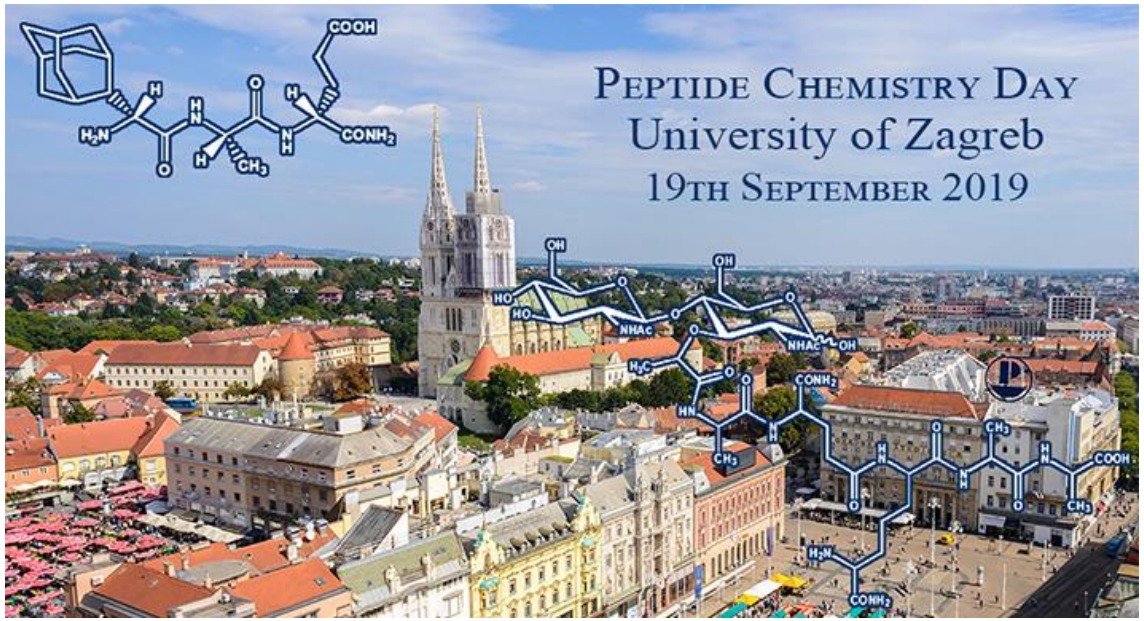
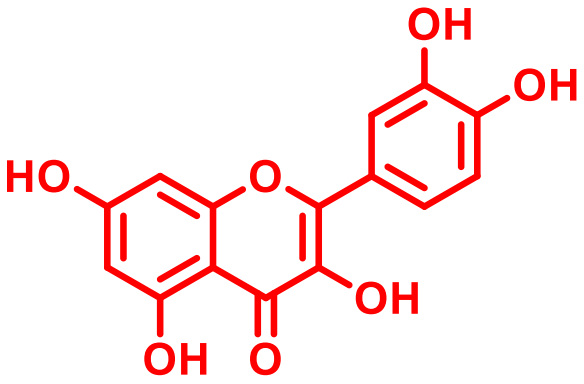


Biološki aktivni peptidi i glikopeptidi kao rezultat kemijske modifikacije prirodnih spojeva

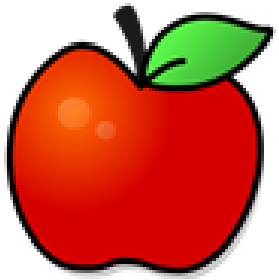
Andreja Jakas, Kristina Vlahoviček-Kahlina, Ljiljana Mrkus, Jelena Batinić, Nina Bjeliš

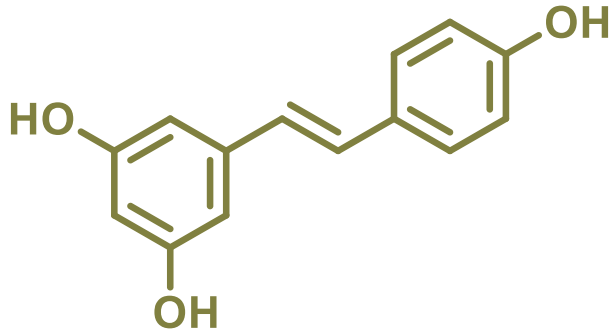
Peptide Chemistry Day, Zagreb, 19. 9. 2019.

Dizajn peptidomimetika s antitumorskim i antioksidacijskim svojstvima



KVERCETIN

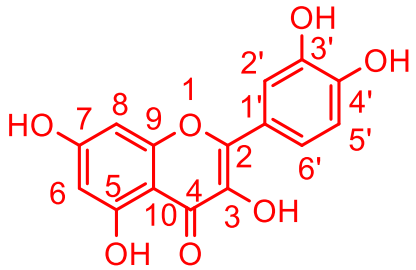




REZVERATROL

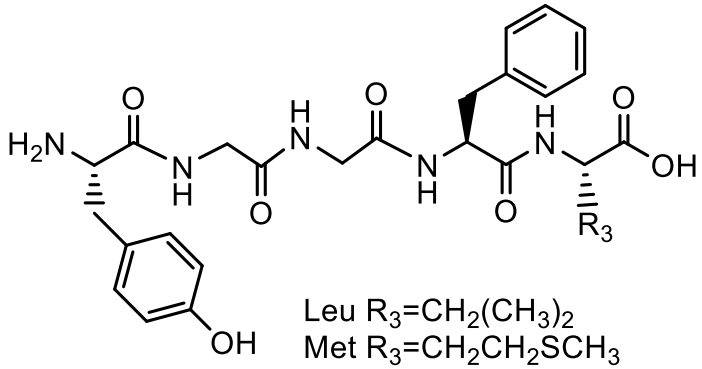
An apple a day keeps the doctor away, but if the doctor is cute, forget the fruit!

Dizajn peptidomimetika s antitumorskim i antioksidacijskim svojstvima

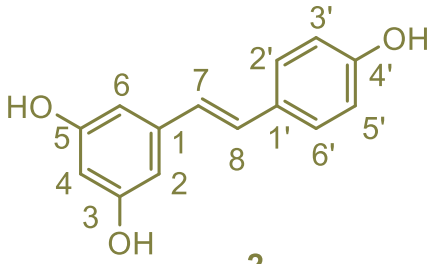


1

ENEKFALINI Leu/Met

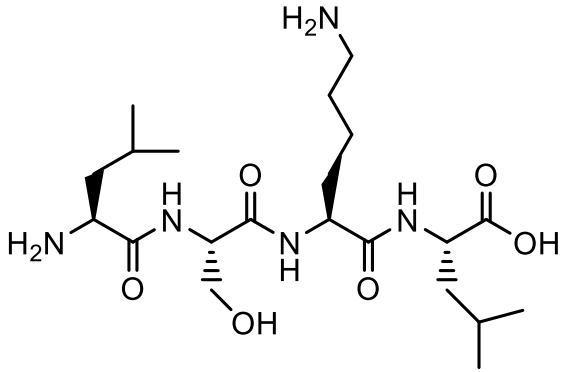


Leu $R_3=CH_2(CH_3)_2$
Met $R_3=CH_2CH_2SCH_3$



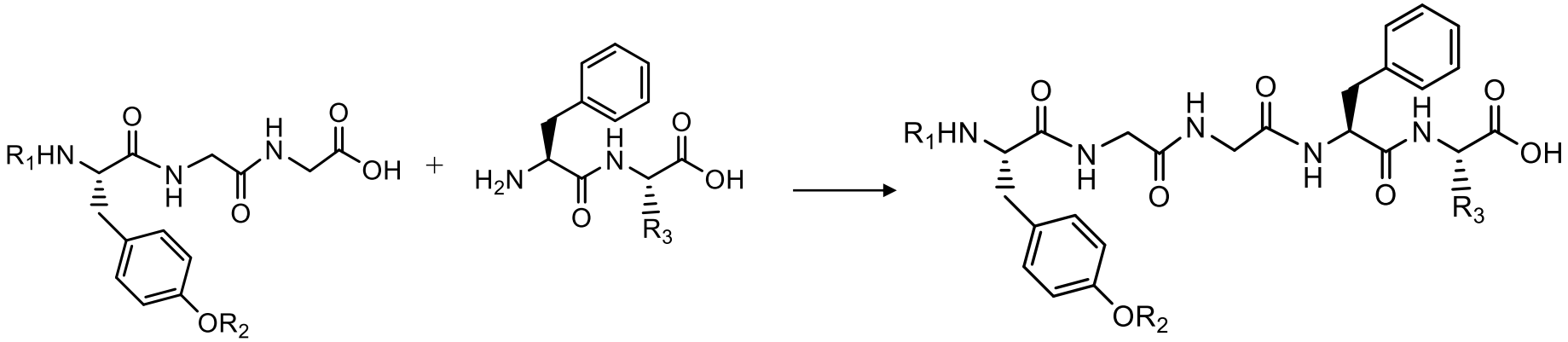
2

Leu-Ser-Lys-Leu (LSKL)

