Supplementary Information

Novel Cyclic Peptides for Targeting EGFR and EGRvIII Mutation for Drug Delivery

Olga Furman [1,2], Alisa Zaporozhets [3], Dror Tobi [4,5], Andrii Bazylevich [3], Michael A. Firer [1,4,6], Leonid Patsenker [3], Gary Gellerman [3,6] and Bat Chen R. Lubin [1,2,*,†]

[1] Department of Chemical Engineering, Biotechnology and Materials, Ariel University, Ariel 40700, Israel; olgaf@ariel.ac.il (O.F.); firer@ariel.ac.il (M.A.F.)
[2] Agriculture and Oenology Department, Eastern Regional R&D Center, Ariel 40700, Israel
[3] Department of Chemical Sciences, Ariel University, Ariel 40700, Israel; aliska.zaporozhets@gmail.com (A.Z.); andriib@ariel.ac.il (A.B.); leonidpa@ariel.ac.il (L.P.); garyg@ariel.ac.il (G.G.)
[4] Adelson School of Medicine, Ariel University, Ariel 40700, Israel; drorto@ariel.ac.il
[5] Department of Molecular Biology, Ariel University, Ariel 40700, Israel
[6] Ariel Center for Applied Cancer Research, Ariel 40700, Israel
* Correspondence: batchenl@ariel.ac.il; Tel.: +972-50-6554655
† Current address: Eastern Regional R&D Center, Room MOP 2.4, Ariel University, Ariel 40700, Israel.

Table S1. Selected C-terminus carboxylate cyclic peptide sequences. .

Peptide	Sequence	Cell lines
P1	c(CLRWRFGRC)	H1299, DKMG, H1975
P2	c(CVRWRFGRC)	
P3	c(CLAVEVRPC)	
P4	c(CSAETVESC)	
P5	c(CPNDSYHQC)	H1299
P6	c(CHVPGSYIC)	H1299
P7	c(CWHSLSLAC)	H1975
P8	c(CSALWASHC)	H1975
P9	c(CVNAMQSYC)	DKMG
P10	c(CNWLSRTEC)	DKMG
P11	c(CAQYTPGRC)	DKMG

Table S2. The numbers of sequences after removing nonspecific peptides that were found in non-EGFR expressed K562 cells.

#	Cell line	Number of common sequences for all 3 cell lines	Number of unique sequences for each line
1	H1299	416	1666
2	H1975		10437
3	DKMG		981

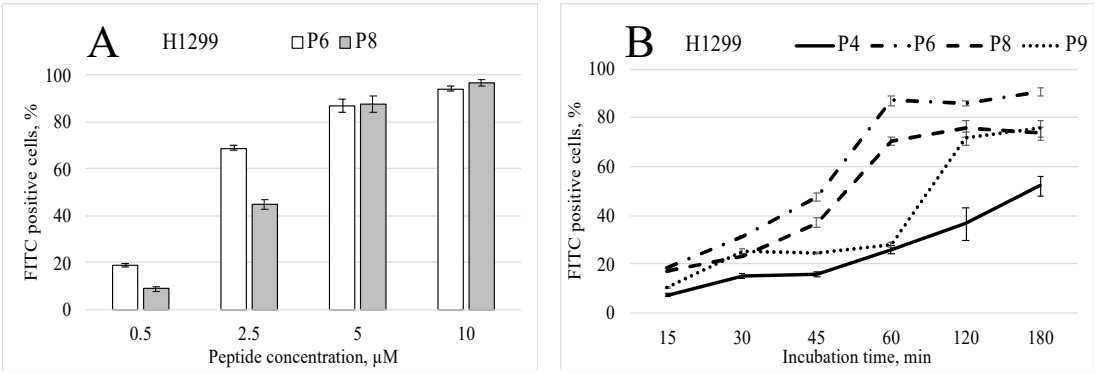


Figure S1. Peptide's titration and time-depended internalization assay.**(A**) H1299 cells were incubated during 1 h with 0.5 μM, 2.5 μM, 5 μM and 10 μM of FITC-labeled P6 and P8 peptides. **(B)** H1299 cells were incubated with 2.5 μM of FITC labeled peptides P4, P6, P8, and P9 for 15, 30, 45,60, 120 and 180 min. Cell analysis was performed using the flow cytometry. Mean ± standard deviation of 2 independent experiments in duplicates is shown.

