Supplementary Data 1

Thiol-to-amine cyclization reaction enables screening of large libraries of macrocyclic compounds and the generation of sub-kDa ligands

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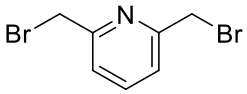
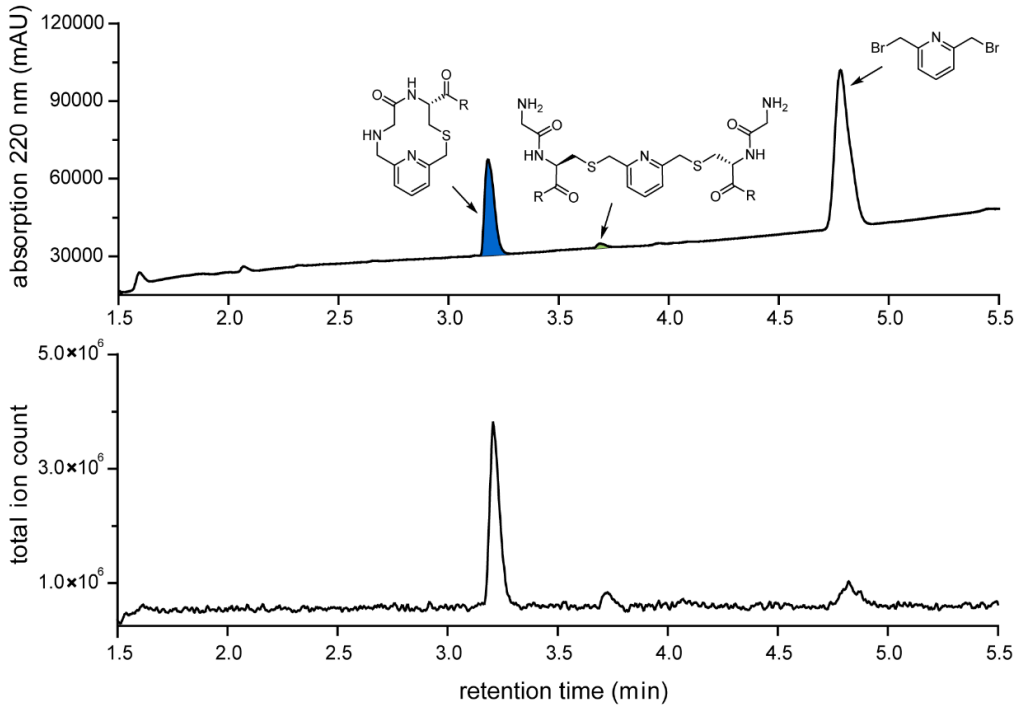
2,6-bis(bromomethyl)pyridine (1)

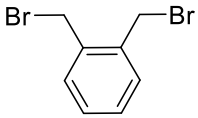


peptide	substrate	product	side products					
	not modified peptide	cyclic peptide	linear peptide-linker conjugate	linear peptide-linker conjugate with OH	linear peptide-linker conjugate with NH_2	linear peptide dimer	other	not identified
Gly-Cys-R	-	96	-	-	-	4	-	-
Ala-Cys-R	-	96	-	-	-	4	-	-
D-Ala-Cys-R	-	73	24	-	-	3	-	-
Val-Cys-R	-	96	-	-	-	4	-	-
β-Ala-Cys-R	-	81	14	-	-	5	-	-
Pro-Cys-R	-	96	-	-	-	4	-	-
Phe-Cys-R	-	96	-	-	-	4	-	-
Thr-Cys-R	-	93	-	-	-	4	-	3
Asn-Cys-R	-	92	-	-	-	4	-	3
Met-Cys-R	-	12	-	-	-	-	74[e]	14
Tyr-Cys-R	-	95	-	-	-	5	-	-
Glu-Cys-R	-	94	-	-	-	6	-	-
Gly-HCys-R_1	-	100	-	-	-	-	-	-
Gly-Mnv- R_1	-	96	-	-	-	4	-	-
Gly-NMeCys-R_1	-	87	-	-	-	-	13[c]	-
$(Gly)_2$-Cys-R	-	96	-	-	-	4	-	-
$(Gly)_3$-Cys-R	-	61	15	-	-	4	-	19
$(Gly)_4$-Cys-R	-	61	32	-	-	3	-	4
$(Gly)_5$-Cys-R	-	89	5	-	4	2	-	-

LC-MS analysis of Gly-Cys-R macrocyclization:

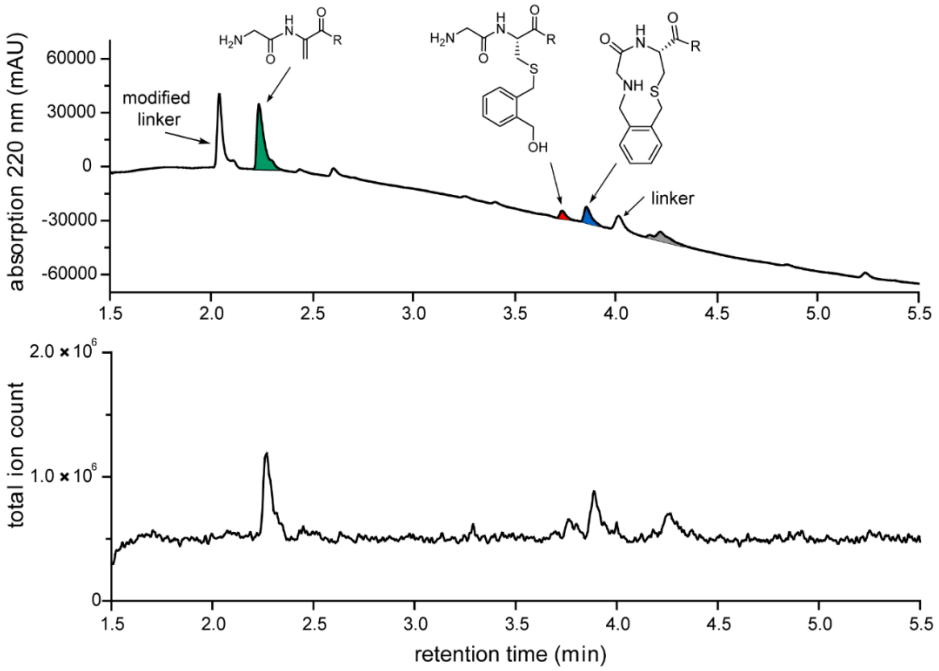


1,2-bis(bromomethyl)benzene (2)

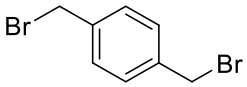


peptide	substrate	product	side products					
	not modified peptide	cyclic peptide	linear peptide-linker conjugate	linear peptide-linker conjugate with OH	linear peptide-linker conjugate with NH_2	linear peptide dimer	other	not identified
Gly-Cys-R	-	16	-	4	-	-	66[a]	14
Ala-Cys-R	-	39	-	-	-	-	44[a], 6[b]	11
D-Ala-Cys-R	-	34	-	-	-	-	54[a], 2[b]	10
Val-Cys-R	-	48	-	-	-	-	46[a], 3[b]	2
β-Ala-Cys-R	-	52	-	-	-	-	23[a], 4[b]	20
Pro-Cys-R	-	51	-	3	-	-	41[a], 5[b]	-
Phe-Cys-R	-	34	-	-	-	-	26[a]	41
Thr-Cys-R	-	41	-	-	-	-	45[a], 6[b]	8
Asn-Cys-R	-	40	-	4	-	-	45[a], 2[b]	9
Met-Cys-R	-	-	-	-	-	-	100[e]	-
Tyr-Cys-R	-	56	-	-	-	-	41[a]	3
Glu-Cys-R	-	56	-	-	-	-	44[a]	-
Gly-HCys-R_1	-	4	-	46	-	-	9[b],40[h]	-
Gly-Mnv- R_1	-	3	-	9	-	-	23[b], 65[h]	-
Gly-NMeCys-R_1	4	38	-	42	-	16	-	-
$(Gly)_2$-Cys-R	-	17	-	8	-	-	59[a]	17
$(Gly)_3$-Cys-R	-	29	-	5	-	-	40[a], 11[b]	15
$(Gly)_4$-Cys-R	-	55	-	4	-	-	34[a]	7
$(Gly)_5$-Cys-R	-	48	-	4	-	-	38[a]	9

LC-MS analysis of Gly-Cys-R macrocyclization:

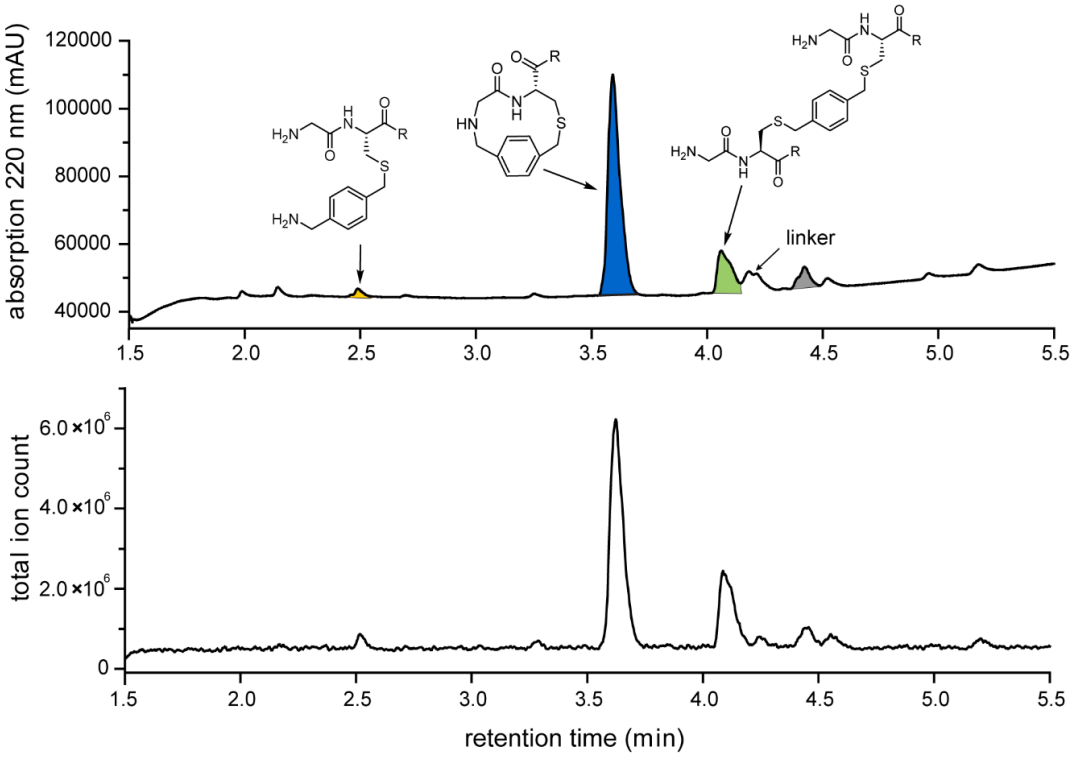


1,4-bis(bromomethyl)benzene (3)

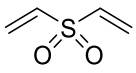


peptide	substrate	product	side products					
	not modified peptide	cyclic peptide	linear peptide-linker conjugate	linear peptide-linker conjugate with OH	linear peptide-linker conjugate with NH_2	linear peptide dimer	other	not identified
Gly-Cys-R	-	79	-	-	2	13	-	6
Ala-Cys-R	-	83	-	-	3	14	-	-
D-Ala-Cys-R	-	32	5	17	3	35	-	8
Val-Cys-R	-	70	-	5	1	21	-	3
β-Ala-Cys-R	-	45	-	15	2	19	-	19
Pro-Cys-R	-	98	-	-	-	2	-	-
Phe-Cys-R	-	73	-	3	2	18	-	4
Thr-Cys-R	-	83	-	-	-	17	-	-
Asn-Cys-R	-	79	-	3	2	16	-	-
Met-Cys-R	-	7	-	2	-	10	81[e]	-
Tyr-Cys-R	-	72	-	3	1	16	-	8
Glu-Cys-R	-	86	-	2	1	11	-	-
Gly-HCys-R_1	23	58	-	12	-	-	2[b]	4, 1
Gly-Mnv- R_1	53	13	-	34	-	-	-	-
Gly-NMeCys-R_1	9	51	-	-	-	24	-	16
$(Gly)_2$-Cys-R	-	30	-	16	3	33	-	18
$(Gly)_3$-Cys-R	-	26	-	12	3	37	-	22
$(Gly)_4$-Cys-R	-	49	-	7	3	27	-	14
$(Gly)_5$-Cys-R	-	44	-	14	3	30	-	9

LC-MS analysis of Gly-Cys-R macrocyclization:

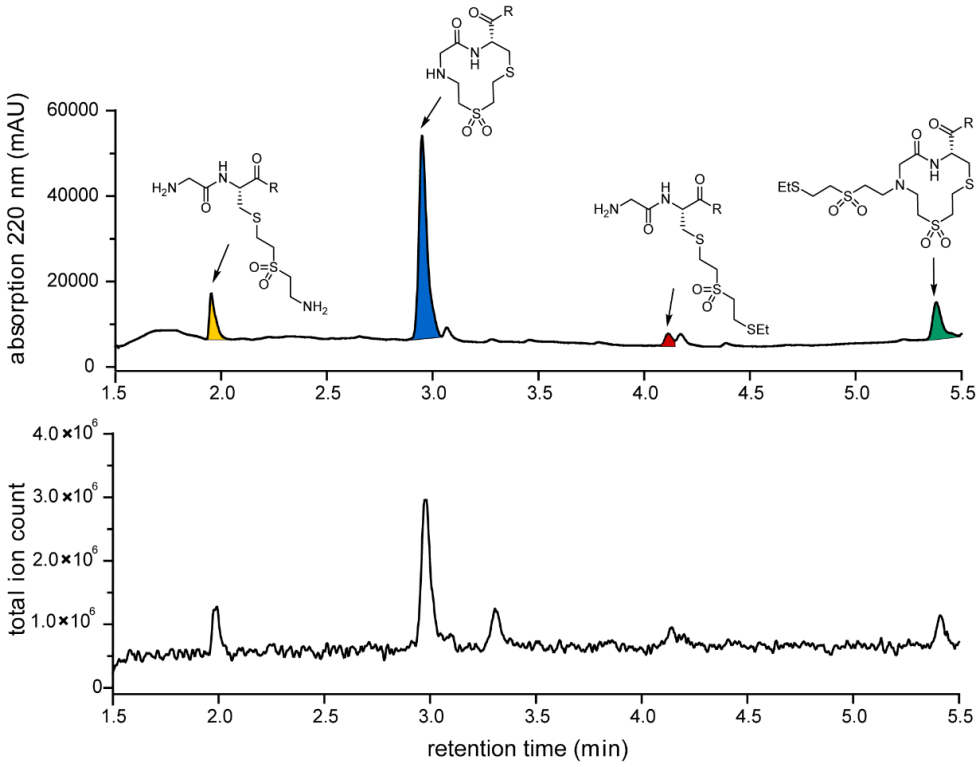


Divinyl sulfone (4)

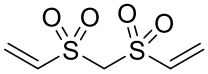


peptide	substrate	product	side products					
	not modified peptide	cyclic peptide	linear peptide-linker conjugate	linear peptide-linker conjugate with OH	linear peptide-linker conjugate with NH_2	linear peptide dimer	other	not identified
Gly-Cys-R	-	74	2	-	12	-	12[c]	-
Ala-Cys-R	-	85	2	-	11	2	-	-
D-Ala-Cys-R	-	54	20	-	26	-	-	-
Val-Cys-R	-	72	8	-	19	1	-	-
β-Ala-Cys-R	-	100	-	-	-	-	-	-
Pro-Cys-R	-	100	-	-	-	-	-	-
Phe-Cys-R	-	65	-	-	15	4	11[d], 5[c]	-
Thr-Cys-R	-	52	10	-	29	2	5[d], 2[c]	-
Asn-Cys-R	-	24	24	-	42	4	6[c]	-
Met-Cys-R	-	65	-	-	26	6	3[b]	-
Tyr-Cys-R	-	62	-	-	16	7	4[d], 3[c],	8
Glu-Cys-R	-	79	-	-	7	1	5[c]	7
Gly-HCys-R_1	-	26	17	-	-	-	16[c]	35, 7
Gly-Mnv- R_1	-	60	5				3[d]	16, 9,7
Gly-NMeCys-R_1	-	41	21	-	-	-	7[d], 5[c],	27
$(Gly)_2$-Cys-R	-	97	-	-	3	-	-	-
$(Gly)_3$-Cys-R	-	95	-	-	2	3	-	-
$(Gly)_4$-Cys-R	-	97	-	-	1	2	-	-
$(Gly)_5$-Cys-R	-	98	-	-	2	-	-	-