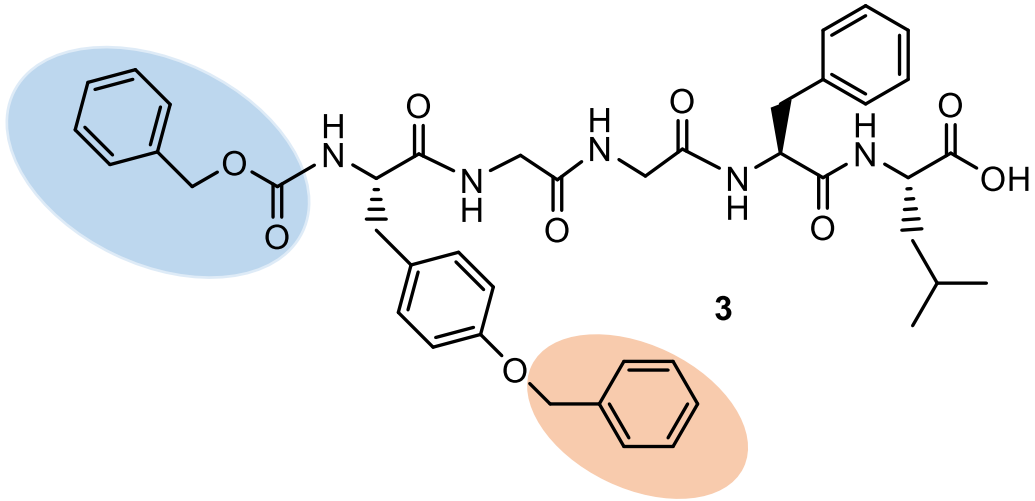


Dizajn peptidomimetika s antitumorskim i antioksidacijskim svojstvima

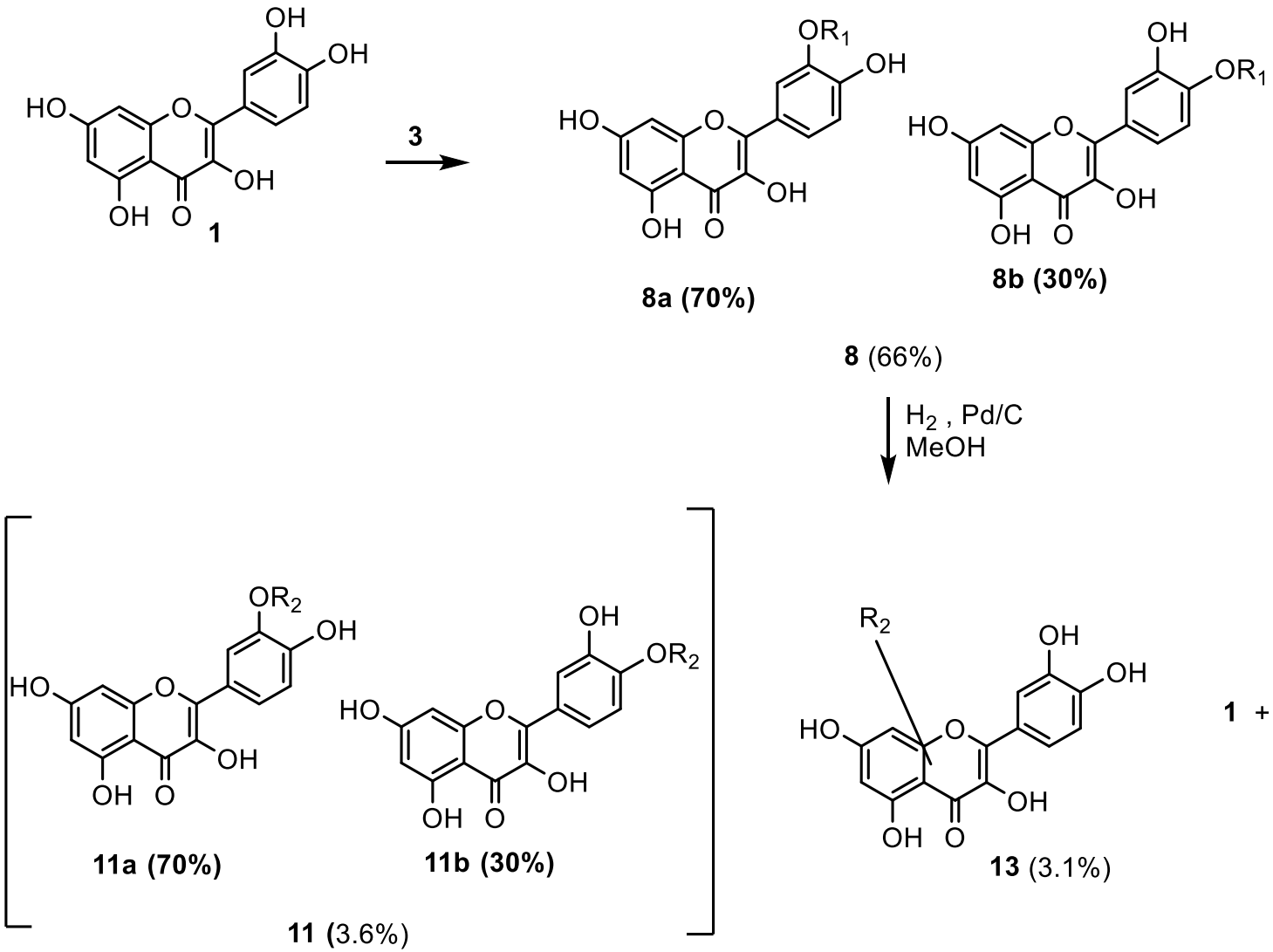
3 + 2 [X-Tyr(X)-Gly-Gly + Phe-Leu/Met)



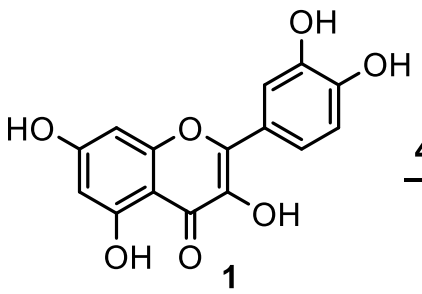
3 R_1 = CBz; R_2 = Bn; R_3 = $-CH_2-CH(CH_3)_2$



Dizajn peptidomimetika s antitumorskim i antioksidacijskim svojstvima

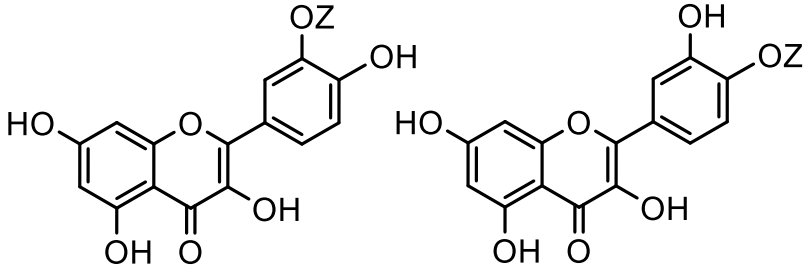


Dizajn peptidomimetika s antitumorskim i antioksidacijskim svojstvima



1

4, 5



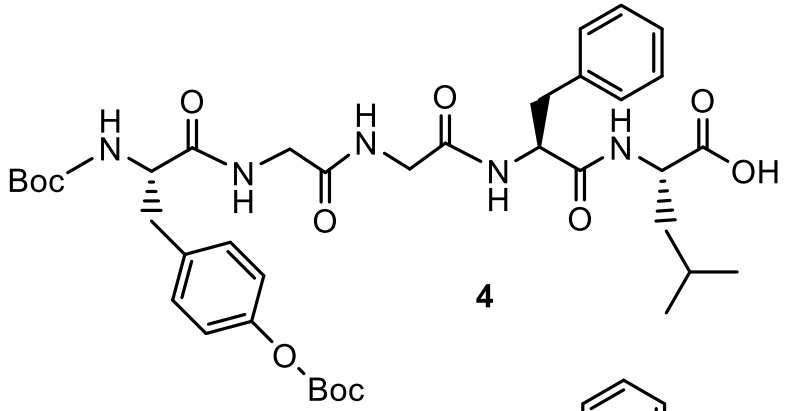
9a (70%)
10a (70%)

9b (30%)
10b (30%)

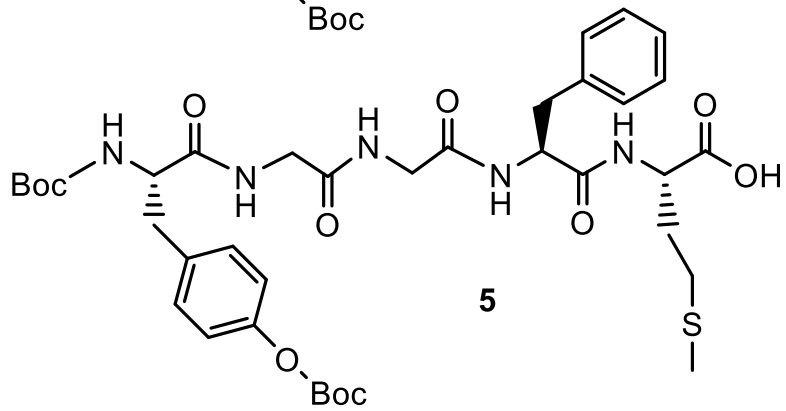
9 Z=Z_1 (43%)
10 Z=Z_2 (51%)

