

Use of an Internal Reference for the Quantitative HPLC-UV Analysis of Solid-Phase Reactions: A Case Study of 2-Chlorotrityl Chloride Resin

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SI-1. Abbreviations:

ACN	acetonitrile
Aib	2-aminoisobutyric acid
BPA	4-biphenylacetic acid
CTC	2-chlorotrityl chloride
DCM	dichloromethane
DIEA	<i>N,N</i> -diisopropylethylamine
DIPCDI	<i>N,N</i> -diisopropylcarbodiimide
DKP	diketopiperazine
DLeu	<i>D</i> -leucine
DMF	<i>N,N</i> -dimethylformamide
Fmoc	9-fluorenylmethyloxycarbonyl

HATU	(O-(7-azabenzotriazol-1-yl)- <i>N,N,N,N</i> -tetramethyluronium hexafluorophosphate)
HBTU	(O-(benzotriazol-1-yl)- <i>N,N,N,N</i> -tetramethyluronium hexafluorophosphate)
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	1-hydroxybenzotriazole
Oxyma	ethyl 2-cyano-2-(hydroxylimino)acetate
PAA	1-pyreneacetic acid
Pbf	2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl
PDA	photodiode array
PyBOP	(benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
Sar	sarcosine (<i>N</i> -methyl glycine)
SPPS	solid phase peptide synthesis
TFA	trifluoroacetic acid
Xaa	<i>L</i> -amino acid (three letter code)
Z	Carboxybenzyl

SI-2. General Information

Apparatus

Analytical HPLC was carried out on a Waters (Ireland) instrument comprising a Sunfire™ C18 reversed-phase analytical column, 3.5 µm, 6 x 100 mm, a separation module (Waters 2695), automatic injector, and photodiode array detector (Waters 2298). Data were managed with Empower 2 software. Linear gradients of ACN (+0.036% TFA) into H₂O (+0.045% TFA) were run at a flow rate of 1.0 mL/min over 8 min. UV detection was performed at 220 nm.

HPLC-MS analyses of peptide samples were carried out on a Waters (Ireland) instrument comprising a Sunfire™ C18 reversed-phase analytical column, 3.5 µm, 6 x 100 mm, a separation module (Waters 2695), automatic injector, photodiode array detector (Waters 2298), and a Waters micromass ZQ unit. Data were managed with MassLynx V4.1 software (Waters). UV detection was performed at 220 nm, and linear gradients of ACN (+0.07% formic acid) into H₂O (+0.1% formic acid) were run at a flow rate of 1.0 mL/min over 8 min.

Compounds

Commercially available chemicals and solvents were used as received. CTC resin with a maximum loading capacity of 1.6 mmol/g was used.

SI-3. Solid-phase chemistry

Solid-phase reactions were performed in polystyrene syringes equipped with a porous polystyrene filter plate. Resins were stirred occasionally. Solvents were removed by vacuum-suction.

Fmoc-groups were cleaved, unless not otherwise specified, by a 10-min treatment of the resin (previously swollen in DCM) with 20% piperidine in DMF, then the resin was washed with DMF and

DCM. Resin functionalization was determined by quantification of the UV-absorbance of dibenzofulvene-piperidide at 290 nm.

Coupling reactions were performed as specified. The completeness of the reaction was eventually checked with the ninhydrine test (Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595). All unreacted material was washed from the resin with DMF followed by DCM (3 x 2 min).

For a test cleavage, a small sample of resin swollen in DCM (approx. 3 - 5 mm in the capillary end of a glass Pasteur pipette) was placed on a glass wool-filter that fits the capillary end of a 150 mm glass Pasteur pipette. With a bulb, a solution of 2% TFA in DCM (approx. 0.5 mL) was sucked in the pipette so that the entire resin sample was dispersed in the solution. The resin showed deep red staining. After 10 min (we observed no variations in the product compositions when longer cleavage times were applied), the cleavage solution was pressed into a 2 mL Eppendorf tube and evaporated with a stream of nitrogen at r.t.

For cleavage with TFA / H₂O (95 : 5) the resulting dry residue was then dissolved in TFA / H₂O (95 : 5) (approx. 0.5 mL) and left for 90 min. Then, it was evaporated with a nitrogen stream at r.t.

The dried residue was re-dissolved in MeOH / H₂O (9 : 1) (approx. 1 mL). The resulting solution was directly used for HPLC-analysis.

Resin 1: CTC resin (0.20 g) was swollen DCM/DMF 3:1 for 30 min. The solvents were removed and a mixture of Fmoc-Phe (39 mg, 0.1 mmol) and PAA (3 mg, 0.01 mmol) dissolved in DCM (1 mL) and DIEA (172 µL, 1 mmol) was added and left for 1 h to react with the resin. MeOH was then added. After 10 min, the resin was washed (DMF, then DCM). The functionalization was found to be 0.37 mmol/g.

Resins 3 and 5: The amino acids Fmoc-Arg(Pbf) (156 mg, 0.24 mmol) and Fmoc-Val (81 mg, 0.24 mmol) were dissolved together with Oxyma (34 mg, 0.24 mmol) in DMF / DCM, and DIPCDI (37 µL, 0.24 mmol) was added. The solution was added to the resins **2** or **4**, respectively, and ninhydrin test was found to be negative after 1 h.

Resin 7: CTC resin (1.0 g) was swollen DCM / DMF (3 : 1) for 30 min. The solvents were removed and a mixture of Fmoc-Pro (280 mg, 0.8 mmol) and BPA (60 mg, 0.3 mmol) dissolved in DCM (1 mL) and DIEA (861 µL, 5 mmol) was added and left for 1 h to react with the resin. MeOH was then added. After 10 min, the resin was washed (DMF, then DCM) and dried.

Resines 9a-c: The Fmoc-group of resin **7** (140 mg, 0.06 mmol) was removed. To Fmoc-amino acid (0.18 mmol) and HBTU (68 mg, 0.18 mmol) dissolved in DMF / DCM (3 : 1) (400 µL), DIEA (62 mL, 0.36 mmol) was added and the solution was added to the resin to give **9**.

Resines 11a-c: The Fmoc-group of resin **9** (30 mg, 0.013 mmol) was removed (5 min treatment with 20% piperidine in DMF). To Fmoc-Aib (21 mg, 0.065 mmol) and HATU (24 mg, 0.065 mmol) dissolved

in DMF (150 μ L), DIEA (22 mL 0.13 mmol) was added and the solution was added to the resin to give **11**.

The products Fmoc-Aib-Xaa-Pro were characterized by HPLC-MS in ES⁺. Fmoc-Aib-Gly-Pro MW: 479.5, found: 480; Fmoc-Aib-DLeu-Pro MW: 535.6, found: 536; Fmoc-Aib-Sar-Pro MW: 493.5, found: 494.

Resin 12: CTC resin (0.5 g) was swollen DCM/DMF 3:1 for 30 min. The solvents were removed and a mixture of Fmoc-Gly (30 mg, 0.1 mmol), Fmoc-Pro (34 mg, 0.1 mmol), Fmoc-Leu ((35 mg, 0.1 mmol), BPA (21 mg, 0.1 mmol) and PAA (26 mg, 0.1 mmol) dissolved in DCM / DMF (2 : 1) (1.5 mL) and DIEA (430 μ L, 2.5 mmol) was added and left for 1 h to react with the resin. MeOH was then added. After 10 min, the resin was washed (DMF, then DCM). The resin was washed and dried.

Resin 13. After removal of the Fmoc-group from aliquots of resin **12** (10 mg), couplings with Fmoc-Gly (18 mg, 0.06 mmol) were performed by activation with HBTU (23 mg, 0.06 mmol) and DIEA (21 μ L, 0.12 mmol); Oxyma (8.5 mg, 0.06 mmol) and DIPCDI (9.3 μ L, 0.06 mmol); HOBt*H₂O (9.3 mg, 0.06 mmol) and DIPCDI (9.3 μ L, 0.06 mmol); or HOAt (8.2 mg, 0.06 mmol) and DIPCDI (9.3 μ L, 0.06 mmol). The solid compounds (Fmoc-Gly, HBTU, Oxyma, HOBt and HOAt) were dissolved in DMF (200 μ L) and the liquid compounds DIEA or DIPCDI were added before the mixture was given to the resin. After cleavage, the residue was dissolved in 0.5 mL of a solution of Z-Gly [80 mg in 20 mL ACN / H₂O (3 : 1)], and diluted to approx. 1 mL with ACN before submitted to HPLC-UV analysis.

SI-4. HPLC-UV analysis

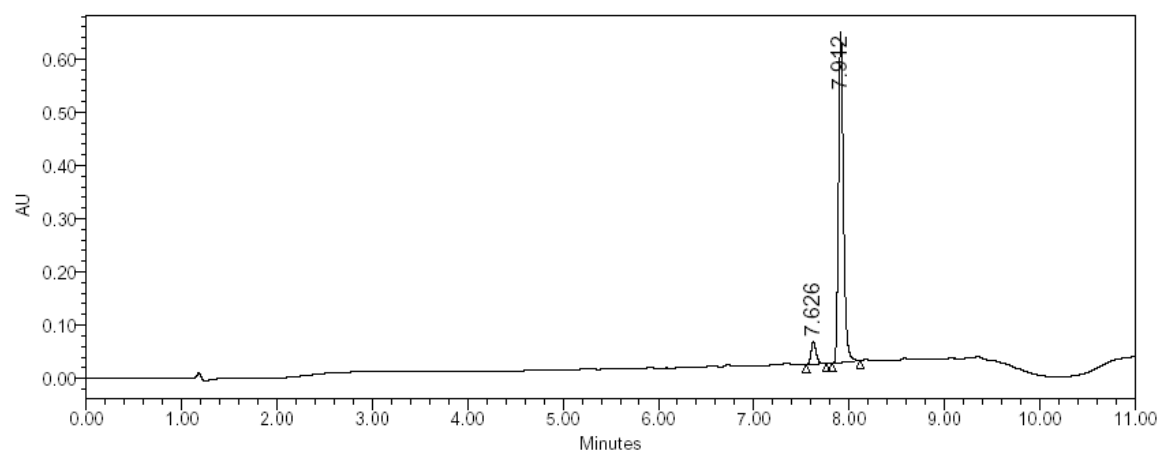
The test sample (1-5 μ L) was injected into the HPLC apparatus. The UV absorbance was observed at 220 nm over an 8 min gradient of 5% - 100% ACN (unless no otherwise indicated), followed by 3 min 100% ACN. A suitable chromatogram has peaks not higher than 1 absorption unit (AU), otherwise the sample was repeated with a smaller volumen. The integration areas of the reference compounds were typically smaller than those of the products (up to 50 times). Note that UV lamps with long running times produce a noisy baseline that may dramatically decrease the exactness of measurement.