LC-MS analysis of Gly-Cys-R macrocyclization:

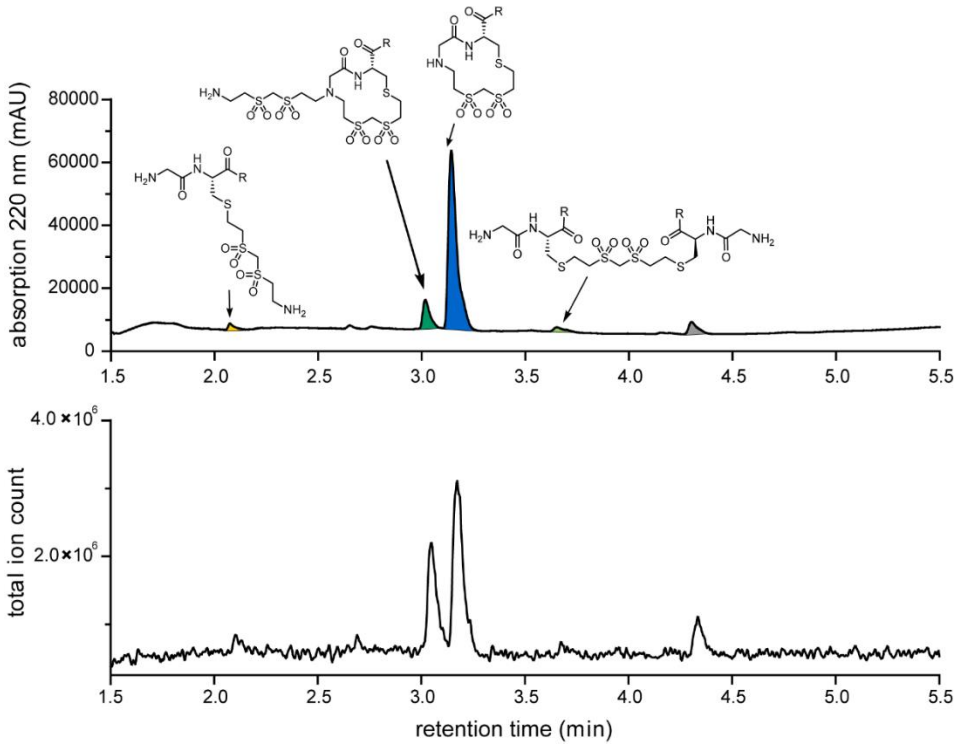


Bis(vinyl sulfonyl)methane (5)

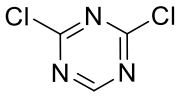


peptide	substrate	product	side products					
	not modified peptide	cyclic peptide	linear peptide-linker conjugate	linear peptide-linker conjugate with OH	linear peptide-linker conjugate with NH_2	linear peptide dimer	other	not identified
Gly-Cys-R	-	80	-	-	2	2	10[d]	6
Ala-Cys-R	-	73	-	-	2	3	17[d]	5
D-Ala-Cys-R	-	47	-	-	16	5	23[d]	9
Val-Cys-R	-	83	-	-	3	4	7[d]	3
β-Ala-Cys-R	-	86	-	-	-	-	4[d]	10
Pro-Cys-R	-	93	-	-	-	-	4[d]	3
Phe-Cys-R	-	82	-	-	3	7	4[d]	4
Thr-Cys-R	-	86	-	-	8	4	-	2
Asn-Cys-R	-	69	-	-	13	8	8[d]	2
Met-Cys-R	-	79	-	-	5	7	6[d]	3
Tyr-Cys-R	-	89	-	-	3	-	5[d]	3
Glu-Cys-R	-	95	-	-	-	-	-	5
Gly-HCys-R_1	-	83	-	-	-	6	2[d]	5, 4
Gly-Mnv- R_1	-	91	4	-	-	2	3[d]	-
Gly-NMeCys-R_1	-	80	-	-	-	3	-	10, 7
$(Gly)_2$-Cys-R	-	87	-	-	1	3	1[d]	8
$(Gly)_3$-Cys-R	-	93	-	-	-	-	-	7
$(Gly)_4$-Cys-R	-	93	-	-	-	-	-	7
$(Gly)_5$-Cys-R	-	95	-	-	-	-	-	5

LC-MS analysis of Gly-Cys-R macrocyclization:

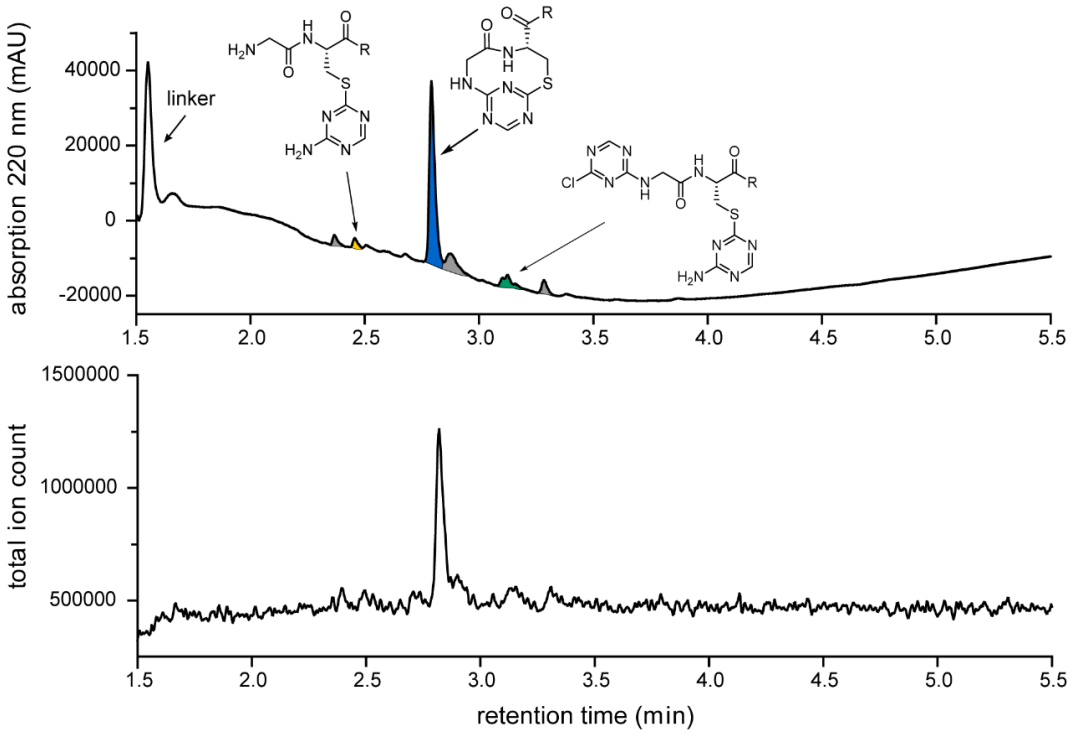


2,4-dichloro-1,3,5-triazine (6)

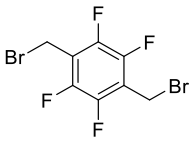


peptide	substrate	product	side products					
	not modified peptide	cyclic peptide	linear peptide-linker conjugate	linear peptide-linker conjugate with OH	linear peptide-linker conjugate with NH_2	linear peptide dimer	other	not identified
Gly-Cys-R	-	77	-	-	3	-	5[g]	15
Ala-Cys-R	-	86	-	-	-	-	-	14
D-Ala-Cys-R	-	88	-	-	-	-	-	12
Val-Cys-R	-	88	-	-	-	-	-	12
β-Ala-Cys-R	-	78	-	-	-	-	7[g]	15
Pro-Cys-R	-	38	-	-	-	-	43[g]	19
Phe-Cys-R	-	72	-	-	-	-	-	28
Thr-Cys-R	-	81	-	-	-	-	5[b]	15
Asn-Cys-R	-	77	-	1	5	-	-	16
Met-Cys-R	-	87	-	-	-	-	-	13
Tyr-Cys-R	-	86	-	-	-	-	-	14
Glu-Cys- R	-	84	-	-	-	-	-	16
Gly-HCys-R_1	-	93	-	-	-	-	-	4, 3
Gly-Mnv- R_1	-	76	-	-	-	-	-	14, 10
Gly-NMeCys-R_1	-	64	-	-	-	-	-	22, 6, 5, 3
$(Gly)_2$-Cys-R	-	86	-	-	-	-	-	14
$(Gly)_3$-Cys-R	-	87	-	-	-	-	-	13
$(Gly)_4$-Cys-R	-	86	-	-	-	-	-	14
$(Gly)_5$-Cys-R	-	88	-	-	-	-	-	12