TFA:voda 9:1

11 Z=Z_3 (30%)
12 Z=Z_4 (25%)

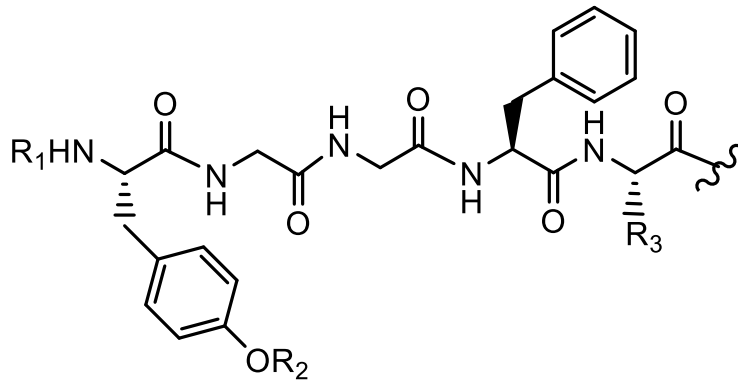


4



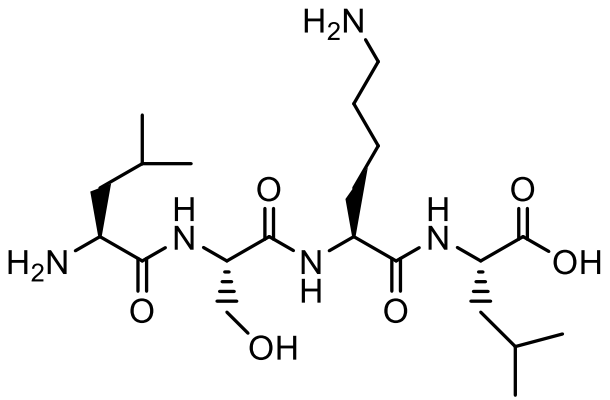
5

Z

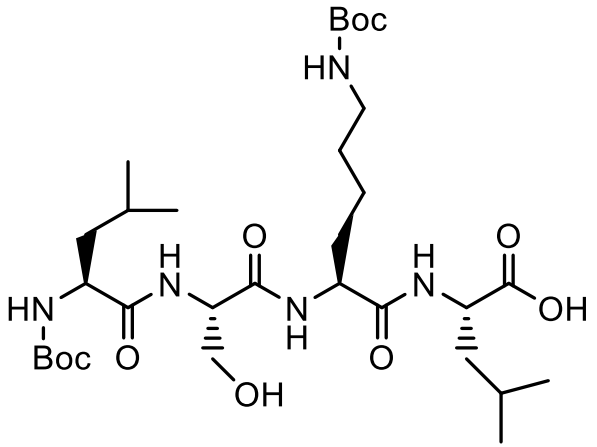


Z_1 R_1=R_2= Boc; R_3= -CH_2-$CH(CH_3)_2$
Z_2 R_1=R_2= Boc; R_3= -CH_2-CH_2-S-CH_3
Z_3 R_1=R_2=H; R_3= -CH_2-$CH(CH_3)_2$
Z_4 R_1=R_2=H; R_3= -CH_2-CH_2-S-CH_3

Dizajn peptidomimetika s antitumorskim i antioksidacijskim svojstvima

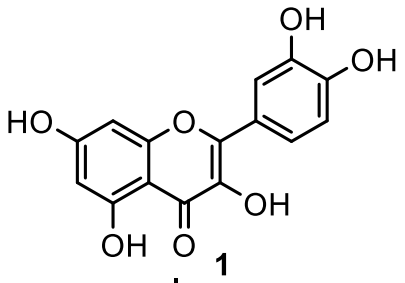


6



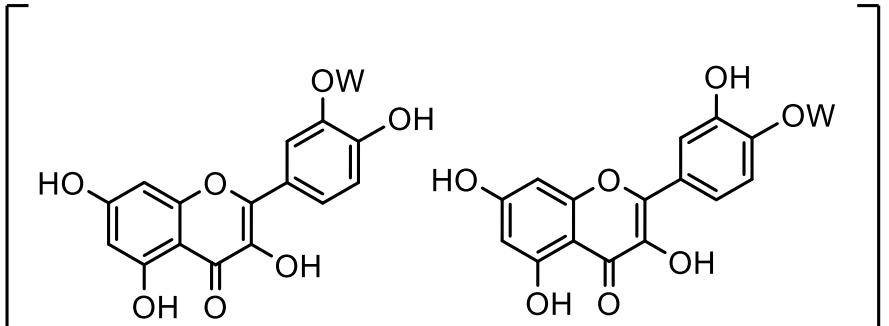
7 (80%)

Dizajn peptidomimetika s antitumorskim i antioksidacijskim svojstvima



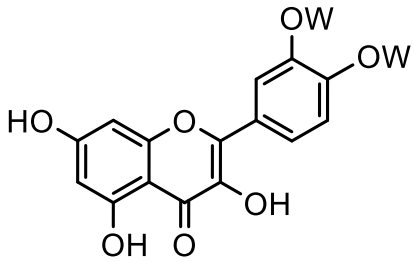
1

7

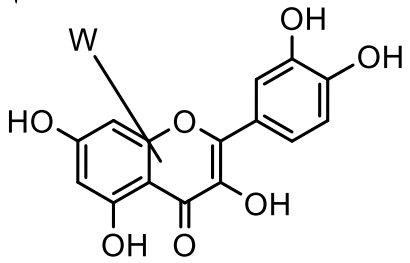


15a (70%) **15b (30%)**

15 W=W_1



16 W=W_1

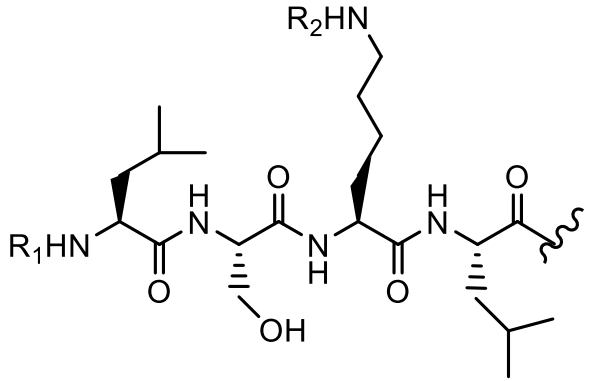


17a:17b (70:30)
17 W=W_2 (58%)

18 W=W_2 (tragovi)

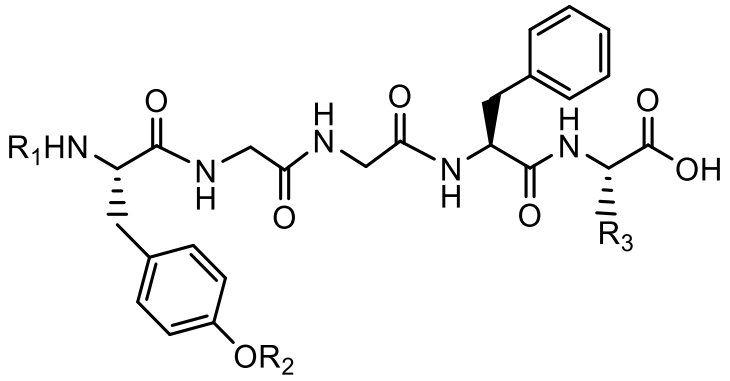
19 W=W_2 (5%)

W

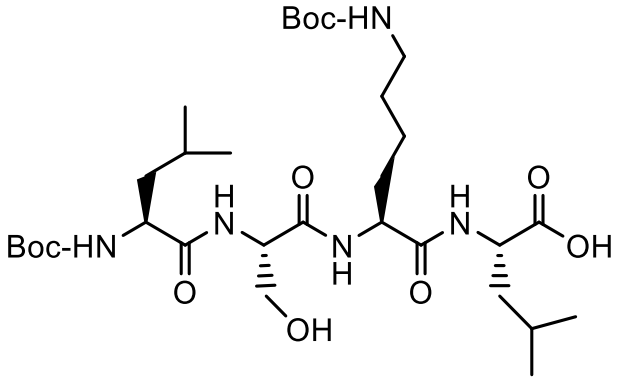


W_1 R_1 = R_2= Boc
W_2 R_1= R_2 = H

Dizajn peptidomimetika s antitumorskim i antioksidacijskim svojstvima



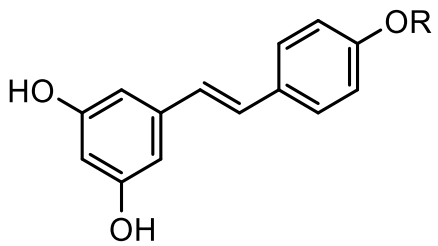
4 $R_1=R_2=Boc$; $R_3= -CH_2-CH(CH_3)_2$
5 $R_1=R_2=Boc$; $R_3= -CH_2-CH_2-S-CH_3$



7

4, **5**

a **2**



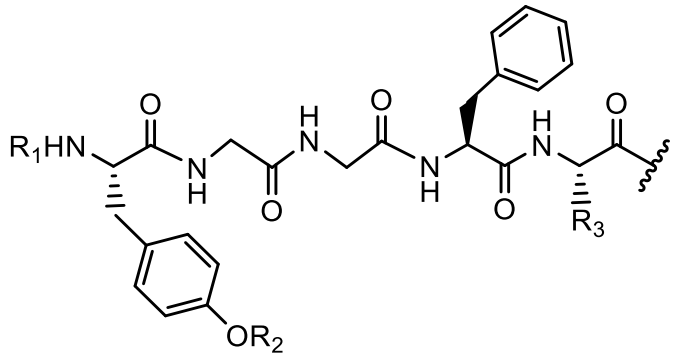
b
20, 21
22 (15%), **23** (30%)

7

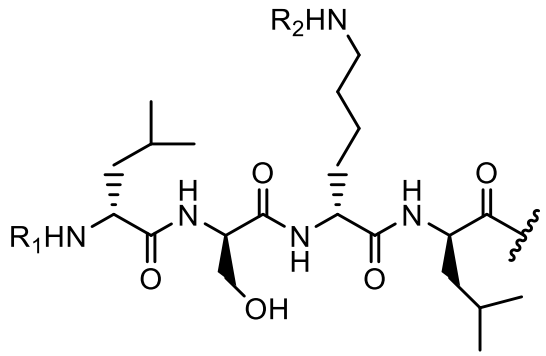
a **2**

b
24
25 (27%)