SI-5. HPLC-UV Chromatograms (Tables 1, 2, 3 and 4)

SI Chromatograms Table SI-1. Solid-phase synthesis of Val-Arg-Phe on resin 1

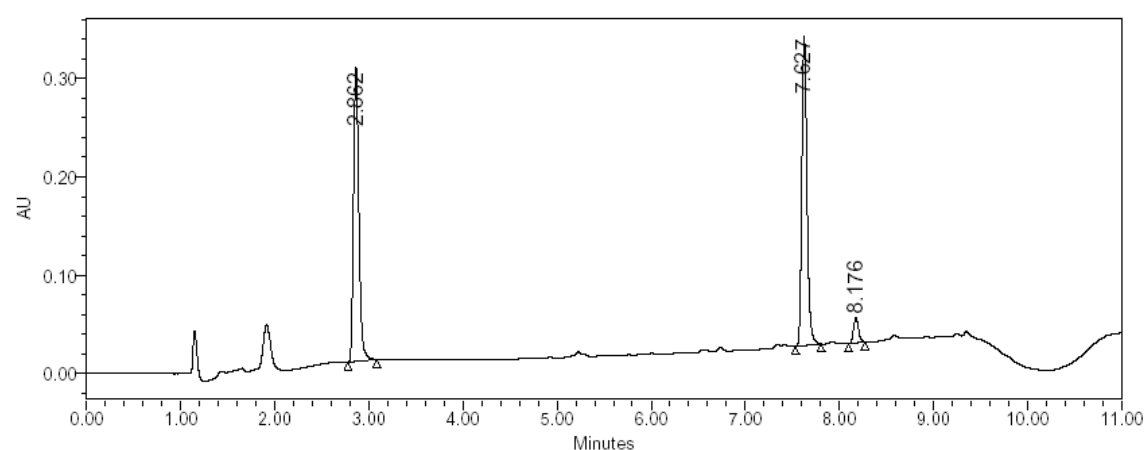
The tripeptide Val-Arg-Phe was synthesized as described above. The entries of Table SI-1 correspond to those in Table 1 in the article.

Table 1, entry 1: Fmoc-Phe: 7.9 min, PAA: 7.6 min.



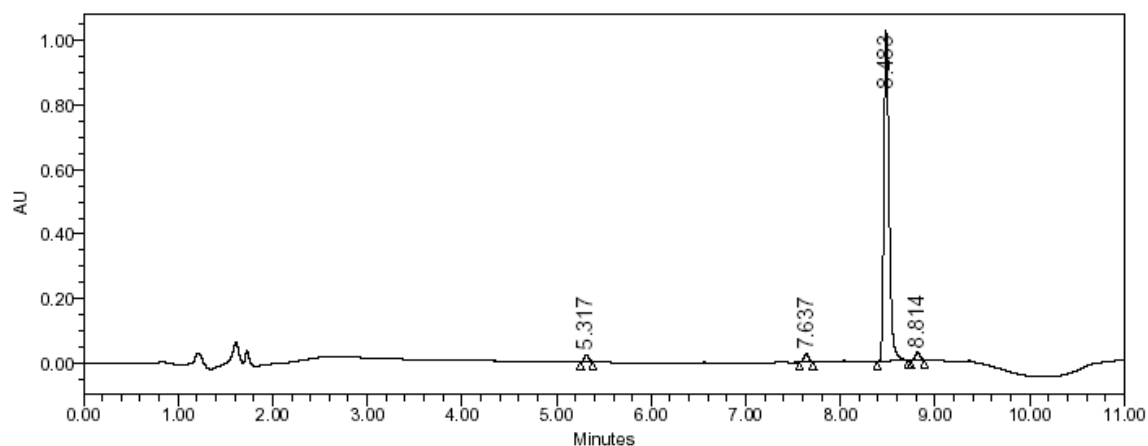
	RT	Area	% Area	Height
1	7.626	154311	6.44	42731
2	7.912	2240344	93.56	620132

Table 1, entry 2: Phe: 2.9 min, PAA: 7.6 min.



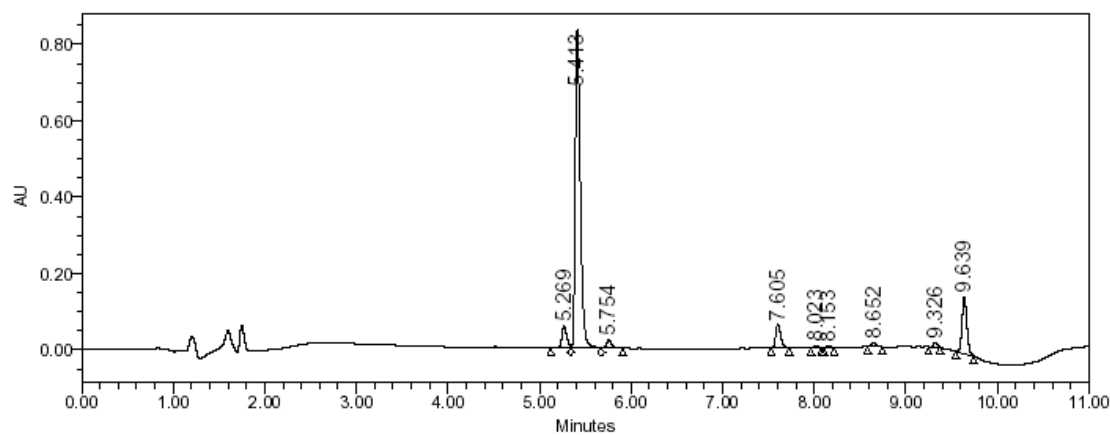
	RT	Area	% Area	Height
1	2.862	1166095	47.77	299202
2	7.627	1183600	48.49	314794
3	8.176	91184	3.74	25603

Table 1, entry 3: Fmoc-Arg(Pbf)-Phe: 8.5 min, PAA: 7.6 min.



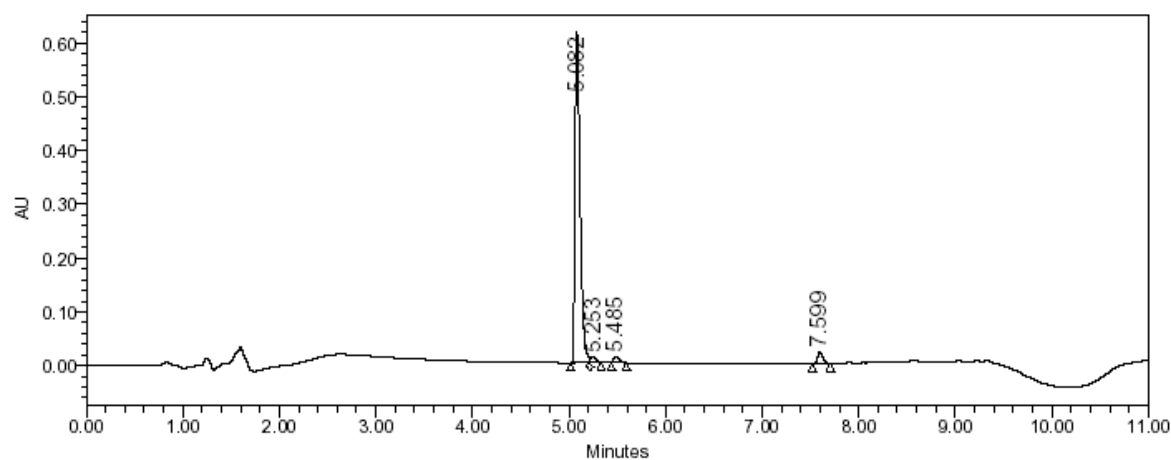
	RT	Area	% Area	Height
1	5.317	69749	1.69	21411
2	7.637	85002	2.06	24858
3	8.483	3898081	94.27	1018172
4	8.814	81992	1.98	23247

Table 1, entry 4: Fmoc-Arg-Phe: 5.4 min. PAA: 7.6 min.



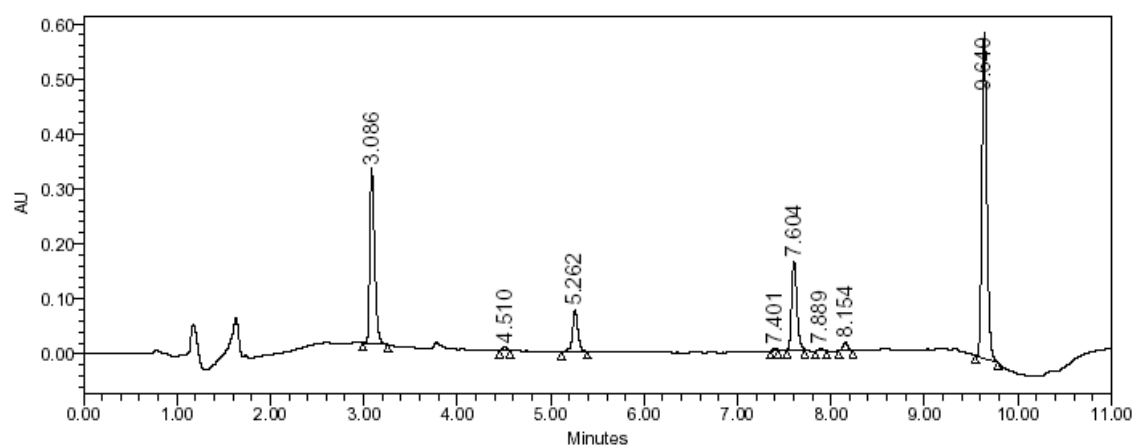
	RT	Area	% Area	Height
1	5.269	227987	5.22	58463
2	5.413	3171359	72.61	833177
3	5.754	91704	2.10	21000
4	7.605	227187	5.20	61275
5	8.023	16934	0.39	5182
6	8.153	17067	0.39	5310
7	8.652	42463	0.97	12039
8	9.326	38413	0.88	12903
9	9.639	534458	12.24	149248

Table 1, entry 5: Arg(Pbf)-Phe: 5.1 min, PAA: 7.6 min.