LC-MS analysis of Gly-Cys-R cyclization:

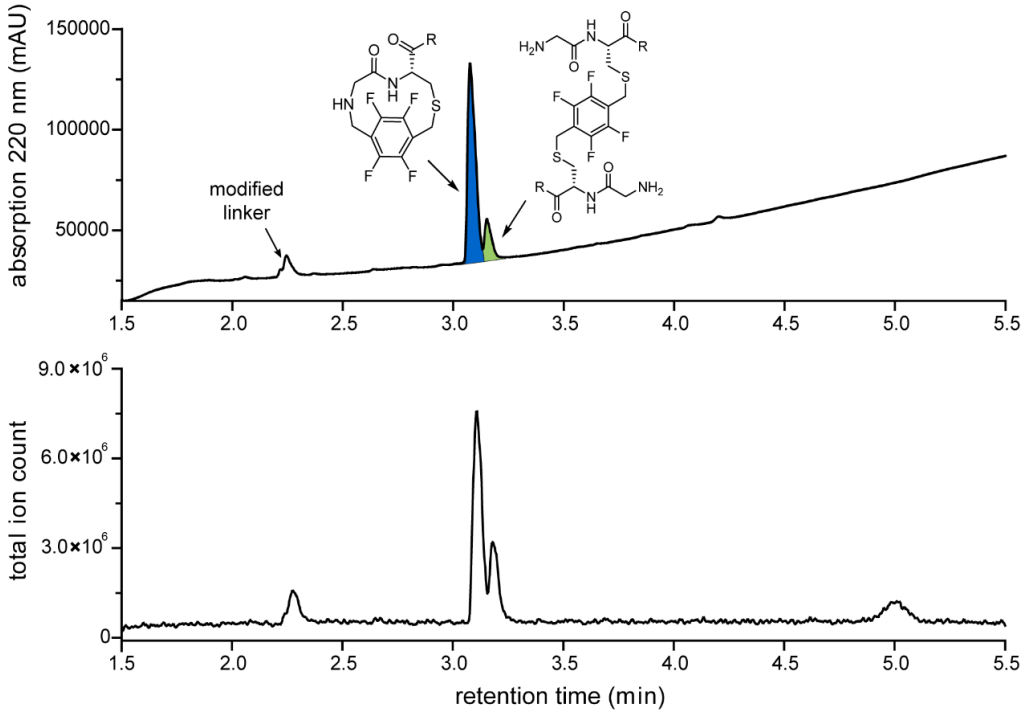


1,4-bis(bromomethyl)-2,3,5,6-tetrafluorobenzene (7)

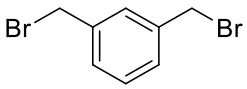


peptide	substrate	product	side products					
	not modified peptide	cyclic peptide	linear peptide-linker conjugate	linear peptide-linker conjugate with OH	linear peptide-linker conjugate with NH_2	linear peptide dimer	other	not identified
Gly-Cys-R	-	85	-	-	-	15	-	-
Ala-Cys-R	-	89	-	-	-	11	-	-
D-Ala-Cys-R	-	49	19	4	9	19	-	-
Val-Cys-R	-	91	-	-	1	8	-	-
β-Ala-Cys-R	-	71	2	2	12	13	-	-
Pro-Cys-R	-	95	-	-	-	5	-	-
Phe-Cys-R	-	89	-	-	2	9	-	-
Thr-Cys-R	-	88	-	-	-	12	-	-
Asn-Cys-R	-	85	-	-	2	13	-	-
Met-Cys-R	-	39	-	-	-	12	49[e]	-
Tyr-Cys-R	-	81	-	-	-	6	-	13
Glu-Cys-R	-	92	-	-	-	8	-	-
Gly-HCys-R_1	-	97	-	-	-	3	-	-
Gly-Mnv- R_1	-	75	-	-	-	16	-	9
Gly-NMeCys-R_1	-	50	-	-	-	22	-	28
$(Gly)_2$-Cys-R	-	59	13	3	9	16	-	-
$(Gly)_3$-Cys-R	-	62	5	3	16	14	-	-
$(Gly)_4$-Cys-R	-	78	-	2	7	13	-	-
$(Gly)_5$-Cys-R	-	78	1	-	9	12	-	-

LC-MS analysis of Gly-Cys-R macrocyclization:

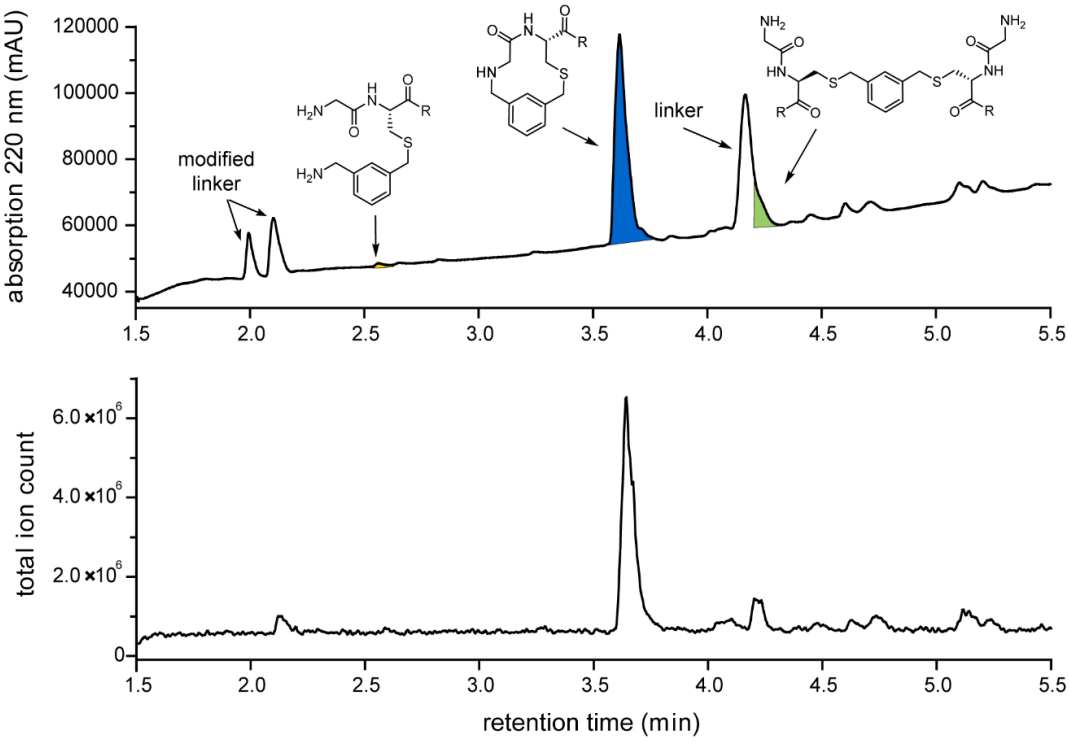


1,3-bis(bromomethyl)benzene (8)

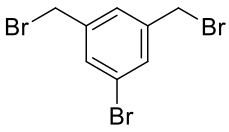


peptide	substrate	product	side products					
	not modified peptide	cyclic peptide	linear peptide-linker conjugate	linear peptide-linker conjugate with OH	linear peptide-linker conjugate with NH_2	linear peptide dimer	other	not identified
Gly-Cys-R	-	86	-	-	1	13	-	-
Ala-Cys-R	-	89	-	-	-	11	-	-
D-Ala-Cys-R	-	69	-	9	3	11	-	8
Val-Cys-R	-	98	-	-	-	2	-	-
β-Ala-Cys-R	-	69	-	7	2	16	-	6
Pro-Cys-R	-	97	-	1	-	2	-	-
Phe-Cys-R	-	97	-	-	-	3	-	-
Thr-Cys-R	-	96	-	-	-	4	-	-
Asn-Cys-R	-	88	-	-	-	12	-	-
Met-Cys-R	-	9	-	-	-	-	91[e]	-
Tyr-Cys-R	-	96	-	-	-	4	-	-
Glu-Cys-R	-	96	-	-	-	4	-	-
Gly-HCys-R_1	-	58	37	-	-	-	5[b]	-
Gly-Mnv- R_1	-	45	-	-	-	-	55[c]	-
Gly-NMeCys-R_1	4	66	-	-	-	8	-	20, 2
$(Gly)_2$-Cys-R	-	43	-	15	5	17	-	20
$(Gly)_3$-Cys-R	-	53	-	6	3	21	2[a]	15
$(Gly)_4$-Cys-R	-	55	-	6	3	17	2[a]	17
$(Gly)_5$-Cys-R	-	79	-	8	4	5	-	4