R

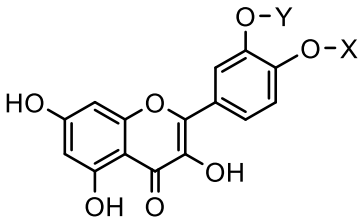


b
20 $R_1=Boc$, $R_2=Boc$, $R_3=-CH_2-CH(CH_3)_2$
b
21 $R_1=Boc$, $R_2=Boc$, $R_3=-CH_2-CH_2-S-CH_3$
22 $R_1=H$, $R_2=H$, $R_3=-CH_2-CH(CH_3)_2$
23 $R_1=H$, $R_2=H$, $R_3=-CH_2-CH_2-S-CH_3$



b
24 $R_1=Boc$, $R_2=Boc$
25 $R_1=H$, $R_2=H$

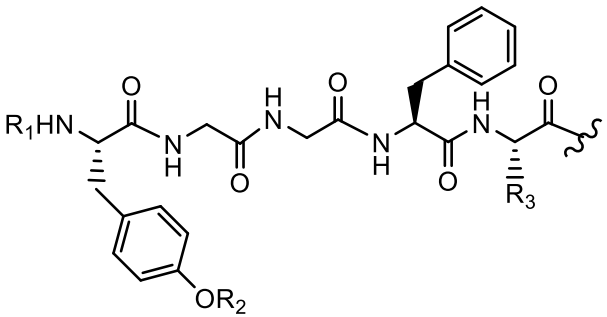
Dizajn peptidomimetika s antitumorskim i antioksidacijskim svojstvima



11 X=Z_4; Y=H/ X=H; Y=Z_4
12 X=Z_5; Y=H/ X=H; Y=Z_5

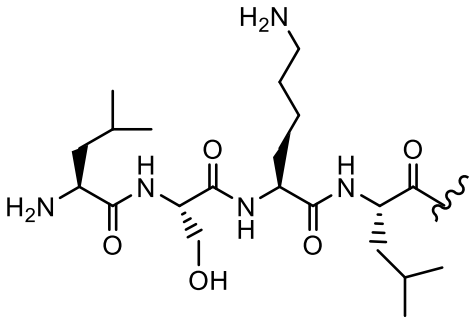
17 X=W; Y=H/ X=H; Y=W

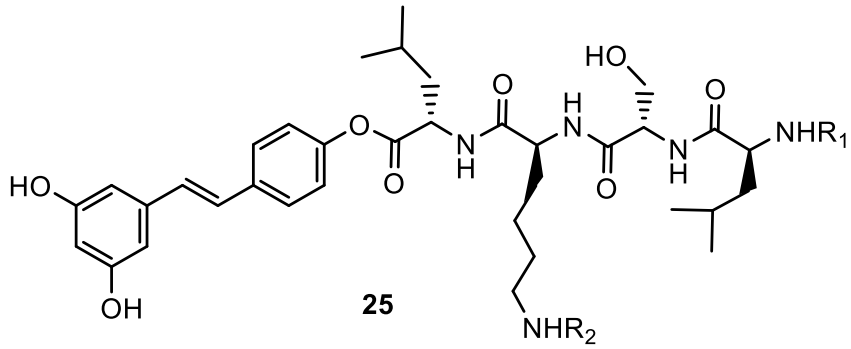
Z



Z_4 R_1=R_2=H; R_3= -CH_2-$CH(CH_3)_2$
Z_5 R_1=R_2=H; R_3= -CH_2-CH_2-S-CH_3

W



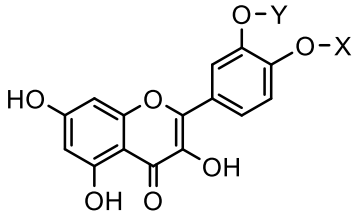


25

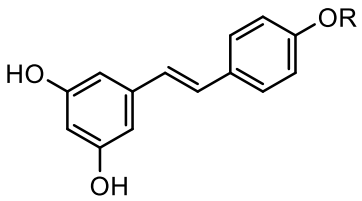
GI_{50}[a] (μM)				
Spoj	**Stanične linije**			
	HCT 116	**SW 620**	**MCF-7**	**H 460**
11	>100	>100	>100	/
12	≥100	>100	>100	/
17	44±2	/	52±20	23±1
25	78±23	/	58±5	41±2

[a] GI_{50}; koncentracija koja uzrokuje 50% inhibiciju

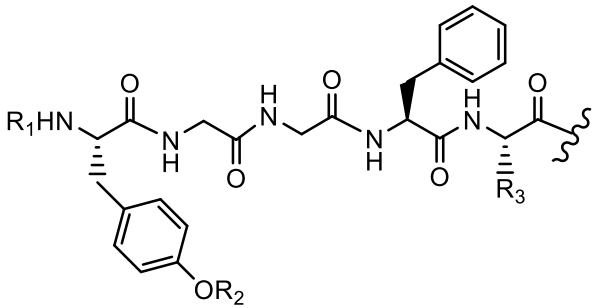
Dizajn peptidomimetika s antitumorskim i antioksidacijskim svojstvima



11 X=Z_4; Y=H/ X=H; Y=Z_4
12 X=Z_5; Y=H/ X=H; Y=Z_5

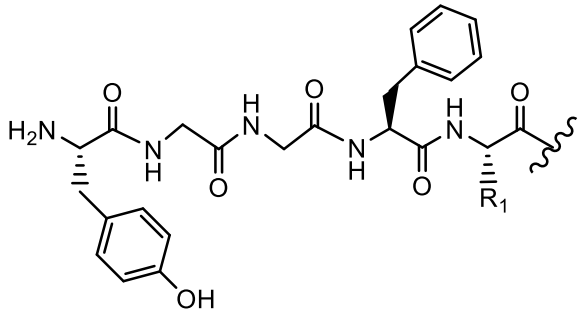


Z



Z_4 R_1=R_2=H; R_3= -CH_2-$CH(CH_3)_2$
Z_5 R_1=R_2=H; R_3= -CH_2-CH_2-S-CH_3

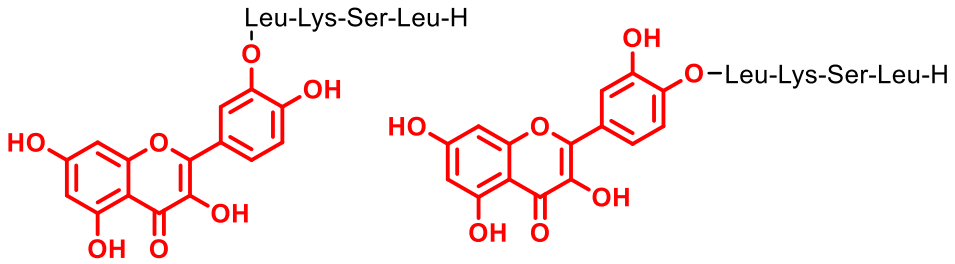
R



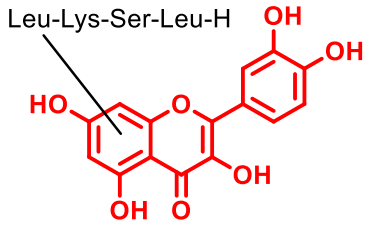
22 R_1=-CH_2-$CH(CH_3)_2$
23 R_1=-CH_2-CH_2-S-CH_3

Spoj	**Trolox eq**	**Omjer** (% aktivnosti Troloxa)
11	0.512971	0.246712 (-75%)
12	3.64279	1.751992 (+75%)
1	2.079227	1
22	1.234359	0.698827 (-30%)
23	1.126212	0.6376 (-36%)
2	1.766331	1

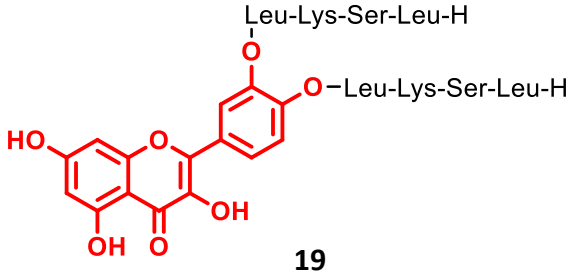
Dizajn peptidomimetika s antitumorskim i antioksidacijskim svojstvima