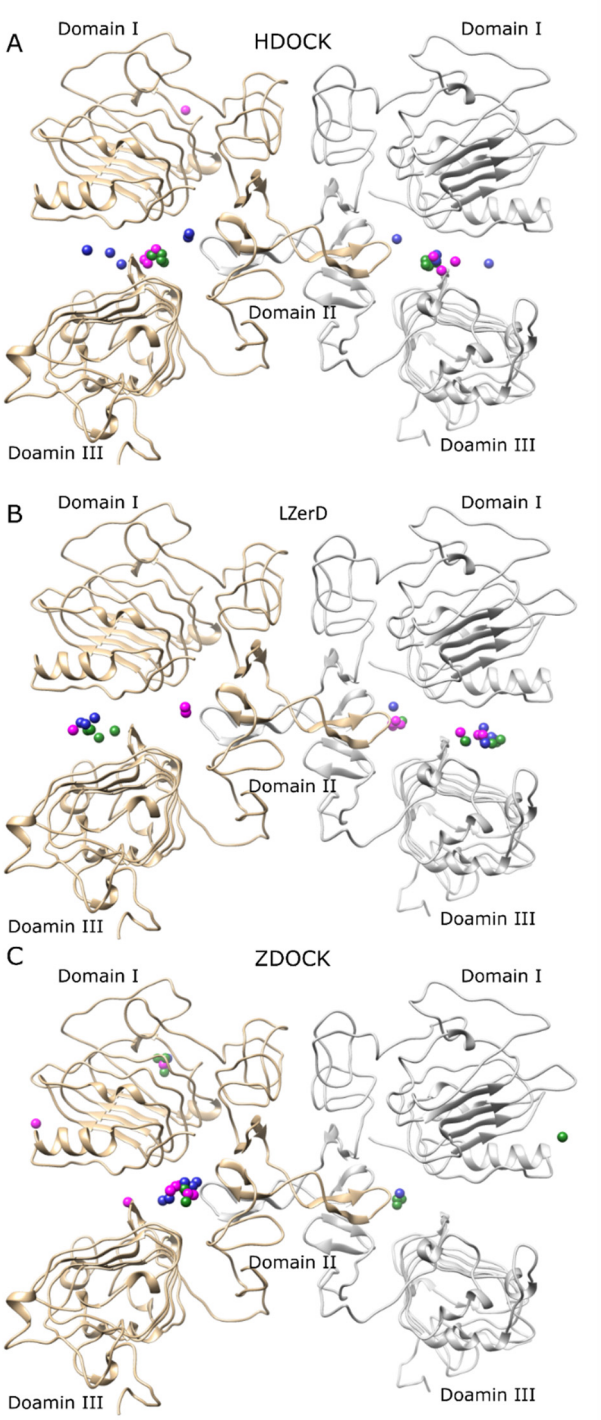
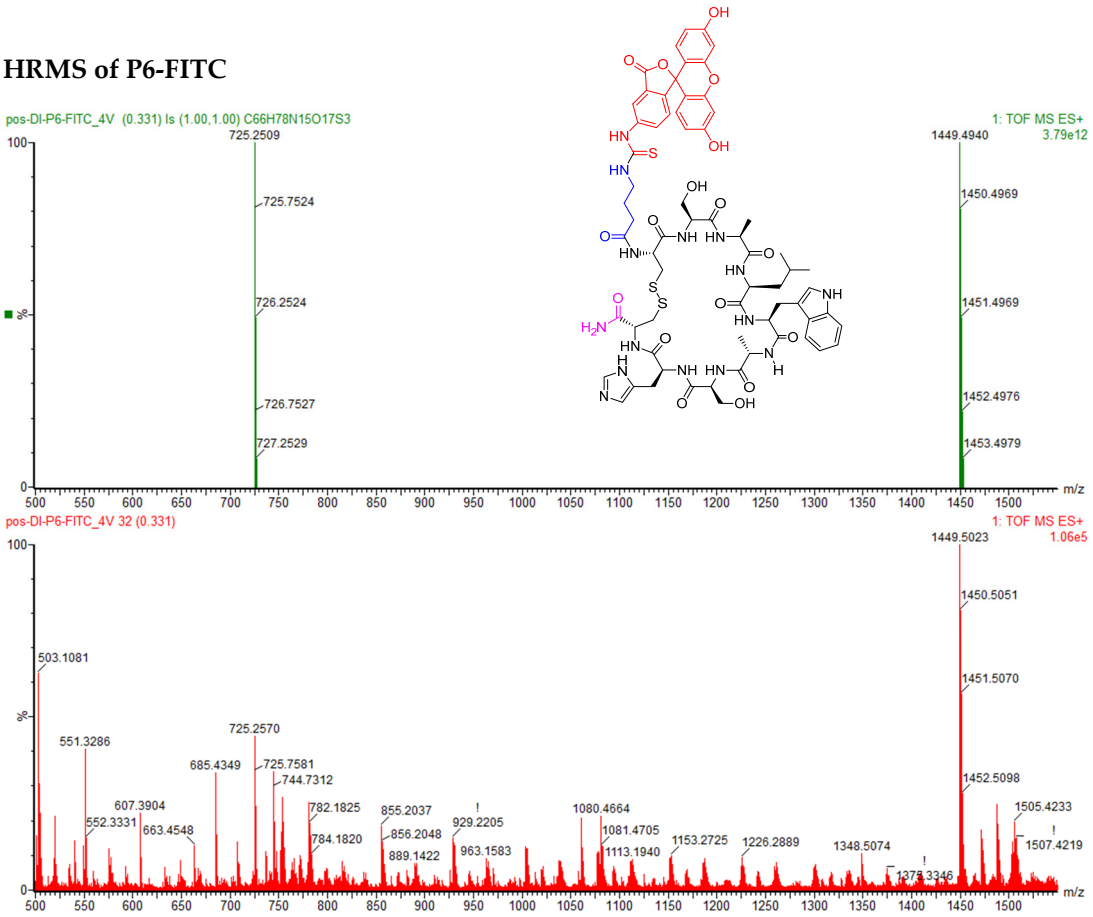