LC-MS analysis of Gly-Cys-R macrocyclization:

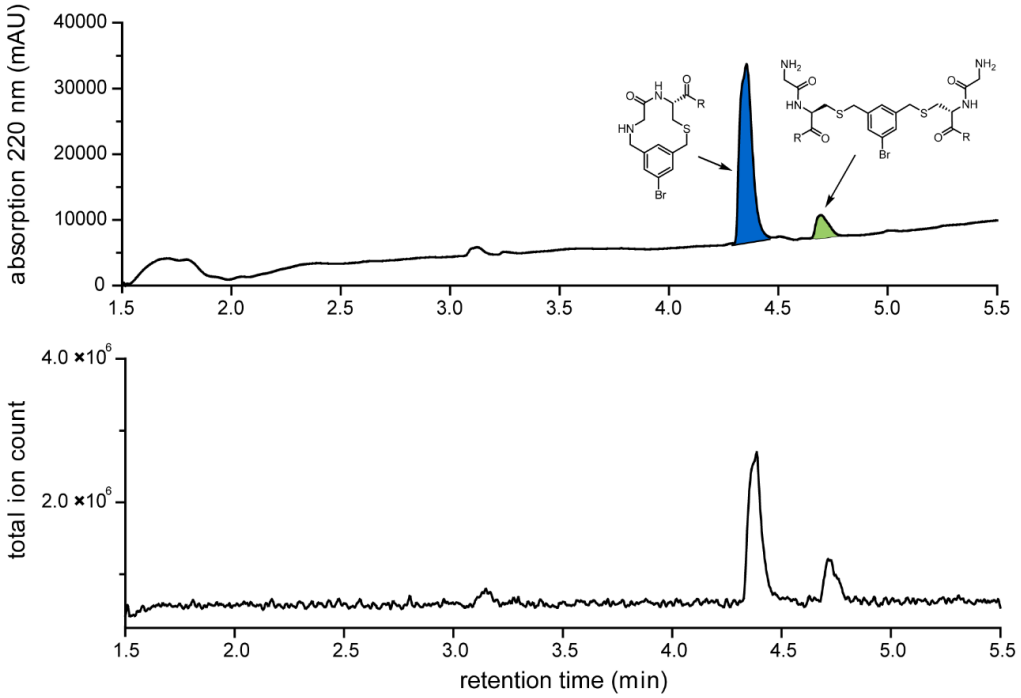


1-bromo-3,5-bis(bromomethyl)benzene (9)

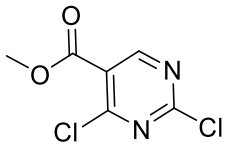


peptide	substrate	product	side products					
	not modified peptide	cyclic peptide	linear peptide-linker conjugate	linear peptide-linker conjugate with OH	linear peptide-linker conjugate with NH_2	linear peptide dimer	other	not identified
Gly-Cys-R	-	89	-	-	-	11	-	-
Ala-Cys-R	-	83	-	-	-	12	6[b]	-
D-Ala-Cys-R	-	73	-	-	-	24	3[a]	-
Val-Cys-R	-	90	-	-	-	10	-	-
β-Ala-Cys-R	-	76	-	-	-	24	-	-
Pro-Cys-R	-	100	-	-	-	-	-	-
Phe-Cys-R	-	90	-	-	-	10	-	-
Thr-Cys-R	-	90	-	-	-	10	-	-
Asn-Cys-R	-	88	-	-	-	12	-	-
Met-Cys-R	-	22	-	-	-	5	73[e]	-
Tyr-Cys-R	-	91	-	-	-	9	-	-
Glu-Cys-R	-	95	-	-	-	5	-	-
Gly-HCys-R_1	9	79	-	-	-	4	-	5, 3
Gly-Mnv- R_1	21	56	-	10	-	-	-	5, 5, 3
Gly-NMeCys-R_1	-	69	-	-	-	-	-	23, 5, 3
$(Gly)_2$-Cys-R	-	58	-	-	-	28	-	14
$(Gly)_3$-Cys-R	-	76	-	-	-	24	-	-
$(Gly)_4$-Cys-R	-	76	-	-	-	24	-	-
$(Gly)_5$-Cys-R	-	77	-	-	5	18	-	-

LC-MS analysis of Gly-Cys-R macrocyclization:

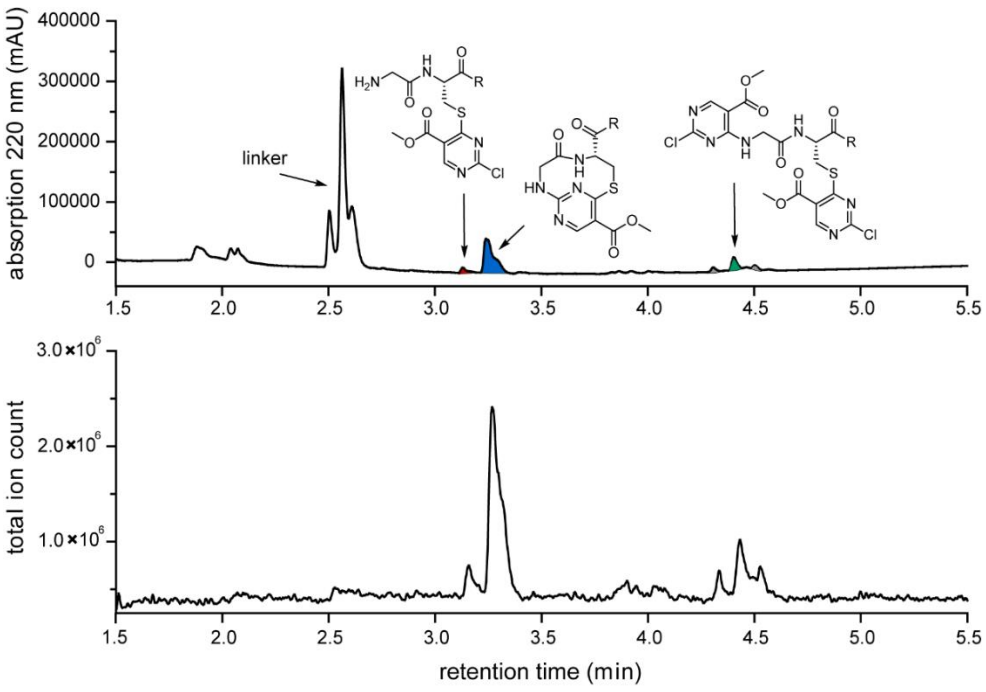


Methyl 2,4-dichloropyrimidine-5-carboxylate (10)

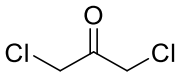


peptide	substrate	product	side products					
	not modified peptide	cyclic peptide	linear peptide-linker conjugate	linear peptide-linker conjugate with OH	linear peptide-linker conjugate with NH_2	linear peptide dimer	other	not identified
Gly-Cys-R	-	64	5	-	-	-	30[f]	-
Ala-Cys-R	-	49, 40	-	-	-	-	9[f], 2[f]	-
D-Ala-Cys-R	-	63, 20	5	-	-	-	12[f]	-
Val-Cys-R	-	51, 33	-	-	-	-	16[f]	-
β-Ala-Cys-R	-	82	-	-	-	-	18[f]	-
Pro-Cys-R	-	8, 5	-	-	-	-	70[f], 11[g]	6
Phe-Cys-R	-	29, 19	6	-	-	-	5[b], 4[f]	36
Thr-Cys-R	-	60, 21	8	-	-	-	11[f]	-
Asn-Cys-R	-	75	16	-	-	-	9[f]	-
Met-Cys-R	-	49, 30	11	-	-	-	10[f]	-
Tyr-Cys-R	-	44, 35	-	-	-	-	-	22
Glu-Cys-R	-	62, 27	-	-	-	-	11[f]	-
Gly-HCys-R_1	-	43, 41	-	-	-	-	13[f]	3
Gly-Mnv- R_1	-	37, 13	-	-	-	-	9[f]	41
Gly-NMeCys-R_1	-	90	3	-	-	-	-	4, 2
$(Gly)_2$-Cys-R	-	48, 37	-	-	-	-	15[f]	-
$(Gly)_3$-Cys-R	-	54	13	-	-	-	32[f]	-
$(Gly)_4$-Cys-R	-	76	-	-	-	-	24[f]	-
$(Gly)_5$-Cys-R	-	56, 26	-	-	-	-	18[f]	-