17

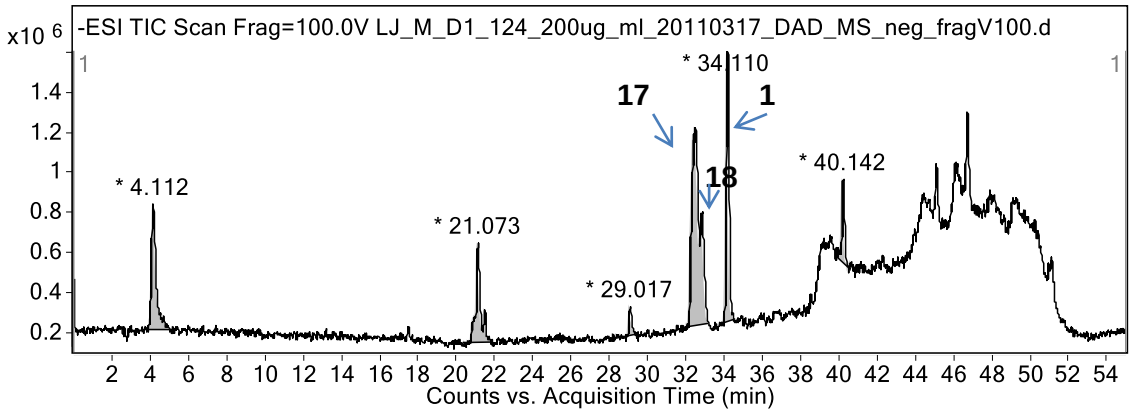


18

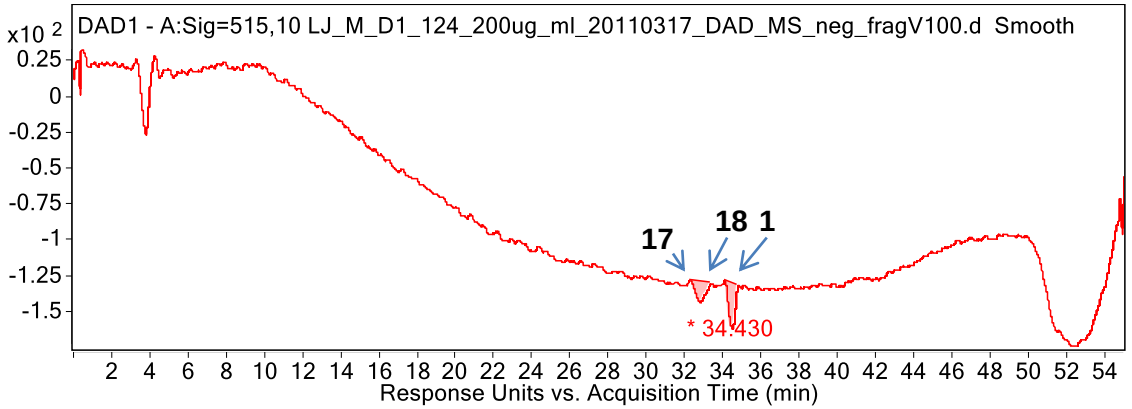


19

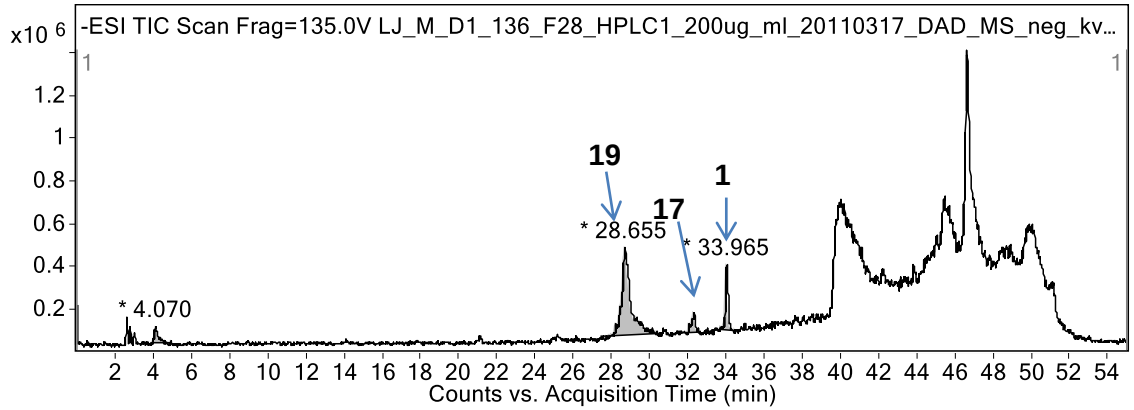
a)



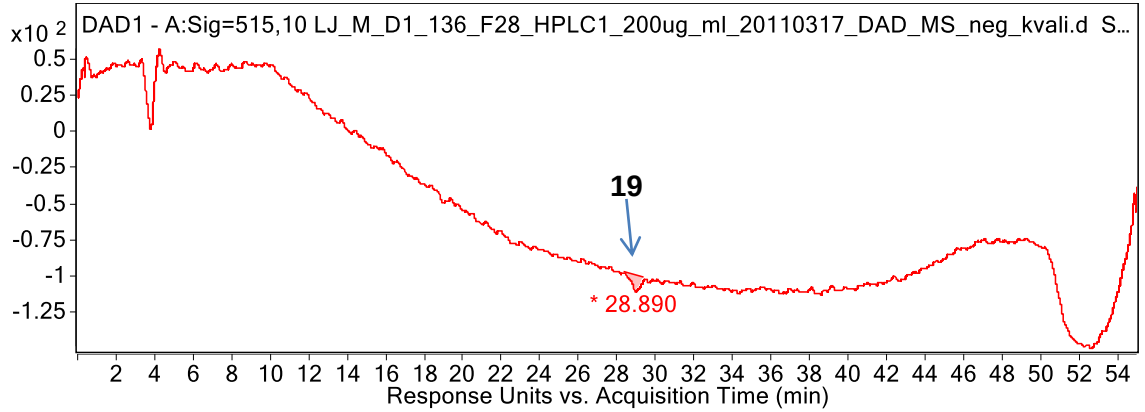
b)



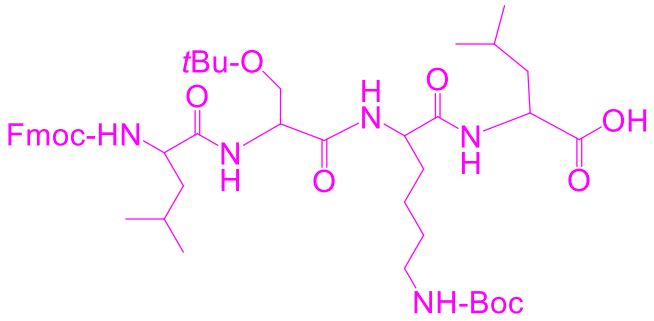
c)

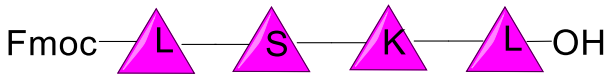


d)

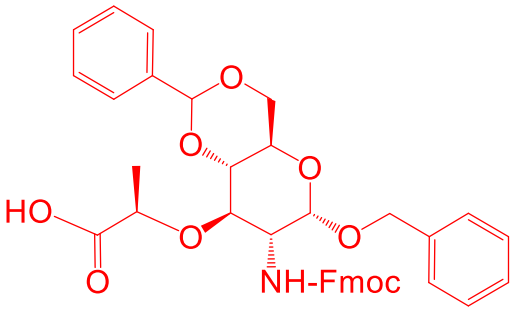


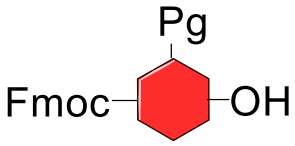
Dizajn antimikrobnih peptida



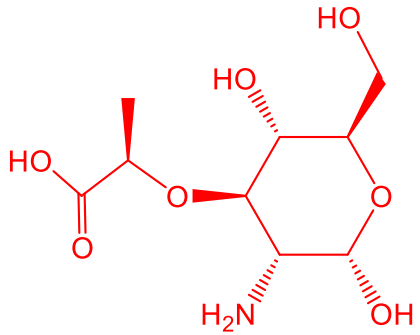


- Biološki aktivan tetrapetid, inhibitor trombospondina 1

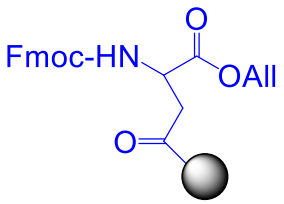


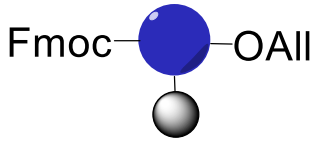


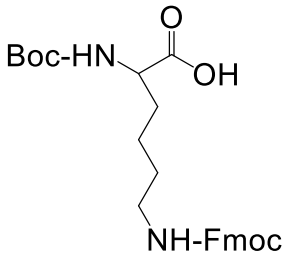
- Muraminska kiselina (Mur) prirodna šećerna aminokiselina (SAA)
- NAM-NAG (N-acetilmuraminska kiselina-N-acetilglukozamin) osnova šećernog dijela peptidoglikana (PG) prisutnog u staničnim stijenkama bakterija
- Struktura Mur omogućuje njenu ugradnju u peptide kao dipeptidnog izoster