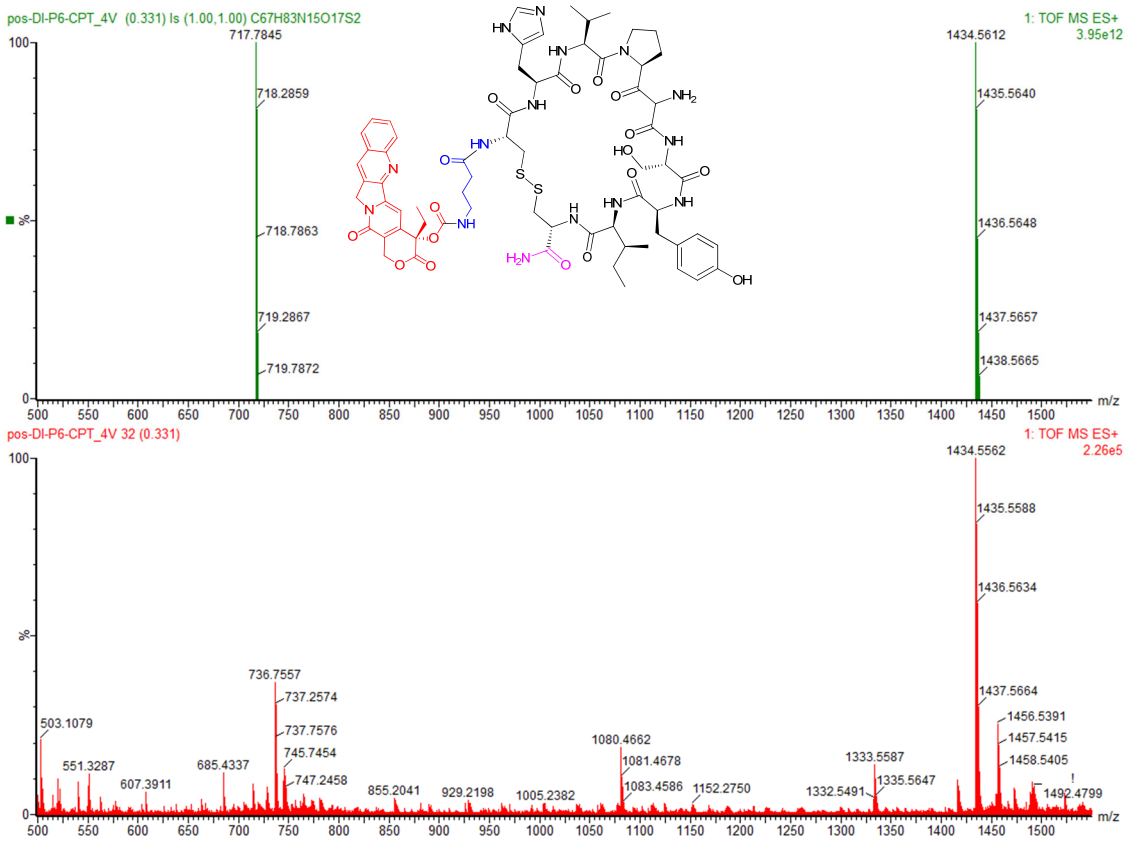
Figure S2. Unbiased rigid body docking of peptides P6, P9, and P11 to the EGFR extracellular region. Unbiased rigid body docking of the peptides to EGFR was carried out with (**A**) HDOCK (**B**) LZerD and (**C**) ZDOCK servers.

The top ten docked poses of each peptides are represented as spheres corresponding to their center of mass and colored green (peptide P6), magenta (peptide P9), and blue (peptide P11). The homodimeric structure of EGFR is shown using ribbon representation with chains A and B colored gray and gold, respectively. The results are similar for all three servers. Most of peptides are bound to the EGFR receptor in the cavity between domains I and III.