LC-MS analysis of Gly-Cys-R macrocyclization:

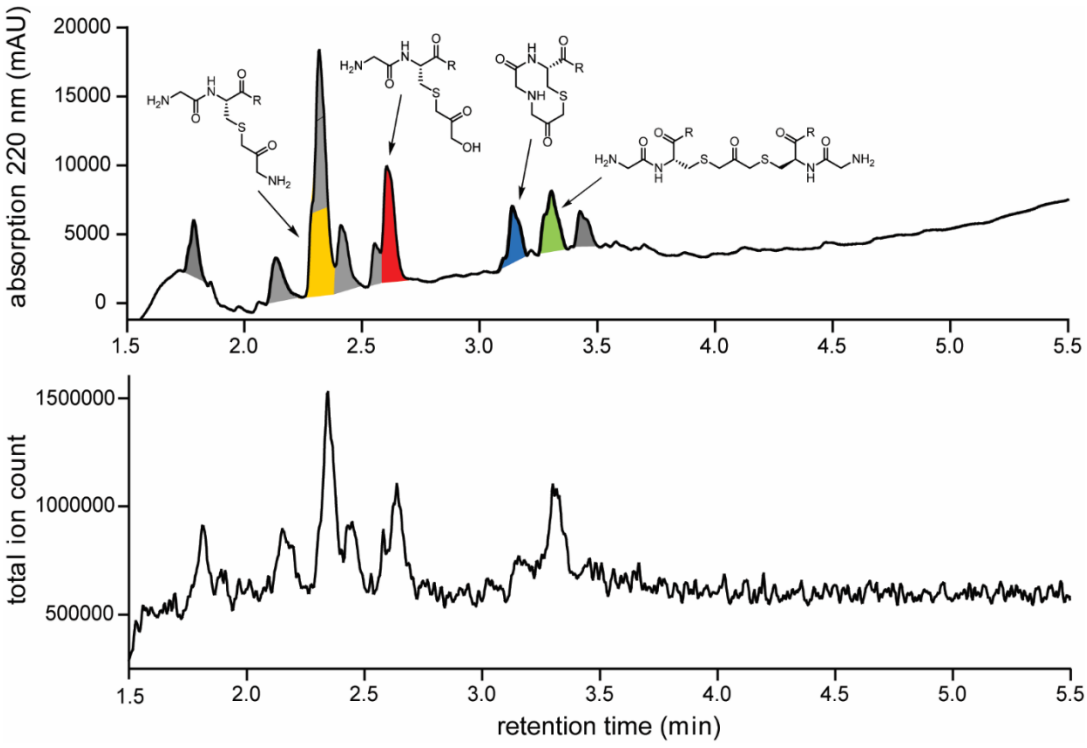


Dichloroacetone (11)

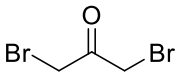


peptide	substrate	product	side products					
	not modified peptide	cyclic peptide	linear peptide-linker conjugate	linear peptide-linker conjugate with OH	linear peptide-linker conjugate with NH_2	linear peptide dimer	other	not identified
Gly-Cys-R	-	9	-	13	16	11	-	13, 9, 8, 6, 7, 6, 2
Ala-Cys-R	-	19	-	13	26	8	-	12, 10, 7, 5
D-Ala-Cys-R	-	19	-	22	39	12	-	8
Val-Cys-R	-	8	-	20	40	7	-	7, 6, 6, 6
β-Ala-Cys-R	-	21	-	13	22	8	-	21, 8, 6, 1
Pro-Cys-R	-	10	-	21	47	3	-	10, 7, 2
Phe-Cys-R	-	18	-	12	20	15	-	12, 8, 6, 4, 3, 2
Thr-Cys-R	-	17	-	9	26	11	-	13, 10, 9, 5
Asn-Cys-R	-	23	-	23	19	16	-	8, 6, 5
Met-Cys-R	-	-	-	4	-	7	89[e]	-
Tyr-Cys-R	-	69	-	-	-	16	-	15
Glu-Cys-R	-	18	-	7	19	12	-	12, 9, 8, 4, 4, 3, 3
Gly-HCys-R_1	-	47	-	10	-	16	-	14, 4, 4, 3, 3
Gly-Mnv-R_1	-	30	-	-	-	5	-	20, 14, 12,10, 3,3,3
Gly-NMeCys-R_1	-	6	-	19	-	6	-	52, 10, 3, 2, 1
$(Gly)_2$-Cys-R	-	33	-	12	-	3	-	27, 22, 3
$(Gly)_3$-Cys-R	-	29	-	8	15	12	-	19, 15, 2
$(Gly)_4$-Cys-R	-	30	-	6	11	4	-	24,23, 2
$(Gly)_5$-Cys-R	-	25	-	6	17	6	-	22, 22, 2

LC-MS analysis of Gly-Cys-R_1 macrocyclization:

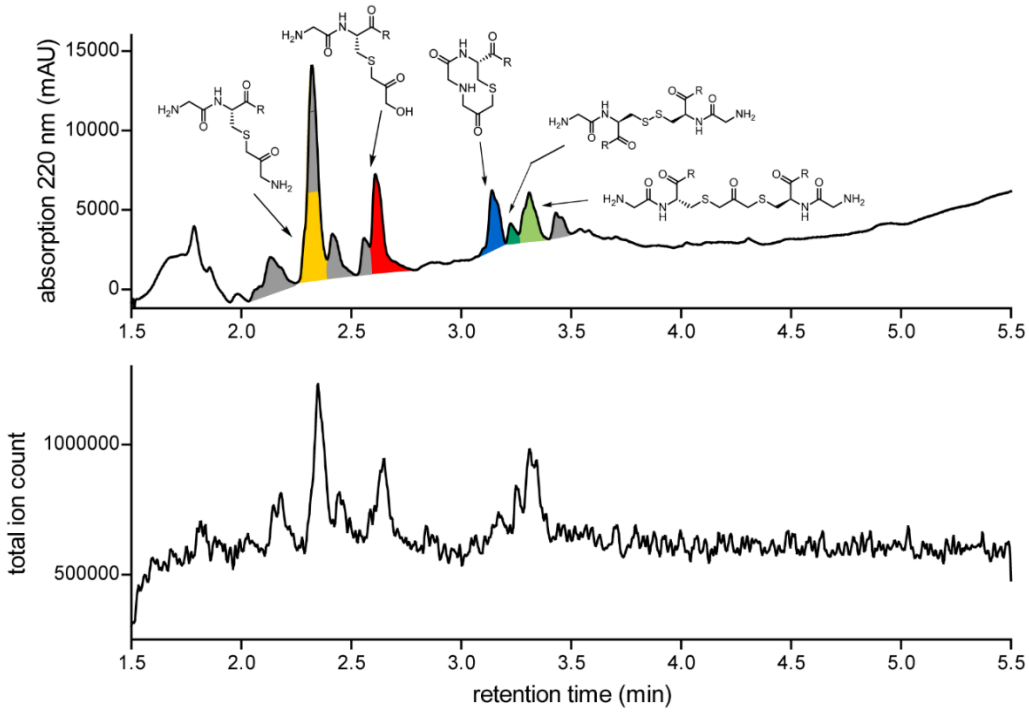


Dibromoacetone (12)

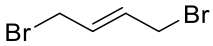


peptide	substrate	product	side products					
	not modified peptide	cyclic peptide	linear peptide-linker conjugate	linear peptide-linker conjugate with OH	linear peptide-linker conjugate with NH_2	linear peptide dimer	other	not identified
Gly-Cys-R	-	10	-	22	13	11	3[b]	15, 8, 7, 6, 5
Ala-Cys-R	-	7	-	21	15	27	10[b]	15, 4
D-Ala-Cys-R	-	5	-	24	24	12	21[b]	15
Val-Cys-R	-	5	-	22	20	10	7[b]	20, 6, 5, 4
β-Ala-Cys-R	-	42	-	10	15	-	12[b]	18, 3
Pro-Cys-R	-	5	-	26	26	-	24[b]	11, 4, 3
Phe-Cys-R	-	16	-	13	13	17	17[b]	10, 6, 6, 2
Thr-Cys-R	-	3	-	12	12	21	21[b]	21, 9
Asn-Cys-R	-	26	-	23	15	5	5[b]	15, 11
Met-Cys-R	-	-	-	8	8	7	7[b], 46[e]	12, 12
Tyr-Cys-R	-	36	-	4	4	-	-	49, 6
Glu-Cys-R	-	21	-	14	14	17	17[b]	7, 4, 3, 3
Gly-HCys-R_1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Gly-Mnv-R_1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Gly-NMeCys-R_1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
$(Gly)_2$-Cys-R	-	38	-	37	-	3	8[b]	14
$(Gly)_3$-Cys-R	-	16	-	16	-	7	7[b]	46, 6
$(Gly)_4$-Cys-R	-	43	-	7	-	-	-	27, 23
$(Gly)_5$-Cys-R	-	38	-	20	-	-	-	16, 15, 9, 2