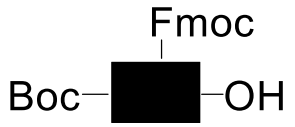


Mur

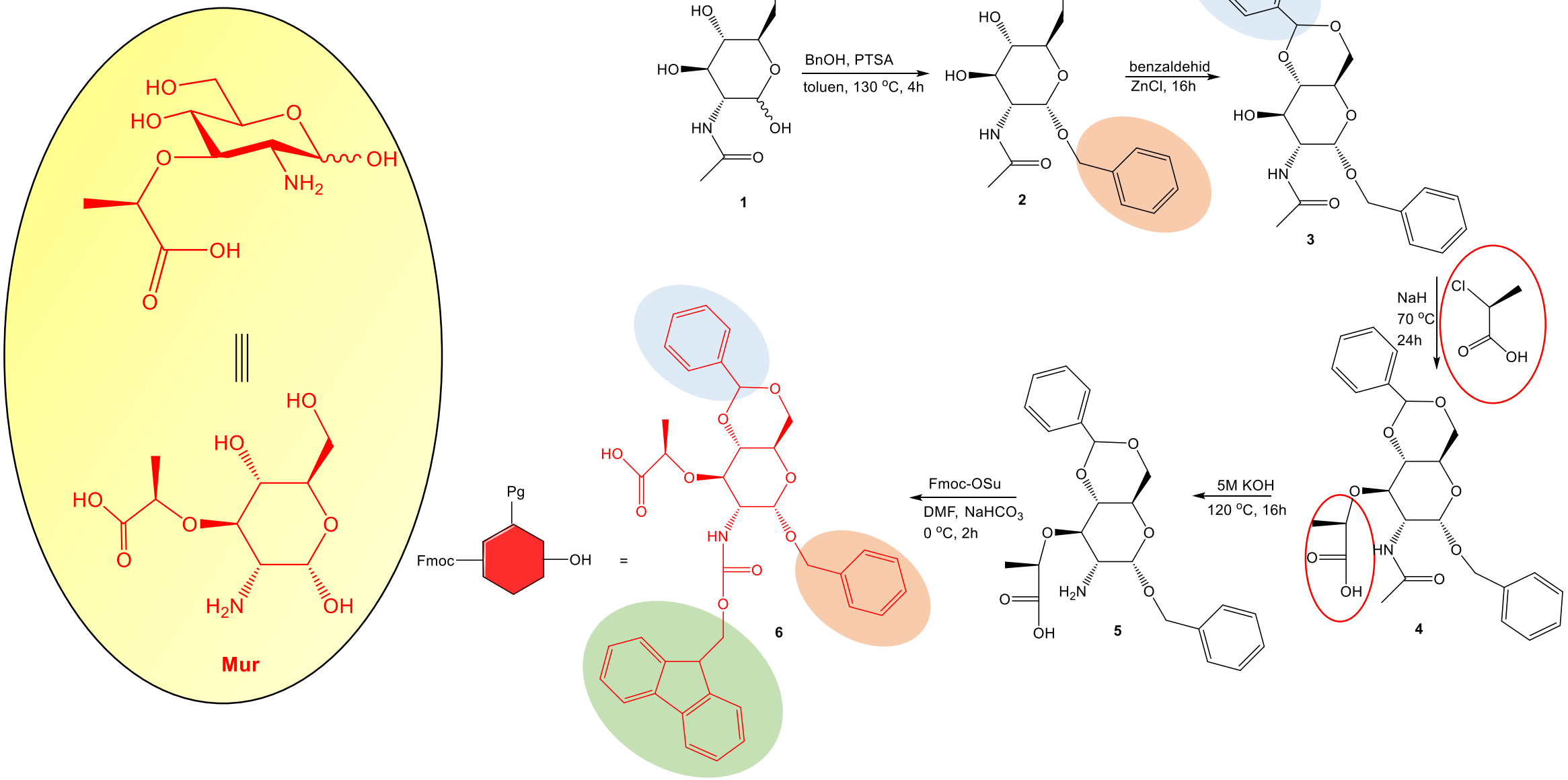








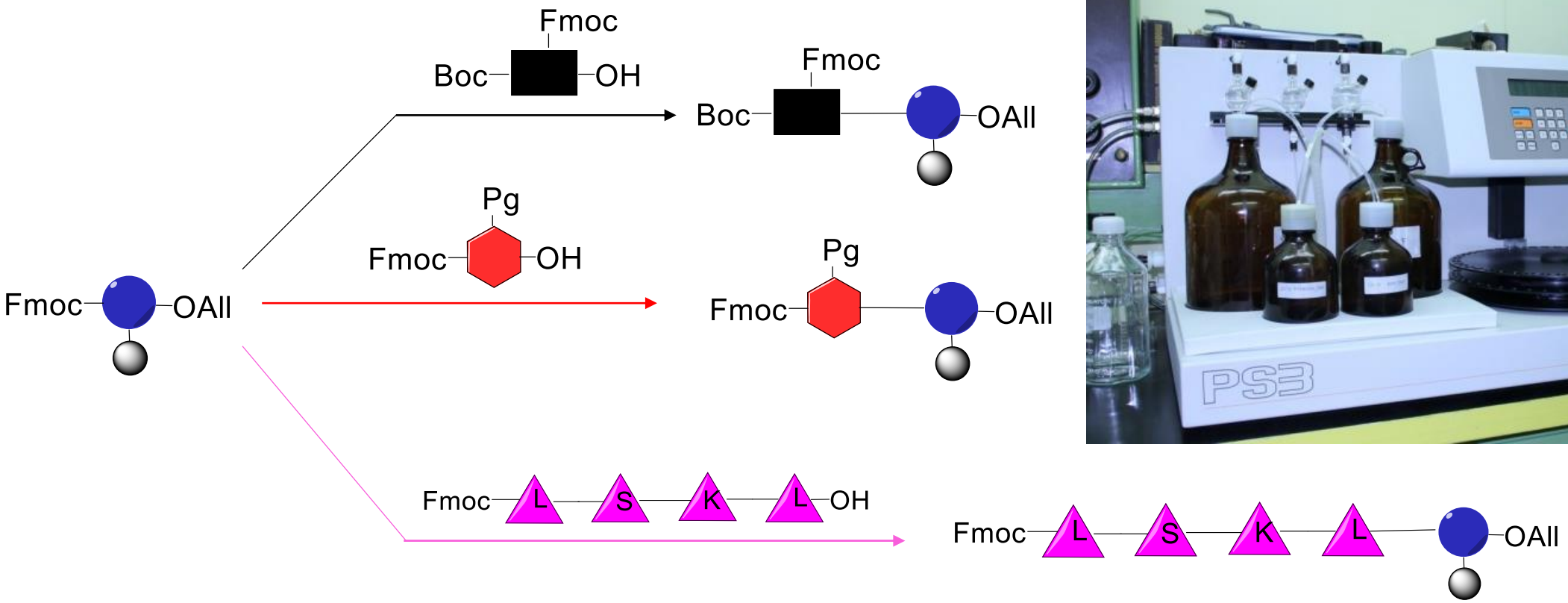
Dizajn antimikrobnih peptida

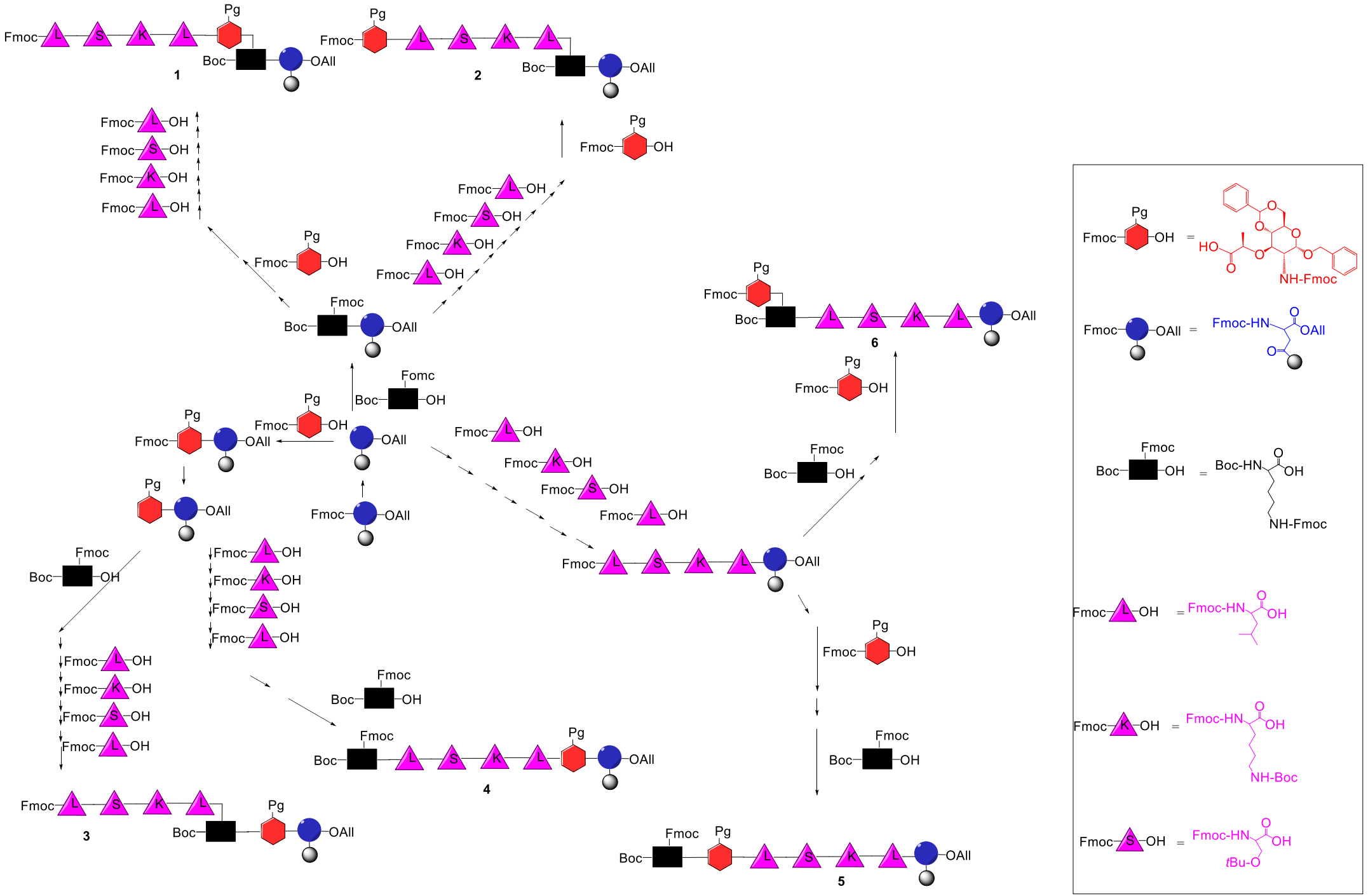


K. Vlahoviček-Kahlina, A. Jakas, *Croat. Chem. Acta* **88** (2015) 151-157

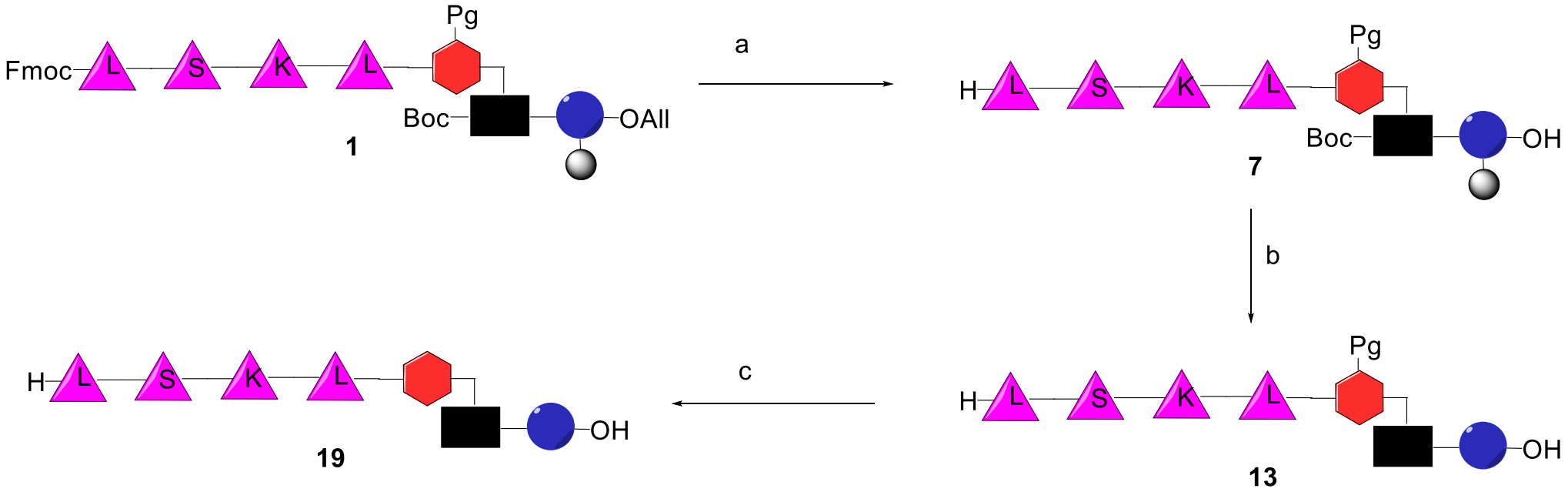
Dizajn antimikrobnih peptida

Metoda sinteze peptidne biblioteke

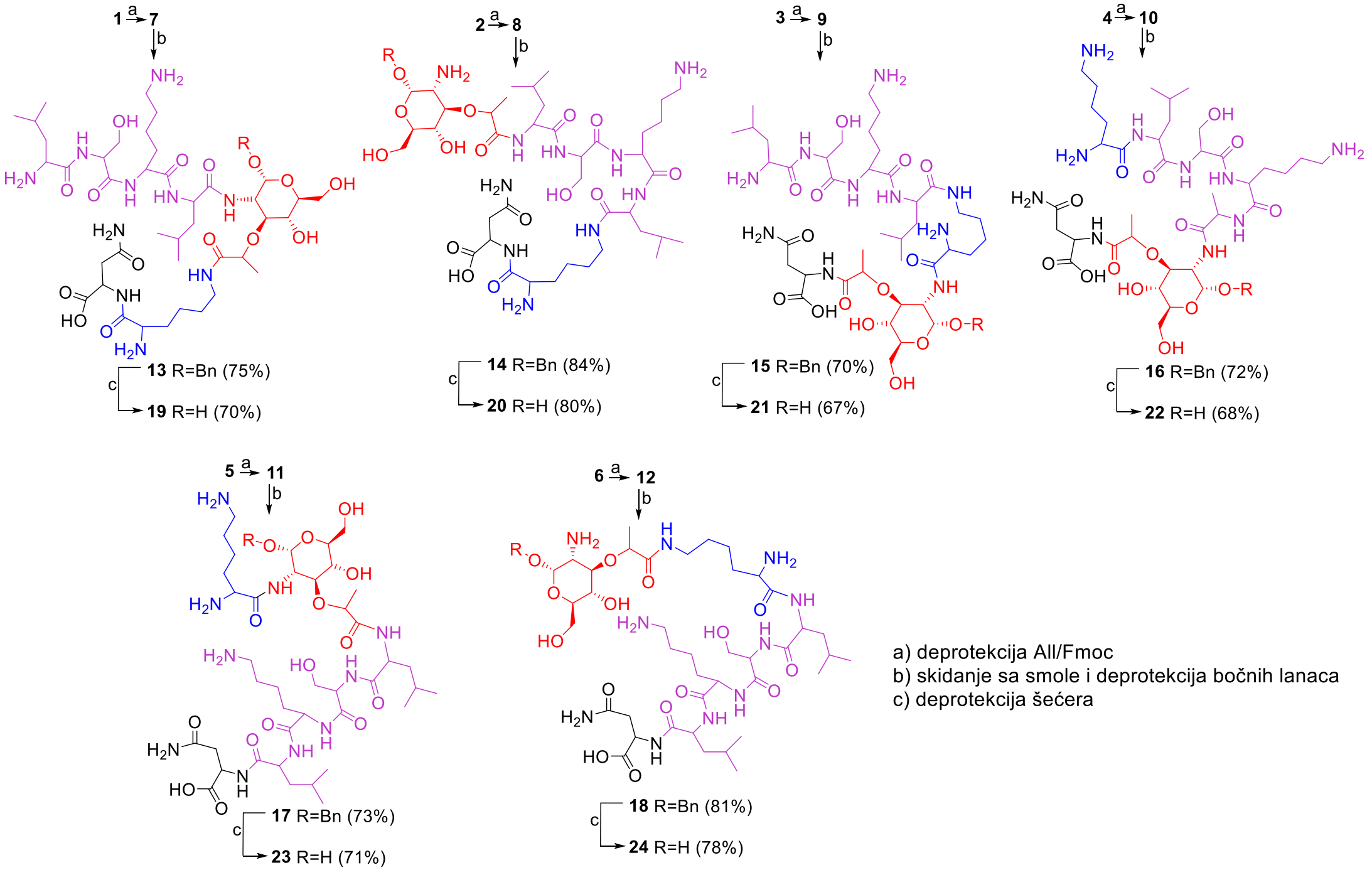



Fmoc L S K L Pg Boc OAll
1
2
3
4
5
6
Fomc
Fmoc L OH
Fmoc S OH
Fmoc K OH
Fmoc L OH
Pg Fmoc OH