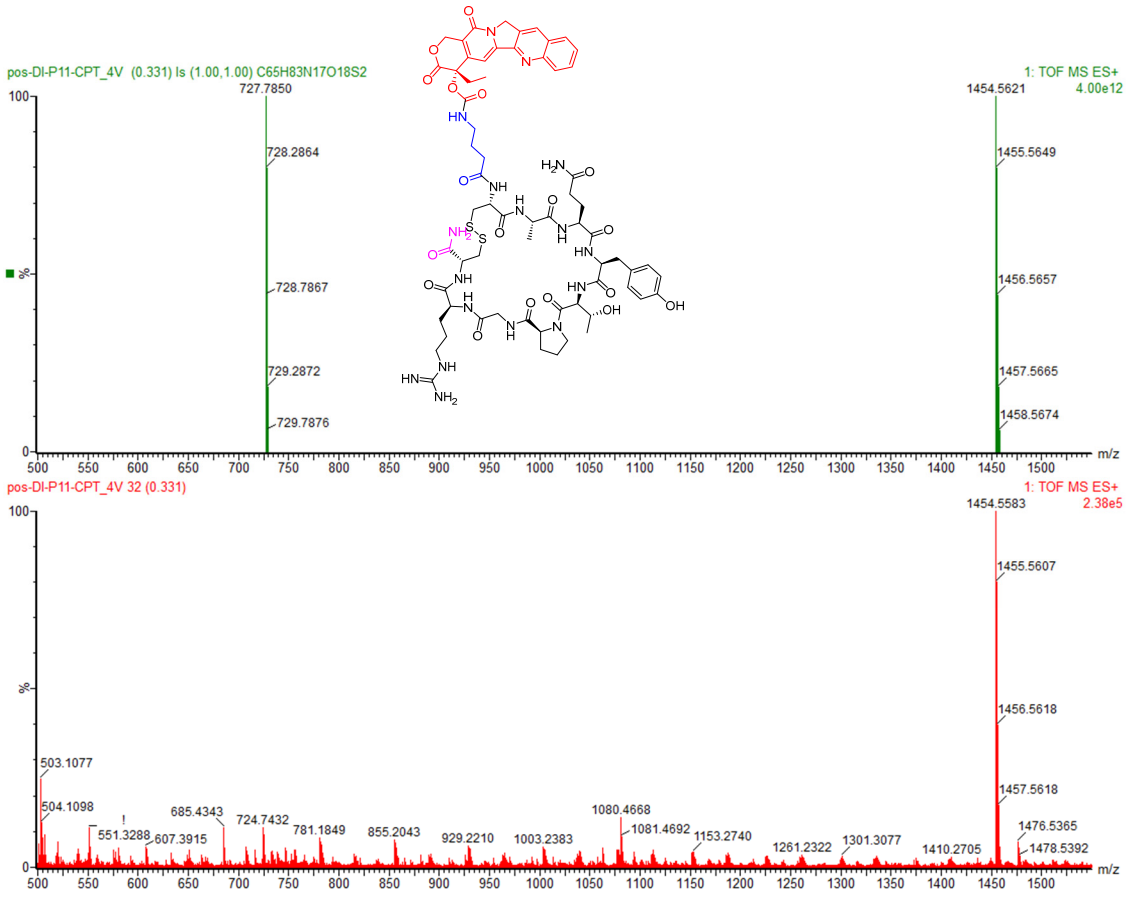
HRMS of P6-FITC



HRMS of P6-CPT

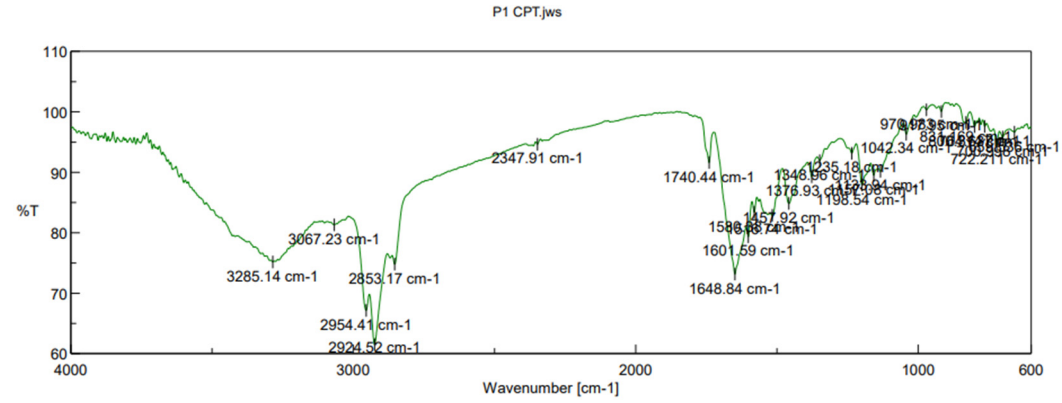


HRMS od P11-CPT



FTIR DATA of the peptide conjugates

P1 FITC



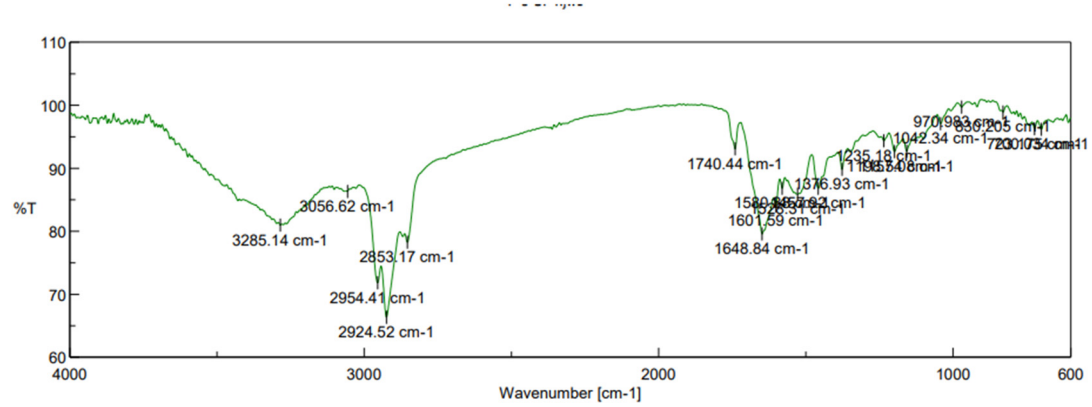
[Comments]	
Sample name	
Comment	
User	
Division	
Company	Ariel University

[Detailed Information]	
Creation date	28/06/2022 12:14
Data array type	Linear data array
Horizontal axis	Wavenumber [cm-1]
Vertical axis	%T
Start	599.753 cm-1
End	4000.6 cm-1
Data interval	0.964233 cm-1