LC-MS analysis of Gly-Cys-R macrocyclization:

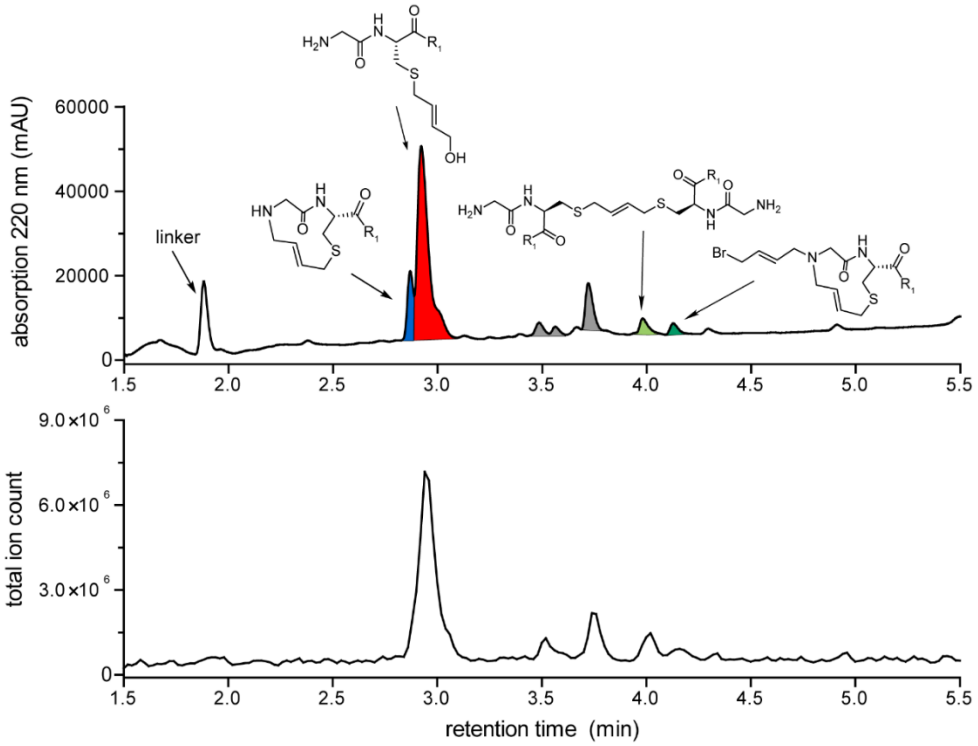


trans-1,4-dibromo-2-butene (13)

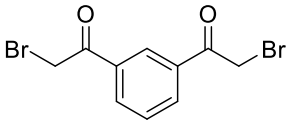


peptide	substrate	product	side products					
	not modified peptide	cyclic peptide	linear peptide-linker conjugate	linear peptide-linker conjugate with OH	linear peptide-linker conjugate with NH_2	linear peptide dimer	other	not identified
Gly-Cys-R_1	-	14	-	63	-	4	3[c]	12, 4
Ala-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
D-Ala-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Val-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
β-Ala-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Pro-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Phe-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Thr-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Asn-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Met-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Tyr-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Glu-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Gly-HCys-R_1	-	82	-	11	-	-	-	7
Gly-Mnv-R_1	-	8	-	88	-	-	-	4
Gly-NMeCys-R_1	-	17	-	79	-	4	-	-
$(Gly)_2$-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
$(Gly)_3$-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
$(Gly)_4$-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
$(Gly)_5$-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

LC-MS analysis of Gly-Cys-R_1 macrocyclization:

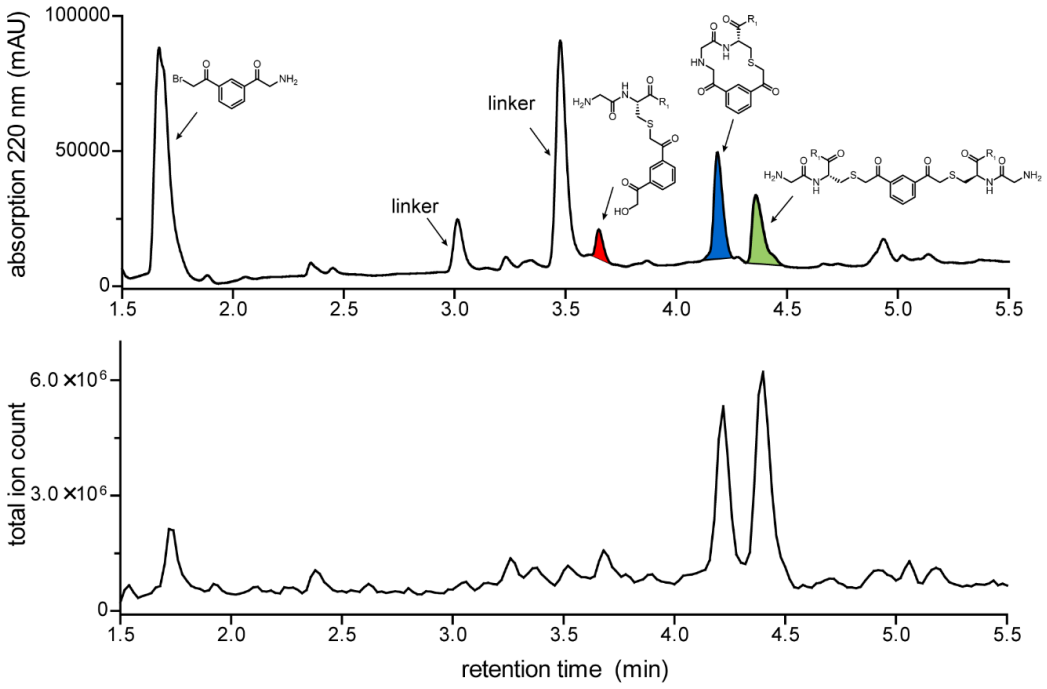


1,3-bis(bromoacetyl)benzene (14)

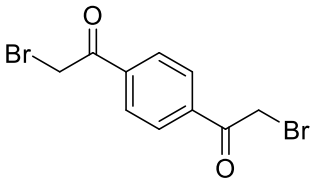


peptide	substrate	product	side products					
	not modified peptide	cyclic peptide	linear peptide-linker conjugate	linear peptide-linker conjugate with OH	linear peptide-linker conjugate with NH_2	linear peptide dimer	other	not identified
Gly-Cys-R_1	-	51	-	9	-	40	-	-
Ala-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
D-Ala-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Val-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
β-Ala-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Pro-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Phe-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Thr-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Asn-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Met-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Tyr-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Glu-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Gly-HCys-R_1	-	5	-	17	-	67	-	11
Gly-Mnv-R_1	-	70	-	8	-	22	-	-
Gly-NMeCys-R_1	-	83	-	-	-	17	-	-
$(Gly)_2$-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
$(Gly)_3$-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
$(Gly)_4$-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
$(Gly)_5$-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

LC-MS analysis of Gly-Cys-R_1 macrocyclization:

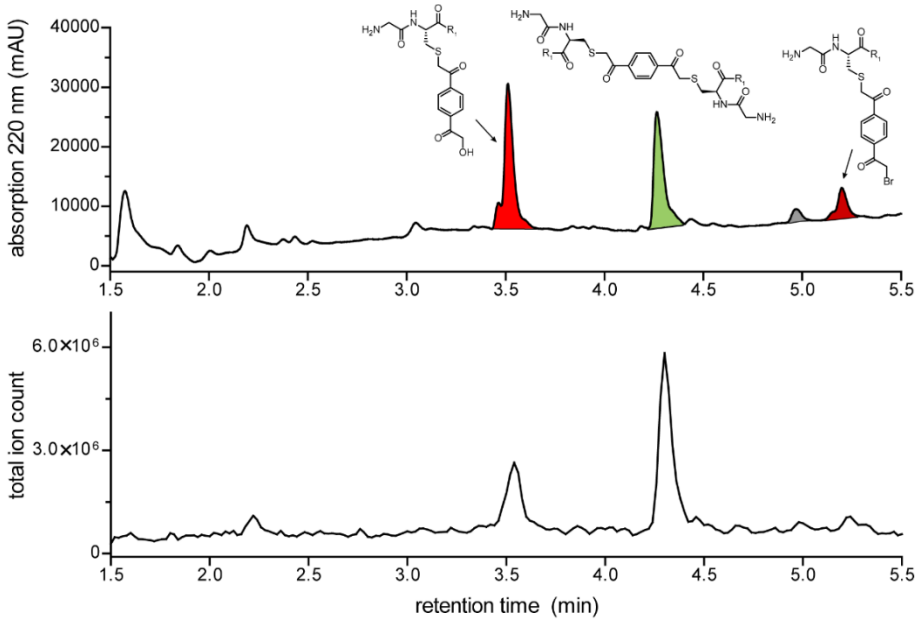


1,4-bis(bromoacetyl)benzene (15)

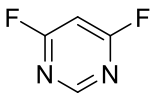


peptide	substrate	product	side products					
	not modified peptide	cyclic peptide	linear peptide-linker conjugate	linear peptide-linker conjugate with OH	linear peptide-linker conjugate with NH_2	linear peptide dimer	other	not identified
Gly-Cys-R_1	-	-	10	48	-	38	-	4
Ala-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
D-Ala-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Val-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
β-Ala-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Pro-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Phe-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Thr-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Asn-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Met-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Tyr-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Glu-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Gly-HCys-R_1	-	-	-	41	-	48	-	11
Gly-Mnv-R_1	-	26	-	8	-	66	-	-
Gly-NMeCys-R_1	10	-	25	18	-	21	5[c], 3[b]	12, 6
$(Gly)_2$-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
$(Gly)_3$-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
$(Gly)_4$-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
$(Gly)_5$-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

LC-MS analysis of Gly-Cys-R_1 macrocyclization:

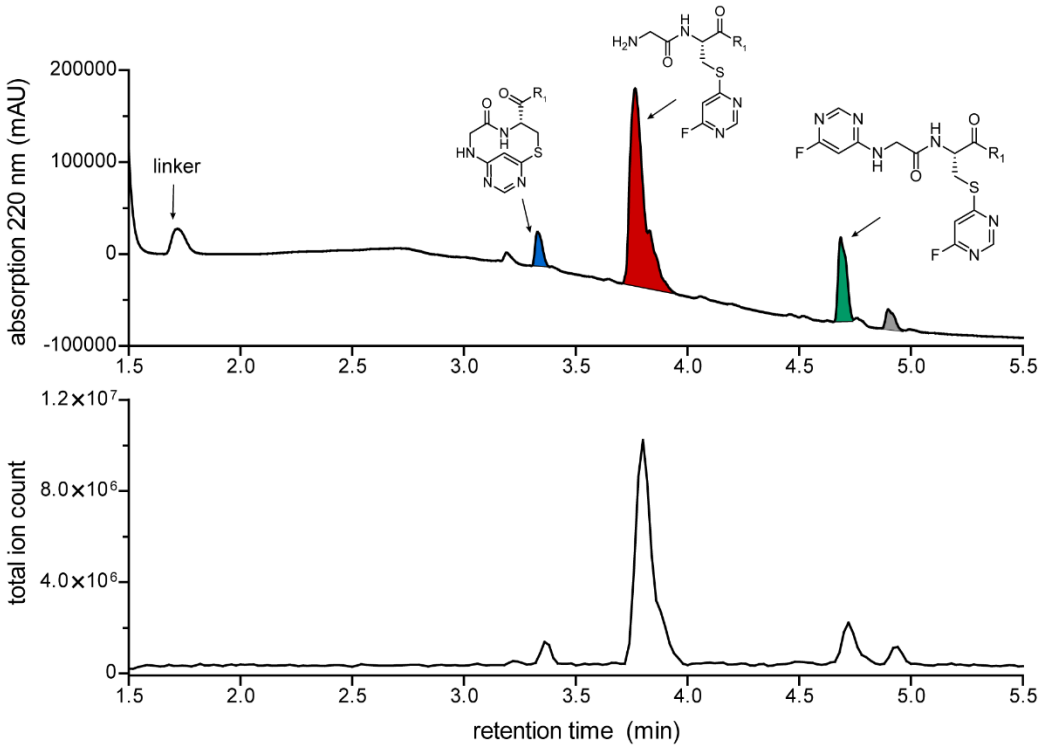


4,6-difluropyrimidine (16)



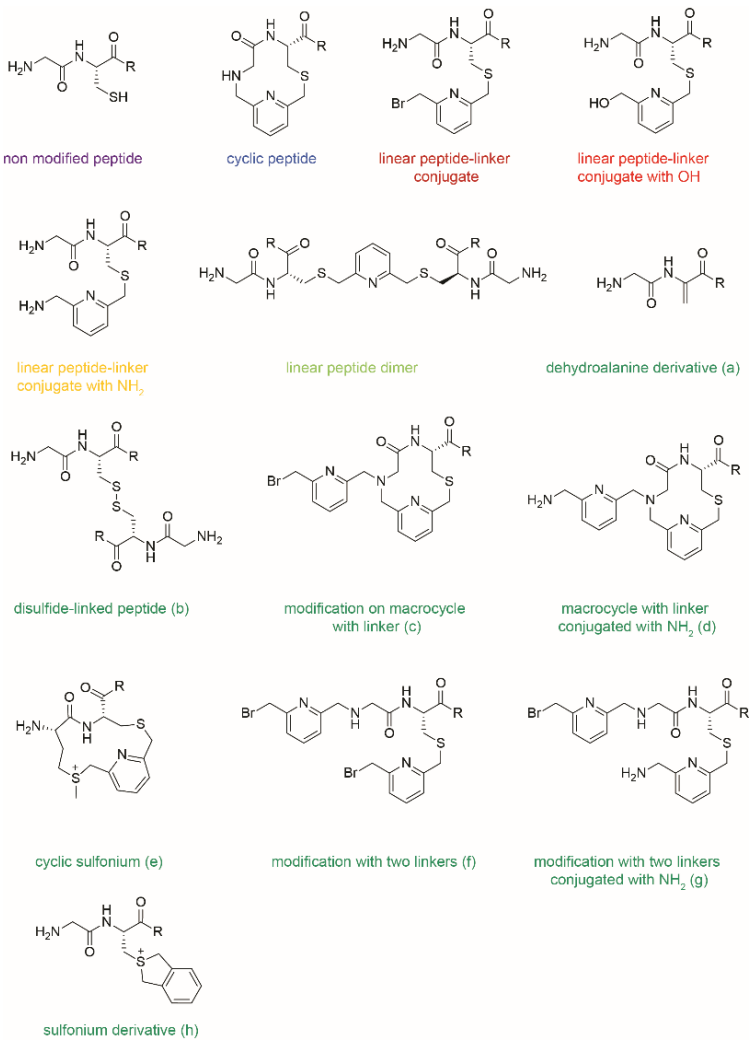
peptide	substrate	product	side products					
	not modified peptide	cyclic peptide	linear peptide-linker conjugate	linear peptide-linker conjugate with OH	linear peptide-linker conjugate with NH_2	linear peptide dimer	other	not identified
Gly-Cys-R_1	-	6	70	-	-	-	19[c]	4
Ala-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
D-Ala-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Val-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
β-Ala-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Pro-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Phe-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Thr-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Asn-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Met-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Tyr-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Glu-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Gly-HCys-R_1	5	25	36	-	-	-	20[c]	10, 4
Gly-Mnv-R_1	9	6	50	-	-	-	17[c]	7,4,4,2
Gly-NMeCys-R_1	36	-	17	-	-	-	-	28, 11, 5, 2
$(Gly)_2$-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
$(Gly)_3$-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
$(Gly)_4$-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
$(Gly)_5$-Cys-R	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

LC-MS analysis of Gly-Cys-R_1 macrocyclization:



Color code of side products:

The peaks of frequently found side products are shown in different colors as illustrated with the example of the peptide Gly-Cys-R and the reagent **1** as follows and in fig. S2C.



The peaks of less often observed side products are shown in green. They are

a) dehydroalanine derivative
b) disulfide-linked peptide
c) modification with two linkers
d) modification with two linkers of which one has reacted with NH_3
e) cyclic sulfonium

The peaks of side products that were not identified but are suspected to contain a peptide moiety (based on the mass) are colored in grey.