Fmoc Boc OH
Fmoc Boc OAll
Pg Fmoc OAll
Pg OAll
Fmoc OAll
Fmoc Pg Boc OAll
Fmoc Boc L S K L OAll
Pg Fmoc = HO O O O O O NH-Fmoc
Fmoc OAll = Fmoc-HN OAll O
Fmoc Boc OH = Boc-HN OH NH-Fmoc
Fmoc L OH = Fmoc-HN OH
Fmoc K OH = Fmoc-HN OH NH-Boc
Fmoc S OH = Fmoc-HN OH tBu-O

Dizajn antimikrobnih peptida

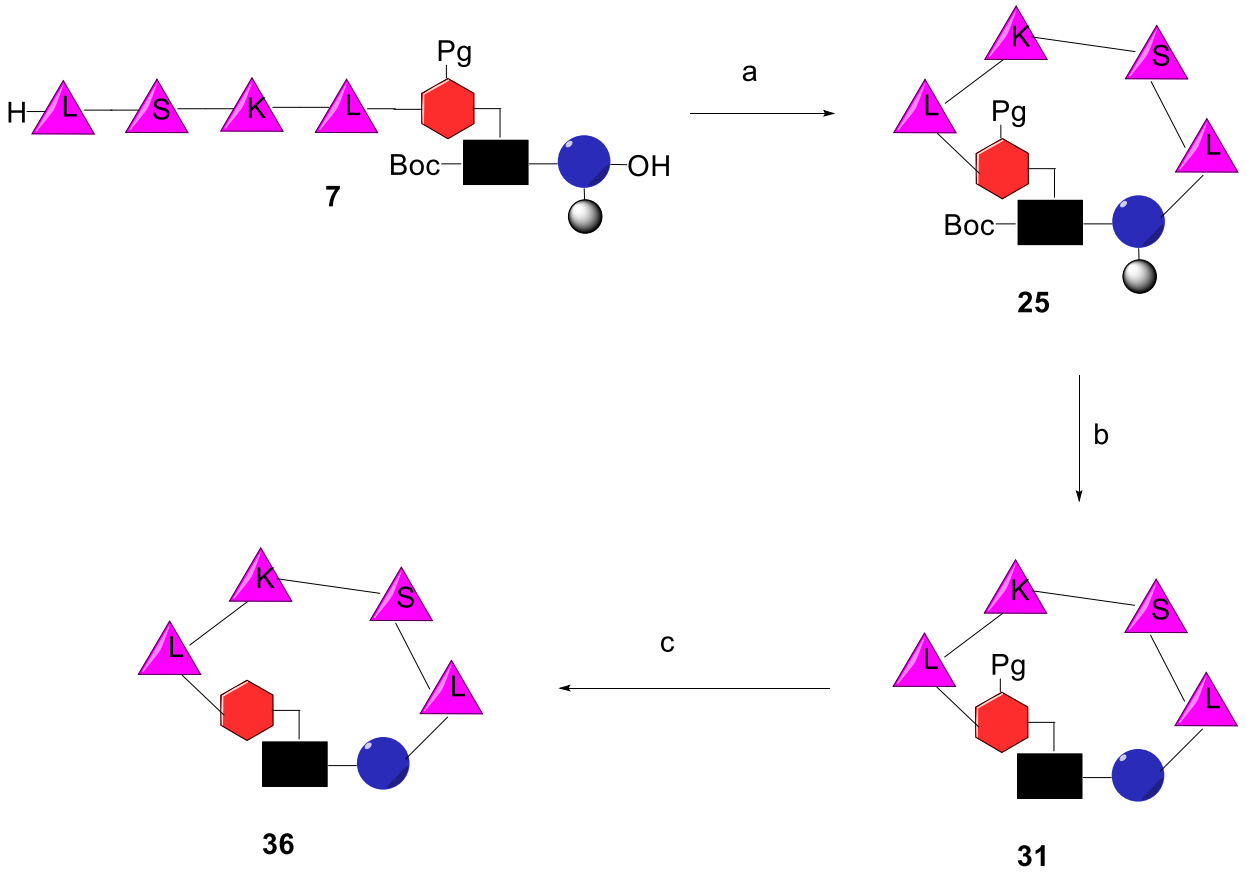


a) deprotekcija All
b) skidanje sa smole i deprotekcija bočnih lanaca
c) deprotekcija šećera



a) deprotekcija All/Fmoc
b) skidanje sa smole i deprotekcija bočnih lanaca
c) deprotekcija šećera