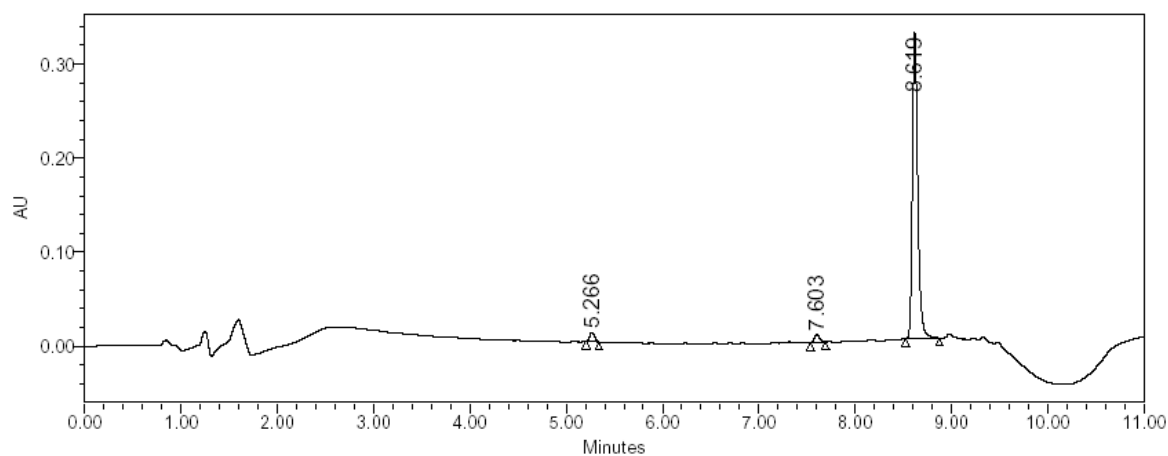
	RT	Area	% Area	Height
1	5.082	2271466	93.39	614515
2	5.253	39106	1.61	9519
3	5.485	47158	1.94	11918
4	7.599	74487	3.06	20699

Table 1, entry 6: Arg-Phe: 3.1 min, PAA: 7.6 min.



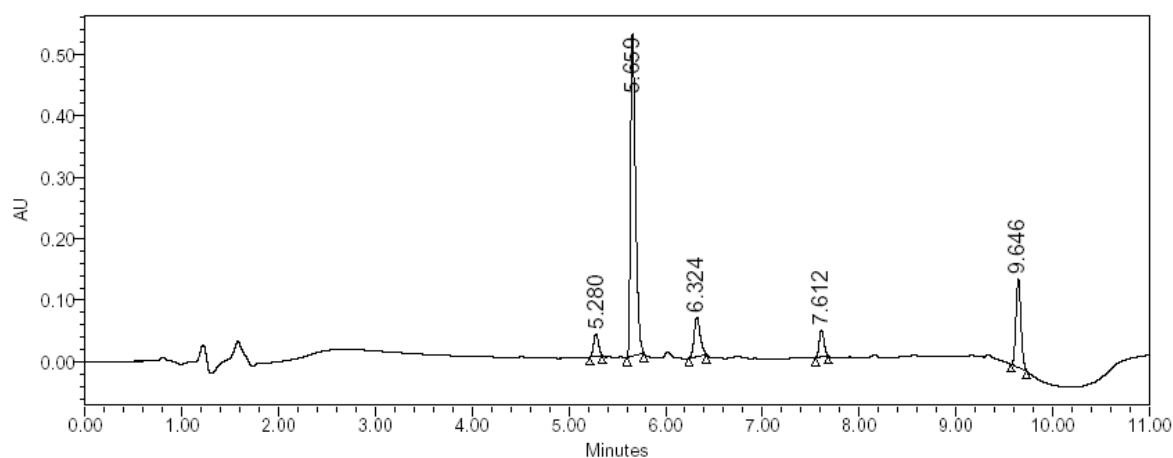
	RT	Area	% Area	Height
1	3.086	1133119	26.35	317652
2	4.510	19415	0.45	6535
3	5.262	291269	6.77	74727
4	7.401	12859	0.30	4671
5	7.604	615000	14.30	162261
6	7.889	12629	0.29	3796
7	8.154	55384	1.29	15692
8	9.640	2161196	50.25	591079

Table 1, entry 7: Fmoc-Val-Arg(Pbf)-Phe: 8.6 min, PAA: 7.7 min.



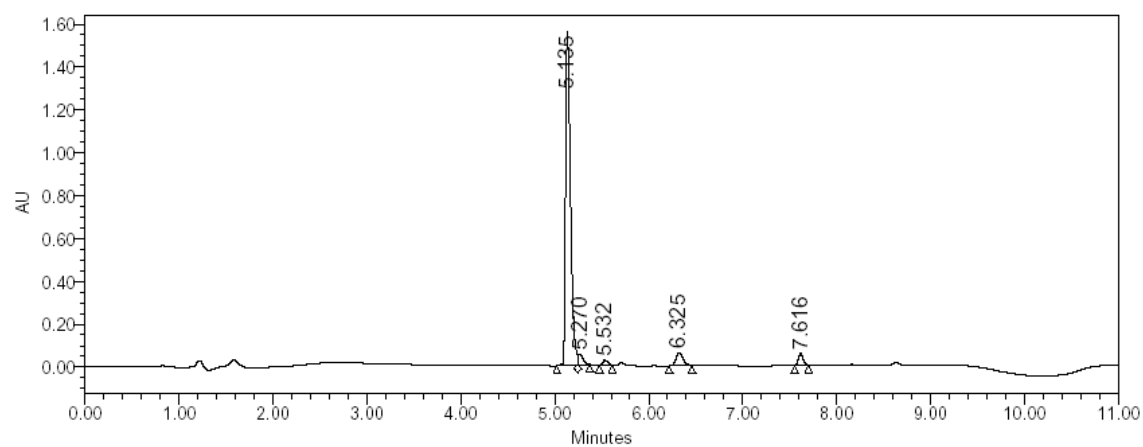
	RT	Area	% Area	Height
1	5.266	32225	2.44	9673
2	7.603	29649	2.24	8578
3	8.619	1258917	95.32	325712

Table 1, entry 8: Fmoc-Val-Arg-Phe: 5.7 min, PAA 7.6 min.



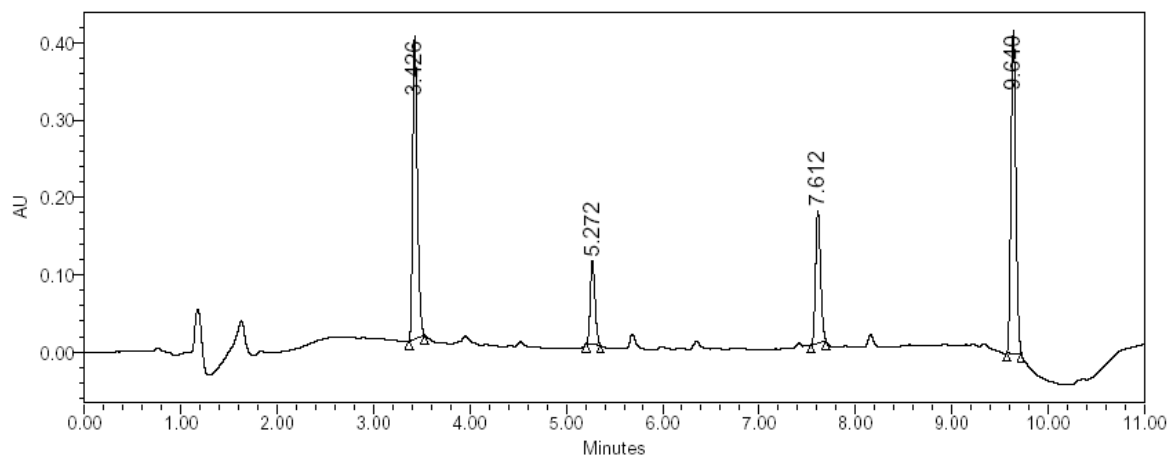
	RT	Area	% Area	Height
1	5.280	125066	4.28	36544
2	5.659	1865667	63.90	524476
3	6.324	282164	9.66	63968
4	7.612	143353	4.91	43179
5	9.646	503506	17.24	143798

Table 1, entry 9: Val-Arg(Pbf)-Phe: 5.1 min, PAA: 7.6 min.



	RT	Area	% Area	Height
1	5.135	5633370	88.03	1554697
2	5.270	180582	2.82	51586
3	5.532	82032	1.28	23222
4	6.325	313767	4.90	59682
5	7.616	189263	2.96	54966

Table 1, entry 10: Val-Arg-Phe: 3.4 min, PAA: 7.6 min.

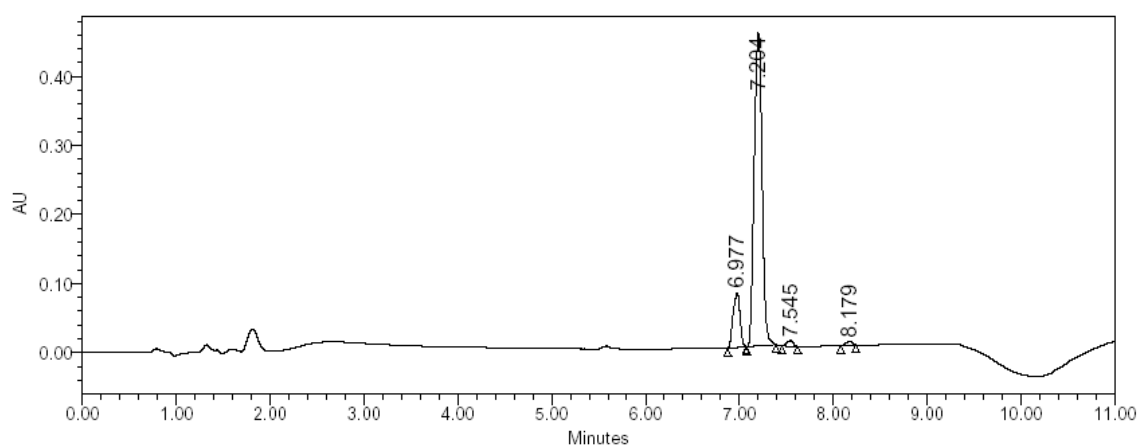


	RT	Area	% Area	Height
1	3.426	1315637	35.25	391528
2	5.272	364329	9.76	107331
3	7.612	605412	16.22	170804
4	9.640	1446856	38.77	417567

