1307.63	DQVVRRNRGGW C*L	267.10	~~~~	y2
b12	1459.7	1459.64	DQVVRRNRGGWC* L	132.10	~~~~	y1
b13	1572.78	~~~~	DQVVRRNRGGWC*L			

[a]The modified residue (Cys68) is designated C*