Data points	3528

[Measurement Information]	
Model Name	FT/IR-4700typeA
Serial Number	F061461788
Measurement Date	28/06/2022 12:13
Light Source	Standard
Detector	TGS
Accumulation	70
Resolution	4 cm-1
Zero Filling	On
Apodization	Cosine
Gain	Auto (8)
Aperture	Auto (7.1 mm)
Scanning Speed	Auto (2 mm/sec)
Filter	Auto (30000 Hz)

P1 CPT.jws

P11 CPT

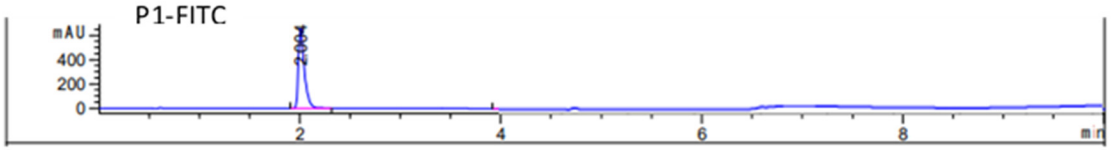


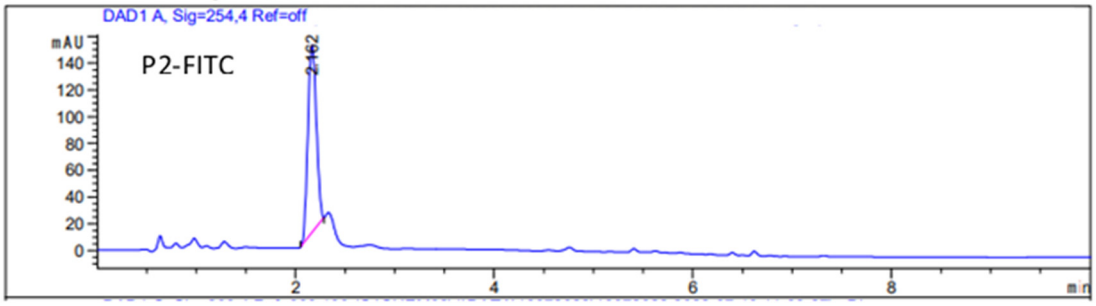
[Comments]	
Sample name	
Comment	
User	
Division	
Company	Ariel University