SI Chromatograms Table SI-2. Solid-phase synthesis of Fmoc-Xaa-Pro on resin 7

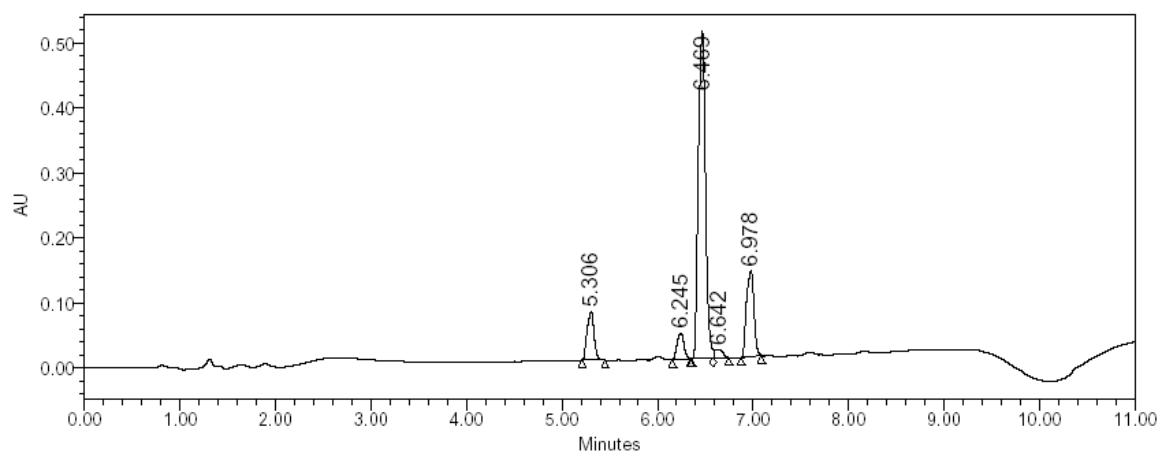
Dipeptides Fmoc-Xaa-Pro (Xaa = Gly, DLeu, Sar) were synthesized as described above. The entries of Table SI-2 correspond to those in Table 2 in the article.

Table 2, entry 1: Fmoc-Pro (loaded resin): 7.2 min, BPA: 7.0 min.



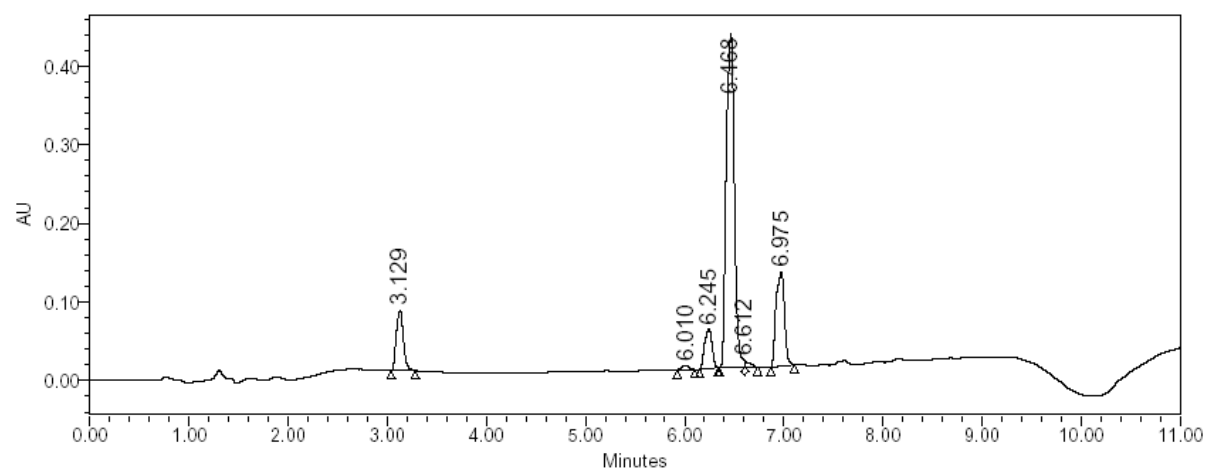
	RT	Area	% Area	Height
1	6.977	404798	13.07	79474
2	7.204	2621982	84.69	452901
3	7.545	40628	1.31	8111
4	8.179	28603	0.92	5473

Table 2, entry 2: Fmoc-Gly-Pro(Oxyma / DIPCDI): 6.5 min, BPA: 7.0 min.



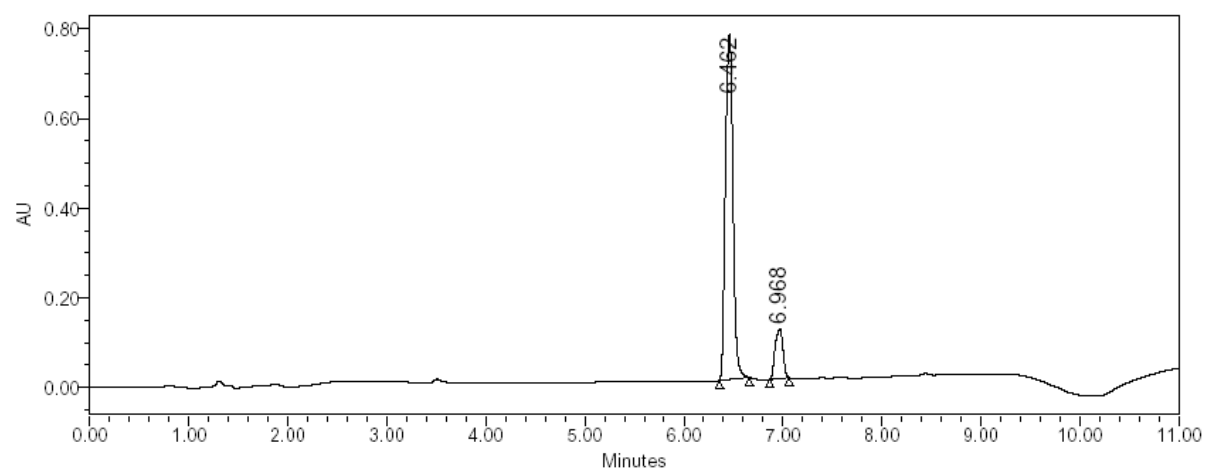
	RT	Area	% Area	Height
1	5.306	370949	9.30	74423
2	6.245	194666	4.88	40186
3	6.469	2635411	66.10	502923
4	6.642	79312	1.99	12865
5	6.978	706814	17.73	131957

Table 2, entry 3: Fmoc-Gly-Pro (HOAt / DIPCDI): 6.5 min, BPA: 7.0 min.



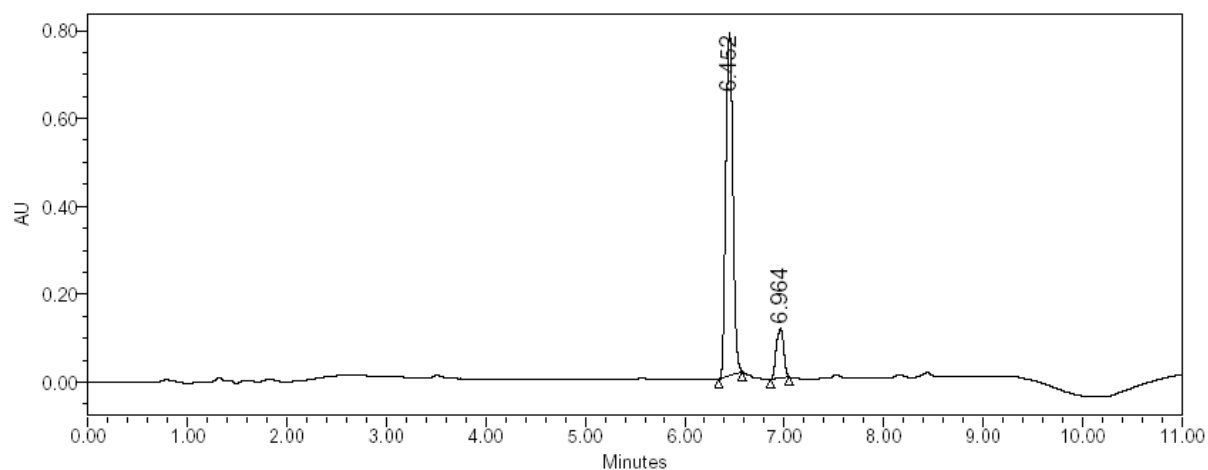
	RT	Area	% Area	Height
1	3.129	371684	9.76	77209
2	6.010	29116	0.76	5568
3	6.245	259247	6.81	49816
4	6.468	2424368	63.64	425000
5	6.612	26788	0.70	7663
6	6.975	698270	18.33	119782

Table 2, entry 4: Fmoc-Gly-Pro (HBTU / DIEA): 6.5 min, BPA: 7.0 min.



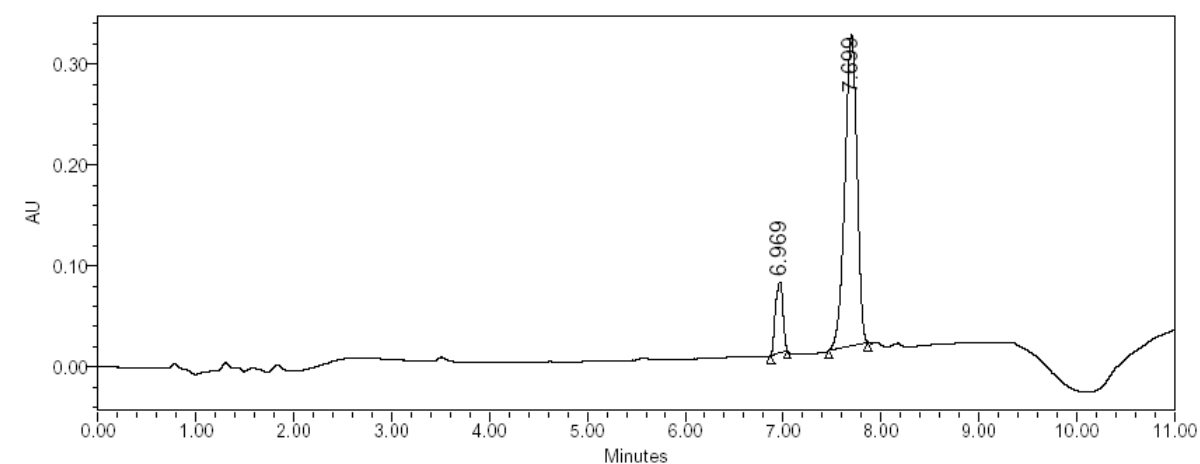
	RT	Area	% Area	Height
1	6.462	4130133	87.24	770445
2	6.968	603977	12.76	111076

Table 2, entry 5: Fmoc-Gly-Pro (HBTU / DIEA, repeated): 6.5 min, BPA: 7.0 min.