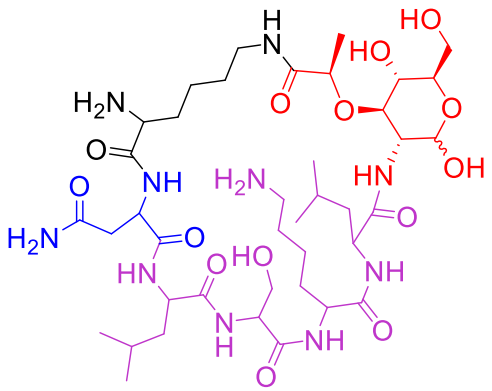
Dizajn antimikrobnih peptida



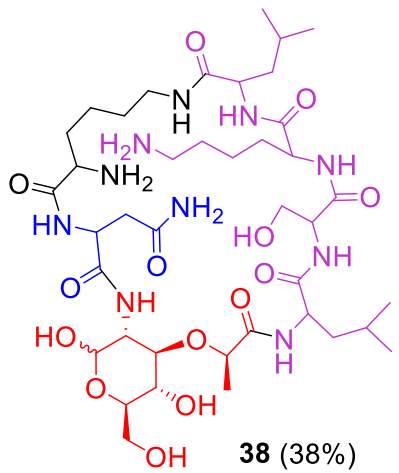
a) ciklizacija
b) skidanje sa smole i deprotekcija bočnih lanaca
c) deprotekcija šećera

Dizajn antimikrobnih peptida

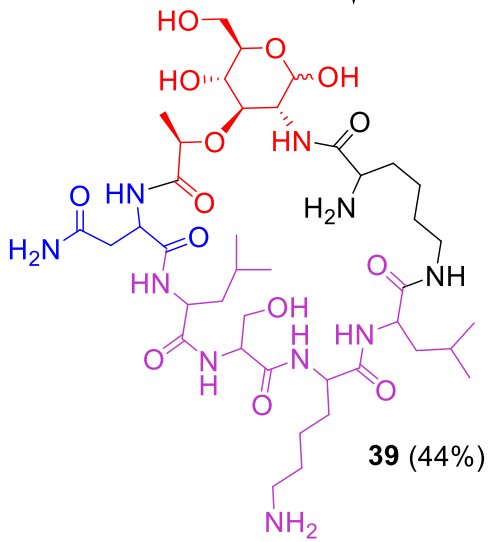
7 —a→ **25** —b→ **31** (37%) —c→ **37** (36%)



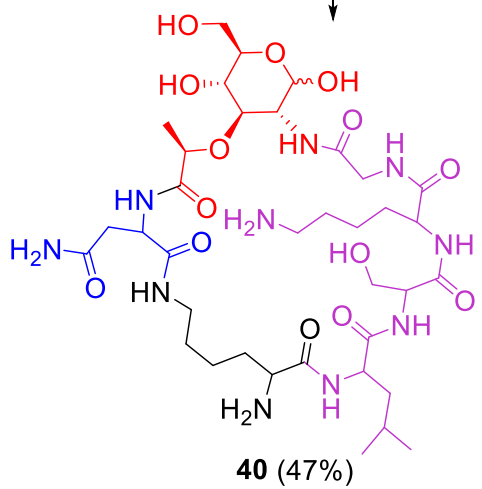
8 —a→ **26** —b→ **32** (44%) —c→ **38** (38%)



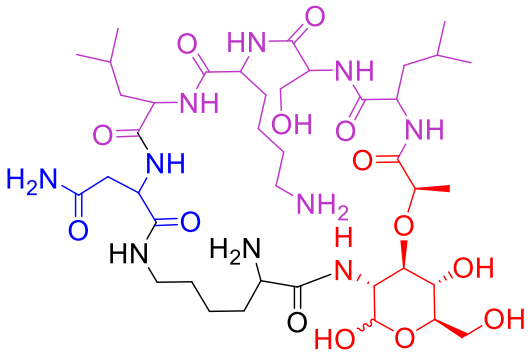
9 —a→ **27** —b→ **33** (50%) —c→ **39** (44%)



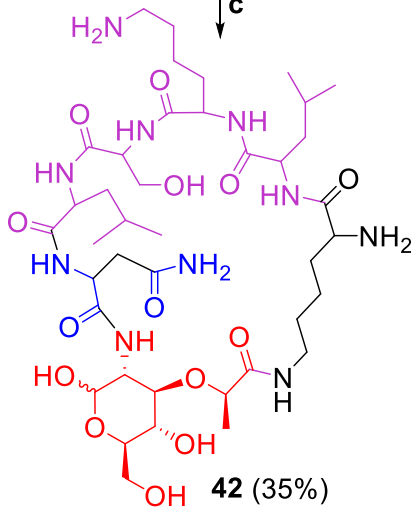
10 —a→ **28** —b→ **34** (54%) —c→ **40** (47%)



11 —a→ **29** —b→ **35** (51%) —c→ **41** (50%)



12 —a→ **30** —b→ **36** (42%) —c→ **42** (35%)

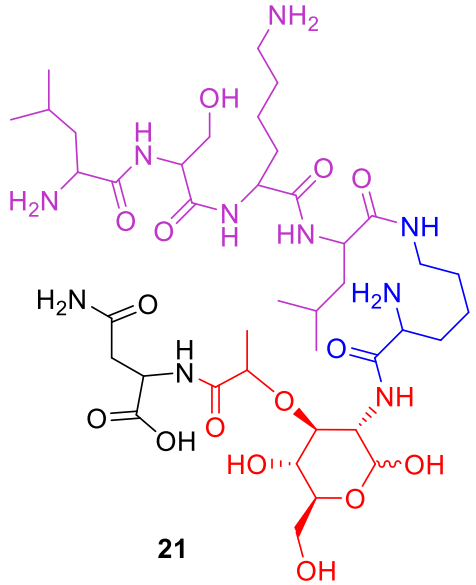


a) ciklizacija
b) skidanje sa smole i deprotekcija bočnih lanaca
c) deprotekcija šećera

Dizajn antimikrobnih peptida

Rezultati mjerenja antimikrobne aktivnosti spojeva mikrodilucijskom metodom

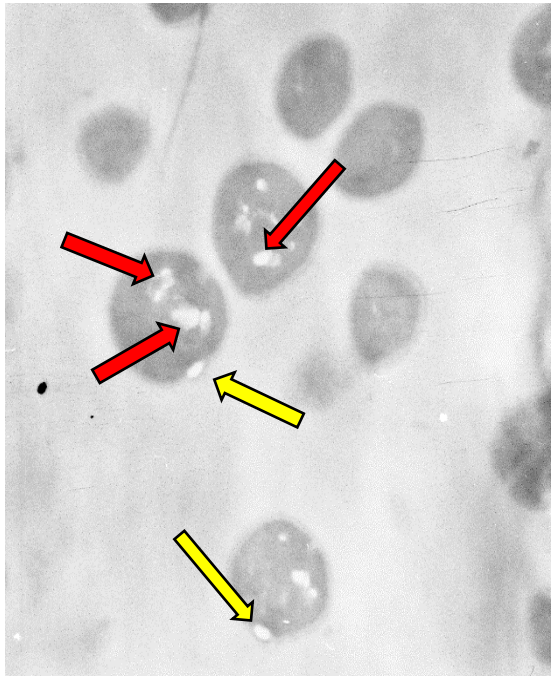
	MIC (μg/mL, N=3[b])				
Glikopeptid	***Staphylococcus aureus* ATCC 6538**	***Escherichia coli* ATCC 10536**	***Candida albicans* ATCC 10231**	***Aspergillus brasiliensis* ATCC 16404**	***Microsporum gypseum* FBF 10570**
LSKL[c]	>800	>800	>800	>800	>800
13-20	>800	>800	>800	>800	>800
21	**533.33±231**	>800	>800	>800	>800
22-24	>800	>800	>800	>800	>800
25-42	>800	>800	>800	>800	>800



21

GI_{50} vrijednosti za linearne i ciklilčke peptide

GI_{50}* (μM)					
Linerni glikopeptid	Cell lines		Ciklički glikopeptid	Cell lines	
	HCT 116	H 460		HCT 116	H 460
13-18	> 100	> 100	**31-36**	> 100	> 100
19-24	> 100	> 100	**37-42**	> 100	> 100



TEM prikaz djelovanja spoja **21** na stanice *Staphylococcus aureus*

Zahvaljujem se:

Svim članovima Laboratorija za biomimetičku kemiju i
Laboratorija za kemiju ugljikohidrata, peptida i glikopeptida

Centru za NMR IRB
Laboratoriju za eksperimentalnu terapiju, Dr. sc. Marijeti Kralj
Laboratoriju za molekularnu biljnu biologiju i biotehnologiju, Luciji Horvat

Dr. sc. Ivanu Koselcu i Vanji Ljoljić-Bilić, Farmaceutsko-biokemijski fakultet, Sveučilište u Zagrebu

Hvala na pažnji!