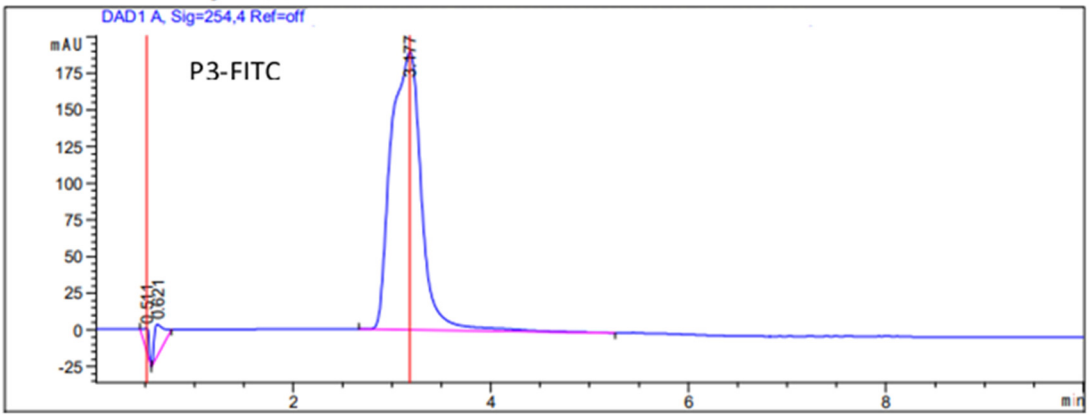
[Detailed Information]	
Creation date	28/06/2022 12:04
Data array type	Linear data array
Horizontal axis	Wavenumber [cm-1]
Vertical axis	%T
Start	599.753 cm-1
End	4000.6 cm-1
Data interval	0.964233 cm-1
Data points	3528

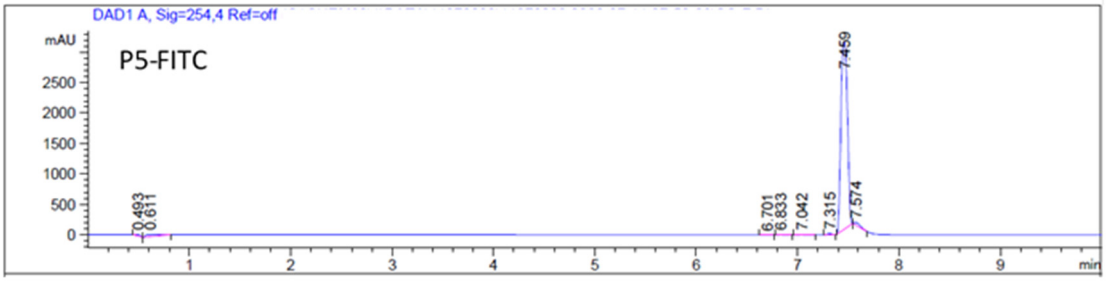
[Measurement Information]	
Model Name	FT/IR-4700typeA
Serial Number	F061461788
Measurement Date	28/06/2022 12:01
Light Source	Standard
Detector	TGS
Accumulation	50
Resolution	4 cm-1
Zero Filling	On
Apodization	Cosine
Gain	Auto (8)
Aperture	Auto (7.1 mm)
Scanning Speed	Auto (2 mm/sec)
Filter	Auto (30000 Hz)

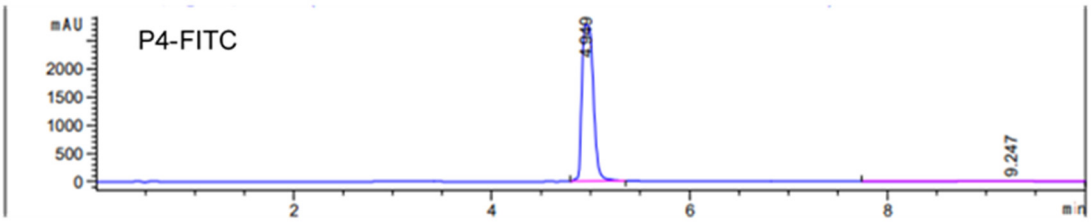
P 11 CPT.jws

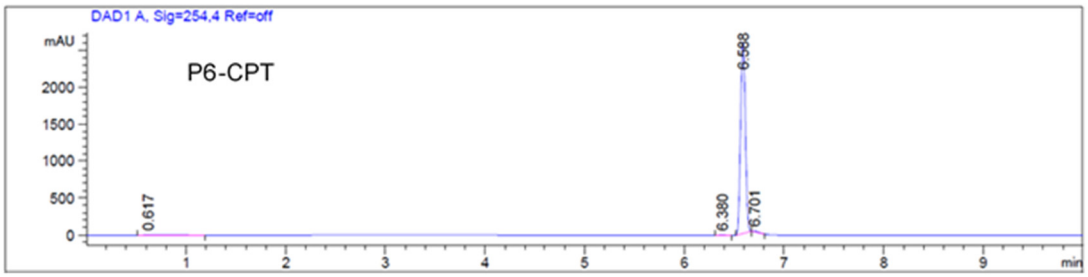

P1-FITC
mAU
400
200
0
2.004
2
4
6
8
min

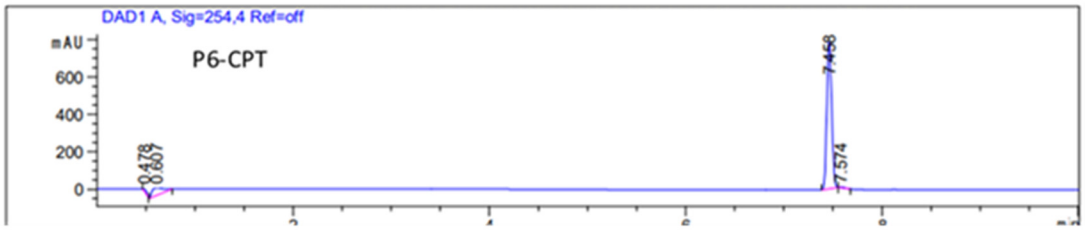

DAD1 A, Sig=254,4 Ref=off
P2-FITC
mAU
140
120
100
80
60
40
20
0
2.162
2
4
6
8
min