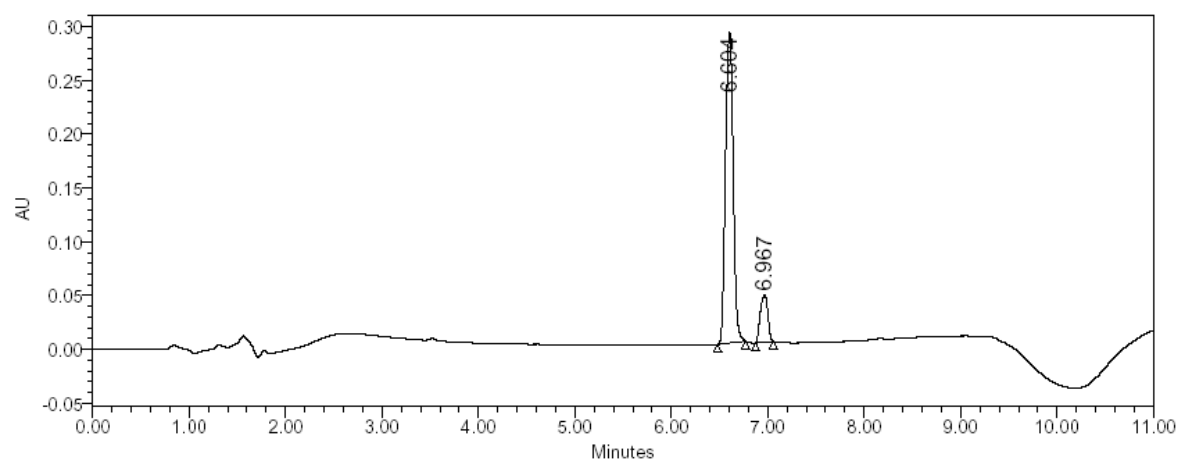
	RT	Area	% Area	Height
1	6.452	3911202	87.16	781738
2	6.964	576175	12.84	112982

Table 2, entry 6: Fmoc-DLeu-Pro (HBTU / DIEA): 7.7 min, BPA: 7.0 min.



	RT	Area	% Area	Height
1	6.969	367224	12.20	70406
2	7.699	2642180	87.80	308962

Table 2, entry 7: Fmoc-Sar-Pro (HBTU / DIEA): 6.6 min, BPA: 7.0 min.

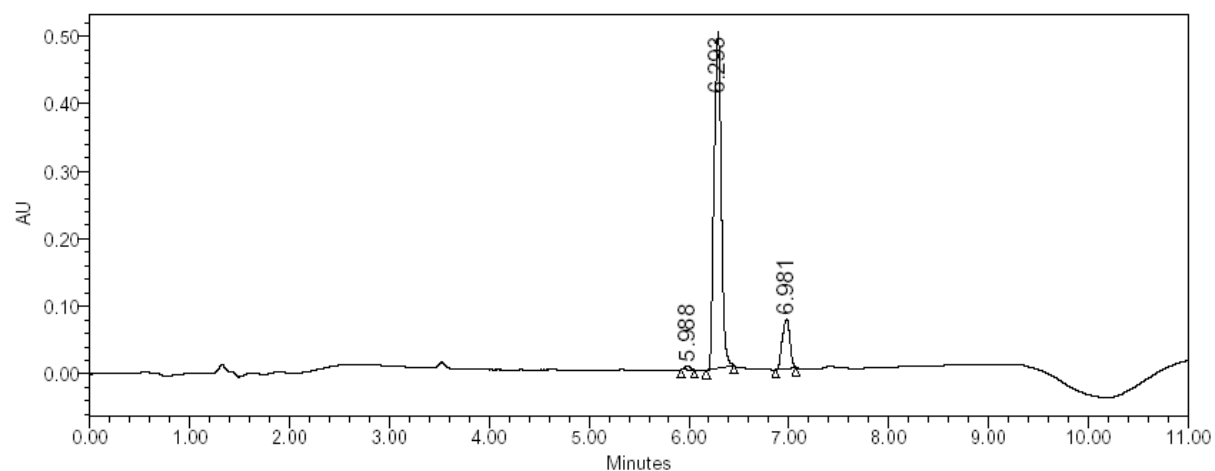


	RT	Area	% Area	Height
1	6.604	1607147	87.32	288309
2	6.967	233462	12.68	43991

SI Chromatograms Table SI-3. Solid-phase synthesis of Fmoc-Aib-Xaa-Pro on resins 10.

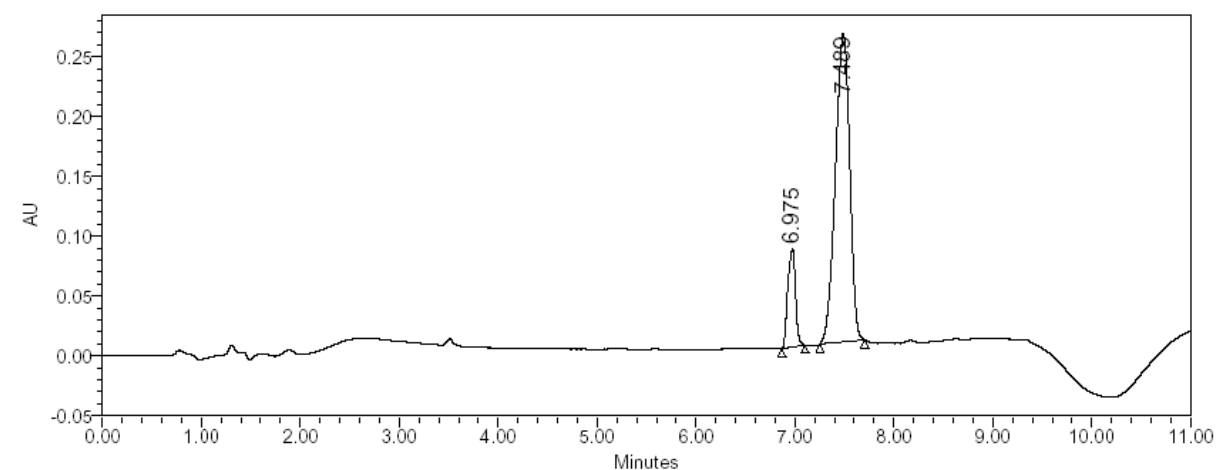
Tripeptides Fmoc-Aib-Xaa-Pro (Xaa = Gly, DLeu, Sar) were synthesized as described above. The entries of Table SI-3 correspond to those in Table 3 in the article.

Table 3, entry 1: Fmoc-Aib-Gly-Pro (HBTU / DIEA): 6.3 min, BPA: 7.0 min.



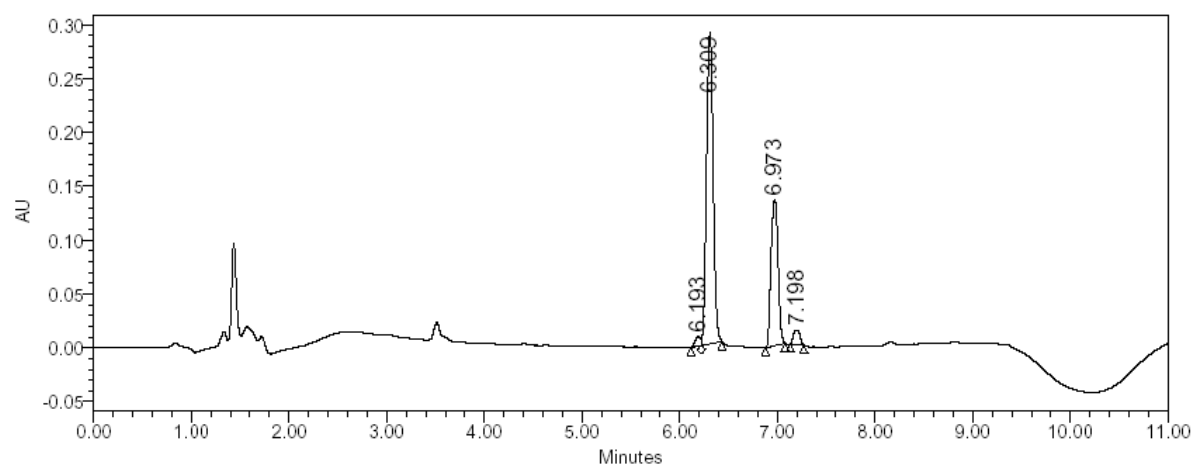
	RT	Area	% Area	Height
1	5.988	24152	0.84	5595
2	6.293	2472066	85.80	497338
3	6.981	384863	13.36	73638

Table 3, entry 2: Fmoc-Aib-DLeu-Pro (HBTU / DIEA): 7.5 min, BPA: 7.0 min.



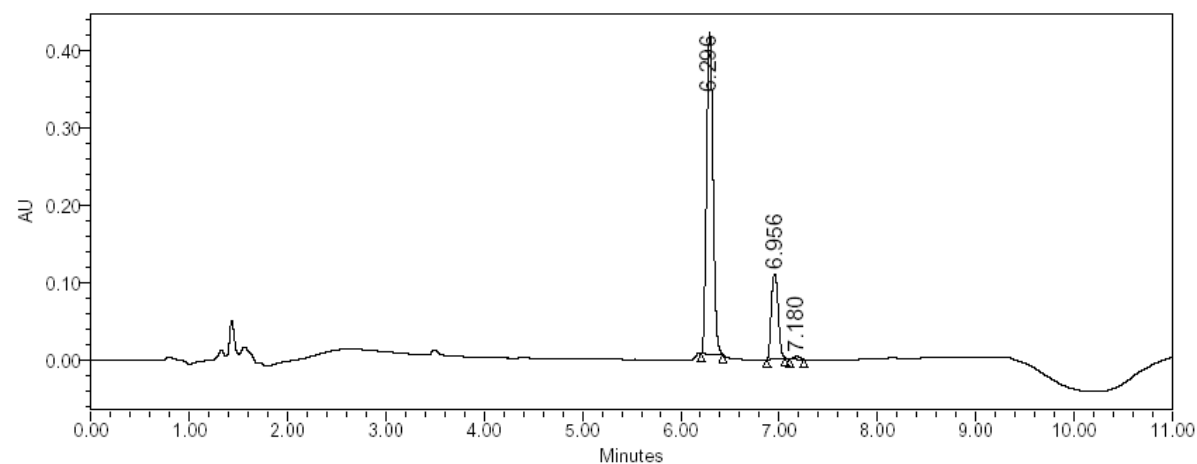
	RT	Area	% Area	Height
1	6.975	439981	14.53	82109
2	7.489	2588629	85.47	257657

Table 3, entry 3: Fmoc-Aib-Sar-Pro (HBTU / DIEA, 40 min): 6.3 min, BPA: 7.0 min.



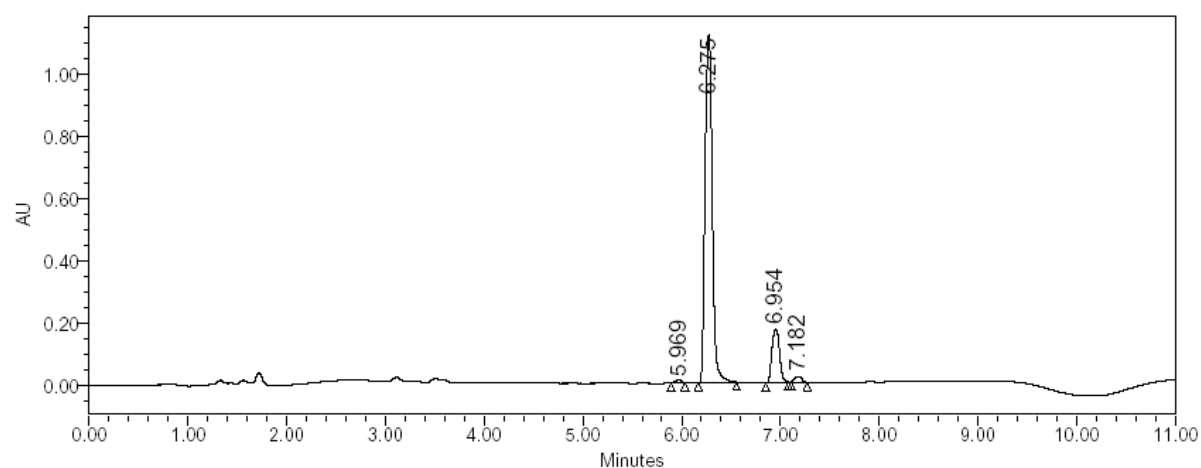
	RT	Area	% Area	Height
1	6.193	32626	1.52	8801
2	6.309	1380606	64.17	288839
3	6.973	675596	31.40	135829
4	7.198	62708	2.91	13813

Table 3, entry 4: Fmoc-Aib-Sar-Pro (HBTU / DIEA, 2 h): 6.3 min, BPA: 7.0 min.



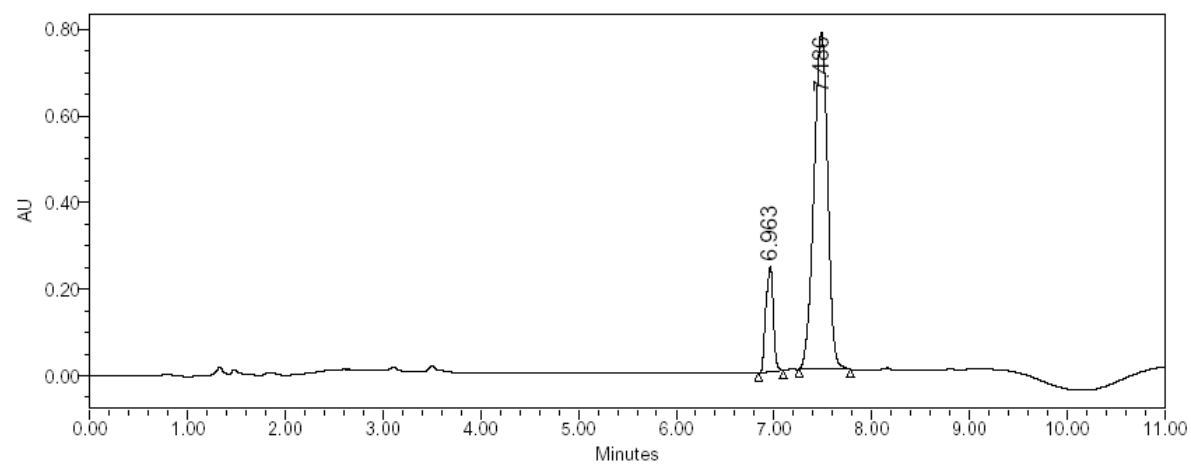
	RT	Area	% Area	Height
1	6.296	1903387	77.74	415728
2	6.956	526693	21.51	109947
3	7.180	18192	0.74	4056

Table 3, entry 5: Fmoc-Aib-Gly-Pro (HATU / DIEA): 6.3 min, BPA: 7.0 min.



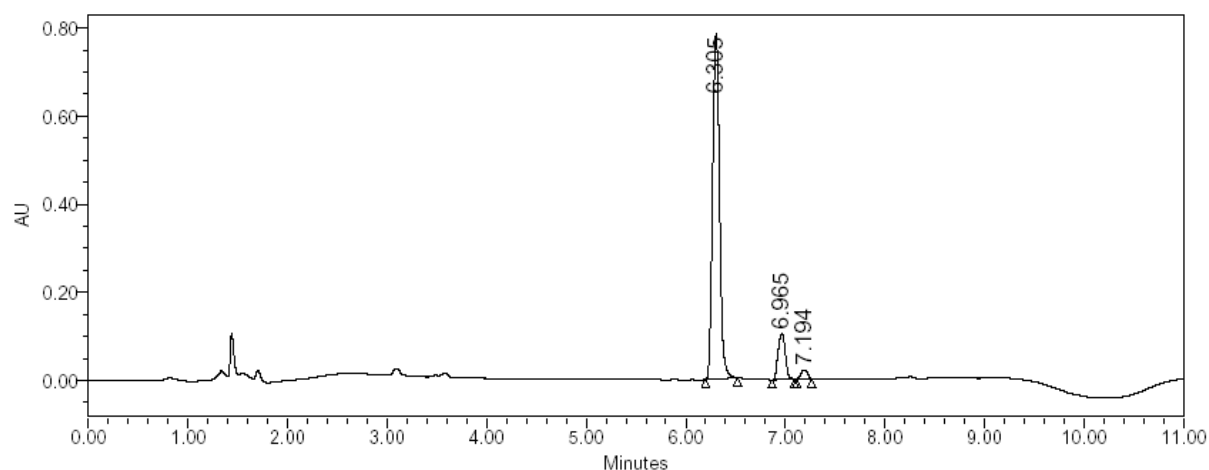
	RT	Area	% Area	Height
1	5.969	42006	0.63	9887
2	6.275	5620724	84.43	1121692
3	6.954	902601	13.56	172006
4	7.182	91614	1.38	18682

Table 3, entry 6: Fmoc-Aib-DLeu-Pro (HATU / DIEA): 7.5 min, BPA: 7.0 min.



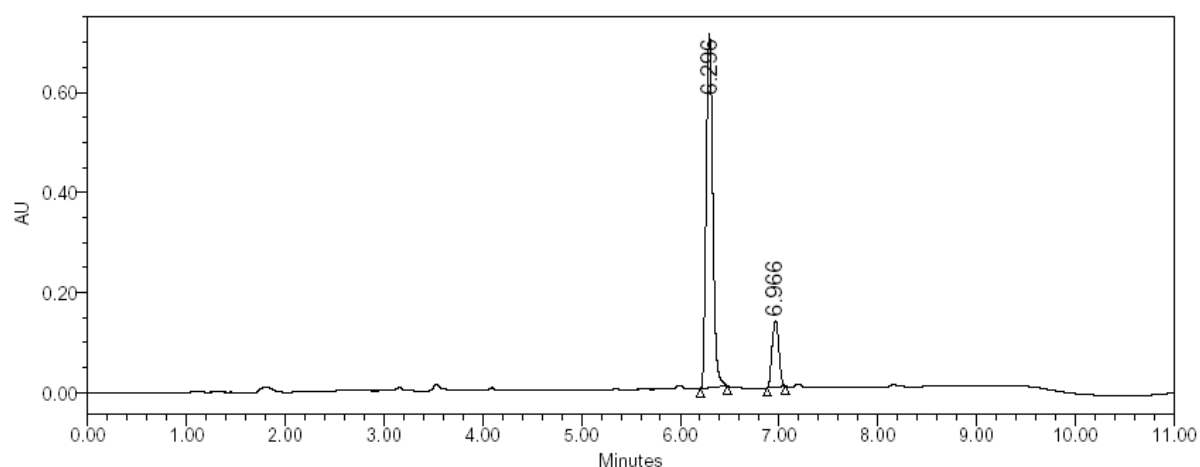
	RT	Area	% Area	Height
1	6.963	1272111	14.39	243912
2	7.486	7567372	85.61	777688

Table 3, entry 7: Fmoc-Aib-Sar-Pro (HATU / DIEA): 6.3 min, BPA: 7.0 min.



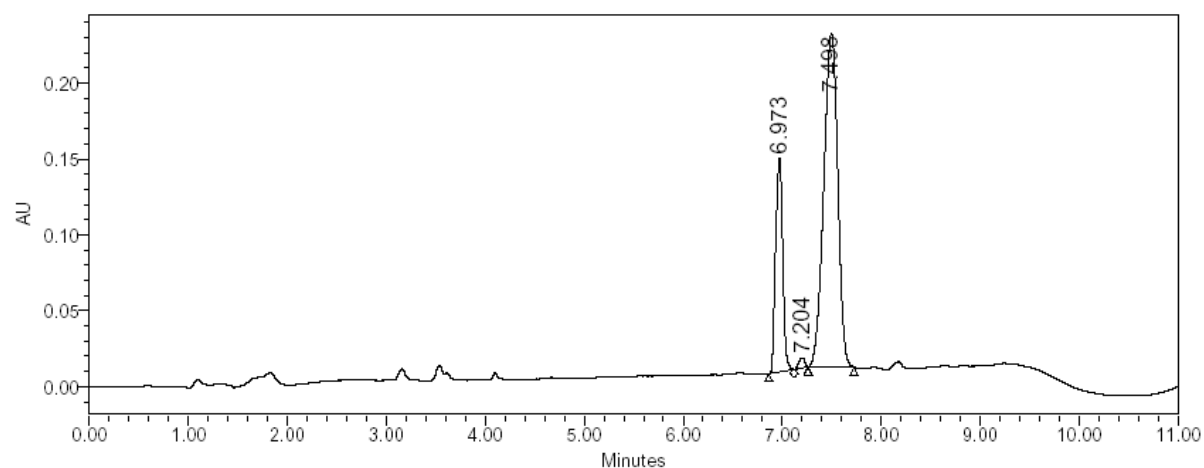
	RT	Area	% Area	Height
1	6.305	3824262	86.17	784570
2	6.965	519210	11.70	104240
3	7.194	94765	2.14	20377

Table 3, entry 8: Fmoc-Aib-Gly-Pro (20 min piperidine, HATU / DIEA): 6.3 min, BPA: 7.0 min.