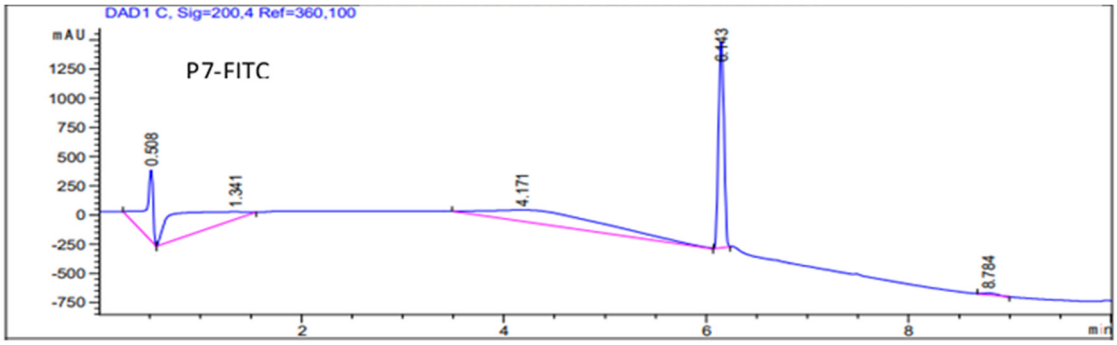

DAD1 A, Sig=254,4 Ref=off
P3-FITC
mAU
175
150
125
100
75
50
25
0
-25
0.511
0.621
3.377
2
4
6
8
min

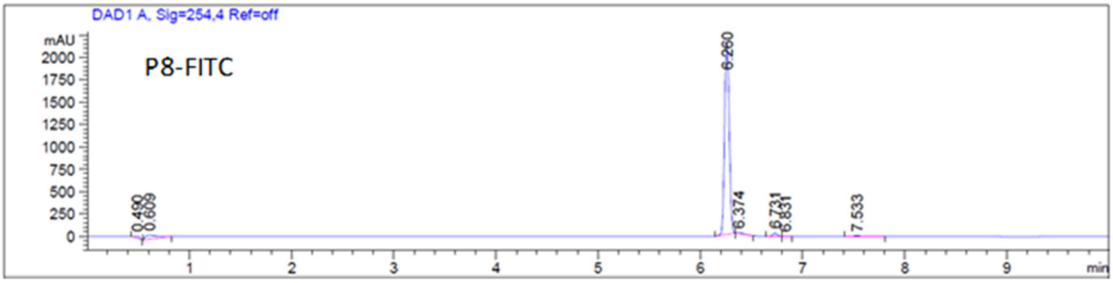

DAD1 A, Sig=254,4 Ref=off
P5-FITC
mAU
2500
2000
1500
1000
500
0
0.493
0.611
6.701
6.833
7.042
7.315
7.459
7.574
1
2
3
4
5
6
7
8
9
min