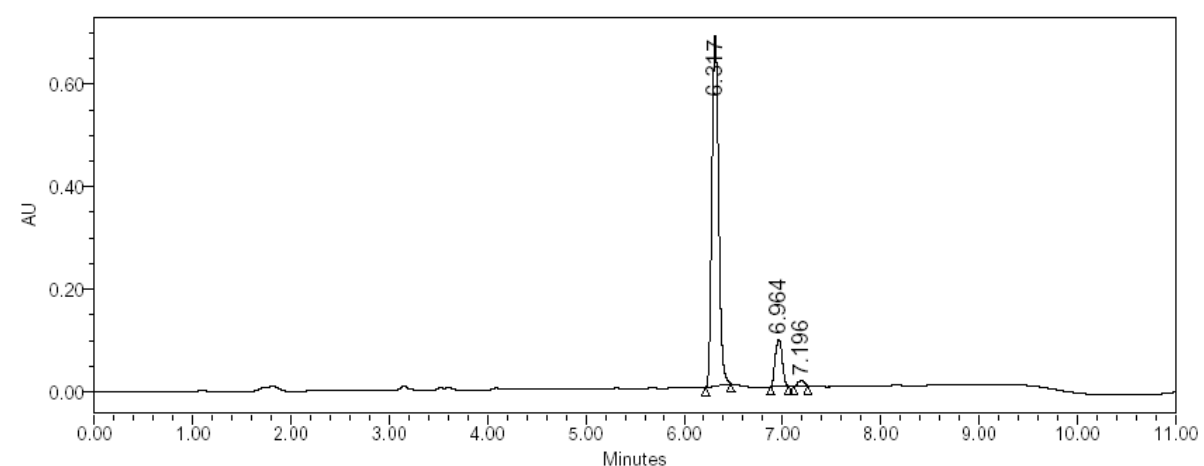
	RT	Area	% Area	Height
1	6.296	3299075	84.11	705771
2	6.966	623342	15.89	132284

Table 3, entry 9: Fmoc-Aib-DLeu-Pro (20 min piperidine, HATU / DIEA): BPA: 7.0 min, 7.5 min.



	RT	Area	% Area	Height
1	6.973	699367	24.21	140977
2	7.204	29287	1.01	6782
3	7.498	2159630	74.77	220101

Table 3, entry 10: Fmoc-Aib-Sar-Pro (20 min piperidine, HATU / DIEA): 6.3 min, BPA: 7.0 min.

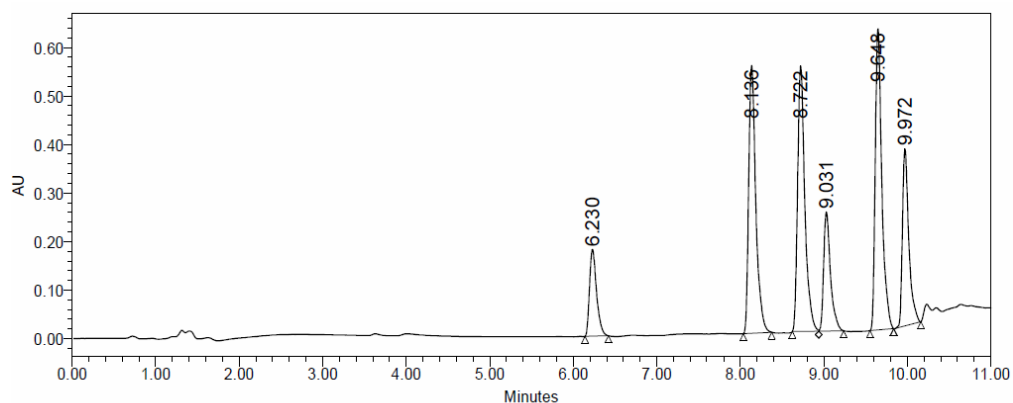


	RT	Area	% Area	Height
1	6.317	3293211	86.87	682803
2	6.964	446104	11.77	92265
3	7.196	51499	1.36	11246

SI Chromatograms Table SI-4. Resins 12 and 13.

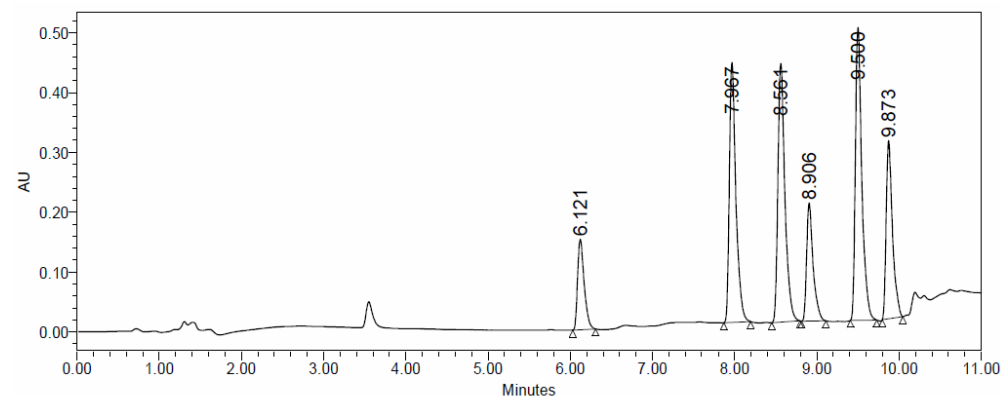
The resins 12 and 13 were synthesized as described above. The entries of Table SI-4 correspond to those in Table 4 in the article.

Table 4, entry 1: (HBTU / DIEA, 40 min) Z-Gly: 6.2 min, Fmoc-Gly-Gly: 8.1 min, Fmoc-Pro-Gly: 8.7 min, BPA: 9.0 min, Fmoc-Leu-Gly: 9.6 min PAA: 10.0 min, (gradient 10% to 80% ACN in 8 min).



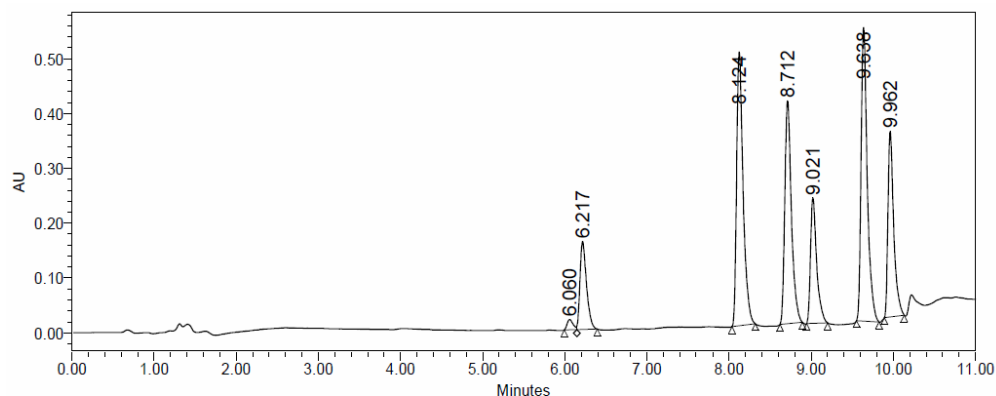
	RT	Area	% Area	Height
1	6.230	1039363	7.27	179361
2	8.136	3242078	22.67	552362
3	8.722	3280730	22.94	547446
4	9.031	1401139	9.80	245414
5	9.648	3394921	23.74	621149
6	9.972	1944798	13.60	364917

Table 4, entry 2: (HBTU / DIEA, 15 h) Z-Gly: 6.1 min, Fmoc-Gly-Gly: 8.0 min, Fmoc-Pro-Gly: 8.6 min, BPA: 8.9 min, Fmoc-Leu-Gly: 9.5 min PAA: 9.9 min, (gradient 10% to 80% ACN in 8 min).



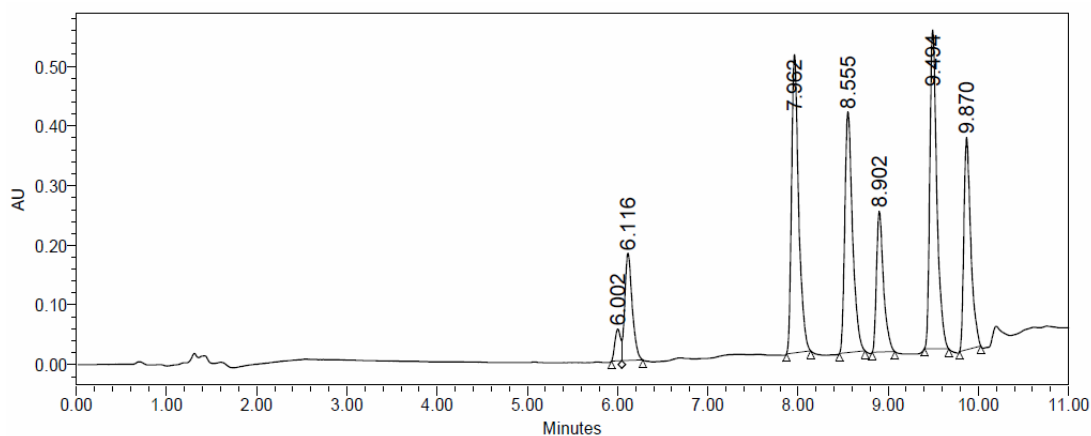
	RT	Area	% Area	Height
1	6.121	836784	7.54	151119
2	7.967	2408823	21.72	434591
3	8.561	2534696	22.85	432395
4	8.906	1081850	9.75	197377
5	9.500	2648671	23.88	489718
6	9.873	1581557	14.26	298156

Table 4, entry 3: (Oxyma / DIPCDI, 40 min) Z-Gly: 6.2 min, Fmoc-Gly-Gly: 8.1 min, Fmoc-Pro-Gly: 8.7 min, BPA: 9.0 min, Fmoc-Leu-Gly: 9.6 min PAA: 10.0 min, (gradient 10% to 80% ACN in 8 min).



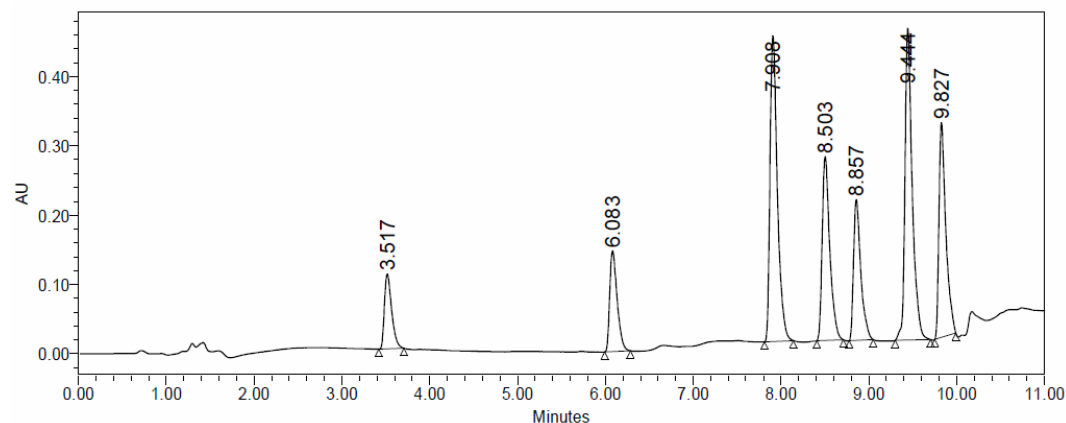
	RT	Area	% Area	Height
1	6.060	94781	0.82	18740
2	6.217	883361	7.63	160467
3	8.124	2718515	23.47	499447
4	8.712	2262785	19.54	406492
5	9.021	1198044	10.34	228733
6	9.638	2767527	23.89	535426
7	9.962	1657421	14.31	338411

Table 4, entry 4: (Oxyma / DIPCDI, 15 h) Z-Gly: 6.1 min, Fmoc-Gly-Gly: 8.0 min, Fmoc-Pro-Gly: 8.6 min, BPA: 8.9 min, Fmoc-Leu-Gly: 9.5 min PAA: 9.9 min, (gradient 10% to 80% ACN in 8 min).



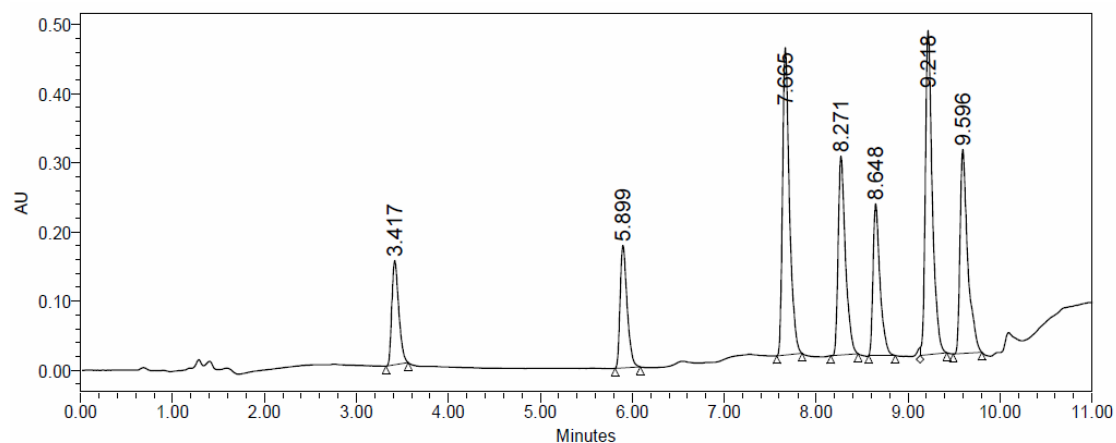
	RT	Area	% Area	Height
1	6.002	231731	1.87	53261
2	6.116	1016634	8.19	179486
3	7.962	2747094	22.14	500233
4	8.555	2383593	19.21	403033
5	8.902	1275188	10.28	235271
6	9.494	2859615	23.05	534387
7	9.870	1891806	15.25	355752

Table 4, entry 5: (HOBt / DIPCDI, 40 min) Z-Gly: 6.1 min, Fmoc-Gly-Gly: 7.9 min, Fmoc-Pro-Gly: 8.5 min, BPA: 8.9 min, Fmoc-Leu-Gly: 9.4 min PAA: 9.8 min, (gradient 10% to 80% ACN in 8 min).



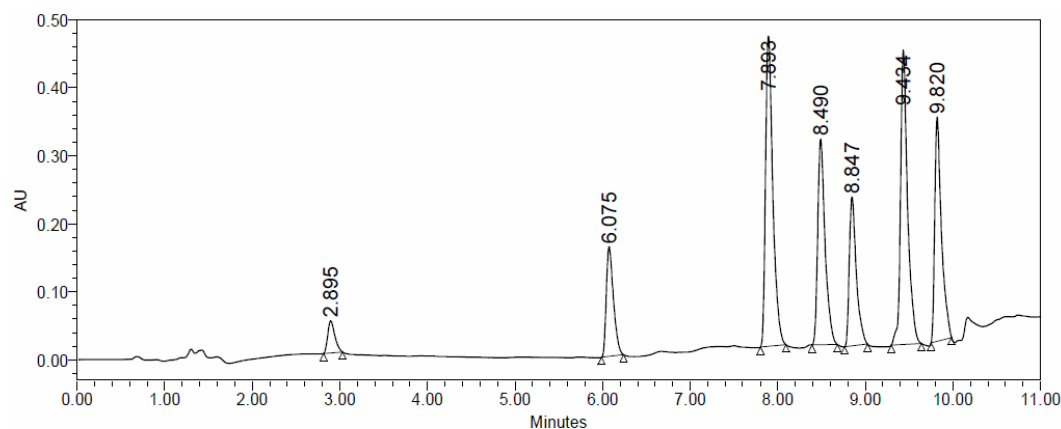
	RT	Area	% Area	Height
1	3.517	614895	5.56	107902
2	6.083	850116	7.69	145525
3	7.908	2556819	23.12	440349
4	8.503	1595576	14.43	265145
5	8.857	1157817	10.47	202781
6	9.444	2588575	23.41	449502
7	9.827	1694963	15.33	309525

Table 4, entry 5: (HOBt / DIPCDI, 15 h) Z-Gly: 5.9 min, Fmoc-Gly-Gly: 7.7 min, Fmoc-Pro-Gly: 8.3 min, BPA: 8.6 min, Fmoc-Leu-Gly: 9.2 min PAA: 9.6 min, (gradient 10% to 80% ACN in 8 min).



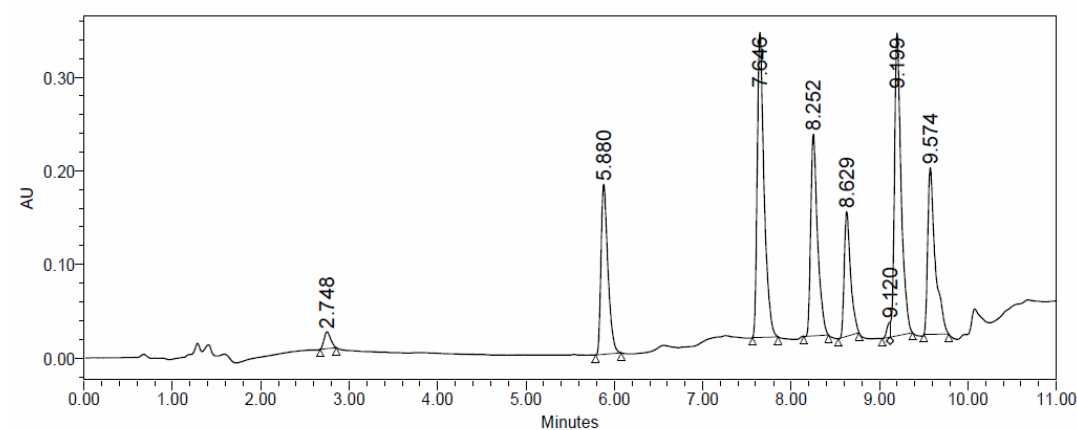
	RT	Area	% Area	Height
1	3.417	787776	7.22	150736
2	5.899	941518	8.62	177020
3	7.665	2319876	21.25	444258
4	8.271	1570721	14.39	287460
5	8.648	1150727	10.54	219330
6	9.218	2443578	22.38	468439
7	9.596	1703686	15.60	294900

Table 4, entry 7: (HOAt / DIPCDI, 40 min) Z-Gly: 6.1 min, Fmoc-Gly-Gly: 7.9 min, Fmoc-Pro-Gly: 8.5 min, BPA: 8.8 min, Fmoc-Leu-Gly: 9.4 min PAA: 9.8 min, (gradient 10% to 80% ACN in 8 min).



	RT	Area	% Area	Height
1	2.895	264914	2.41	46993
2	6.075	911392	8.30	161294
3	7.893	2621235	23.87	455998
4	8.490	1766441	16.09	301996
5	8.847	1214000	11.06	219057
6	9.434	2432174	22.15	432674
7	9.820	1769048	16.11	330042

Table 4, entry 8: (HOAt / DIPCDI, 15 h) Z-Gly: 5.9 min, Fmoc-Gly-Gly: 7.6 min, Fmoc-Pro-Gly: 8.3 min, BPA: 8.6 min, Fmoc-Leu-Gly: 9.1 min PAA: 9.6 min, (gradient 10% to 80% ACN in 8 min).



	RT	Area	% Area	Height
1	2.748	92181	1.24	17863
2	5.880	986703	13.29	181681
3	7.646	1751267	23.59	325904
4	8.252	1156823	15.58	215236
5	8.629	667018	8.99	133175
6	9.120	38194	0.51	15914
7	9.199	1685294	22.70	323525
8	9.574	1046117	14.09	177877