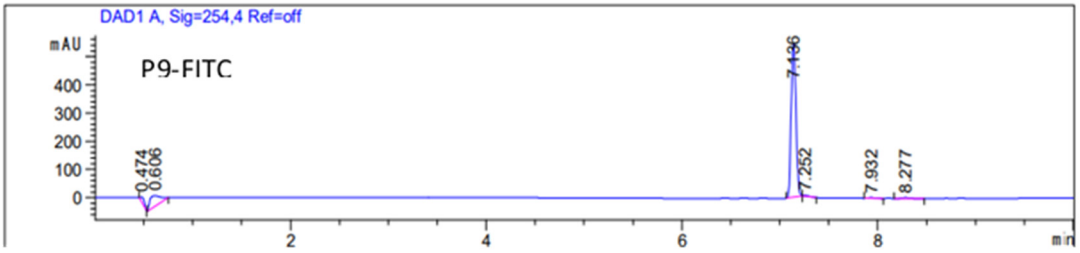

P4-FITC
mAU
2000
1500
1000
500
0
4.949
9.247
2
4
6
8
min

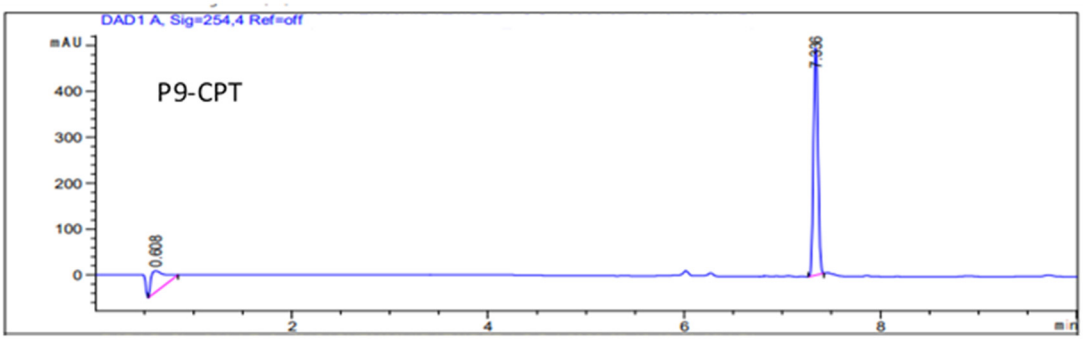

DAD1 A, Sig=254,4 Ref=off
mAU
P6-CPT
6.588
0.617
6.380
6.701
min

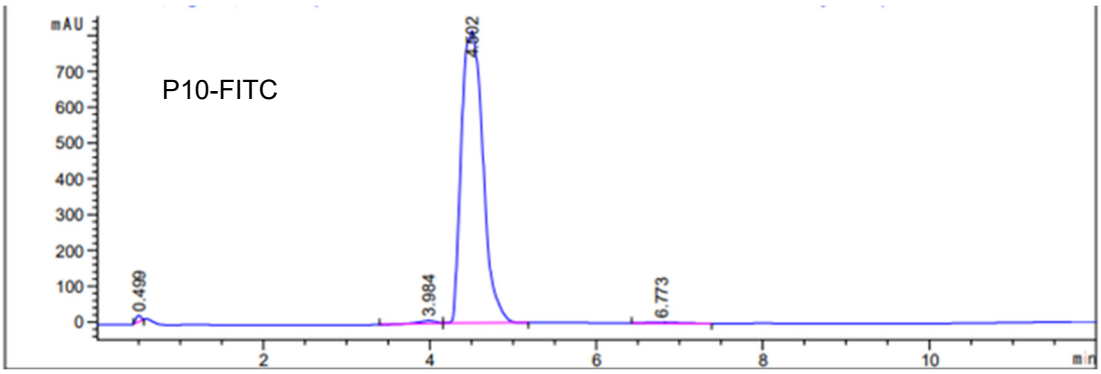

DAD1 A, Sig=254,4 Ref=off
mAU
P6-CPT
7.468
0.478
0.607
7.574

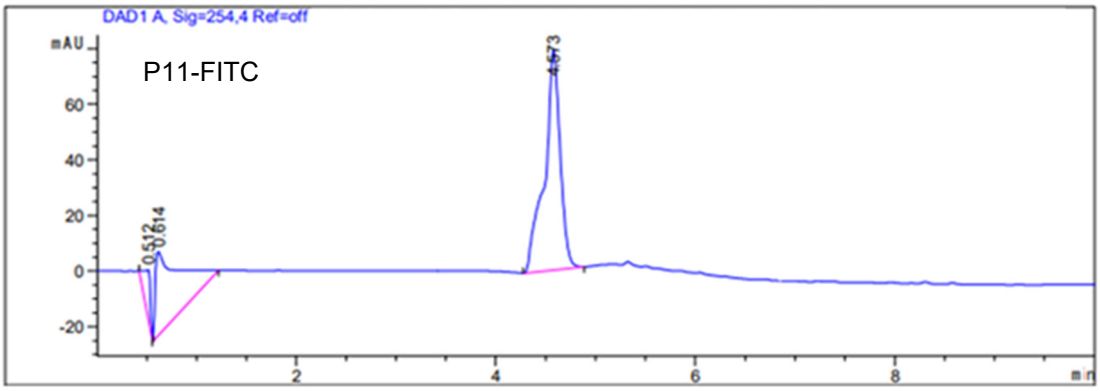

DAD1 C, Sig=200,4 Ref=360,100
mAU
P7-FITC
6.143
0.508
1.341
4.171
8.784
min


DAD1 A, Sig=254,4 Ref=off
mAU
P8-FITC
6.260
0.490
0.609
6.374
6.731
6.831
7.533
min


DAD1 A, Sig=254,4 Ref=off
mAU
P9-FITC
7.196
0.474
0.606
7.252
7.932
8.277


DAD1 A, Sig=254,4 Ref=off
mAU
P9-CPT
7.936
0.608
min


P10-FITC
mAU
700
600
500
400
300
200
100
0
0.499
3.984
4.562
6.773
2
4
6
8
10


DAD1 A, Sig=254,4 Ref=off
P11-FITC
mAU
60
40
20
0
-20
0.512
0.614
4.573
2
4
6
8