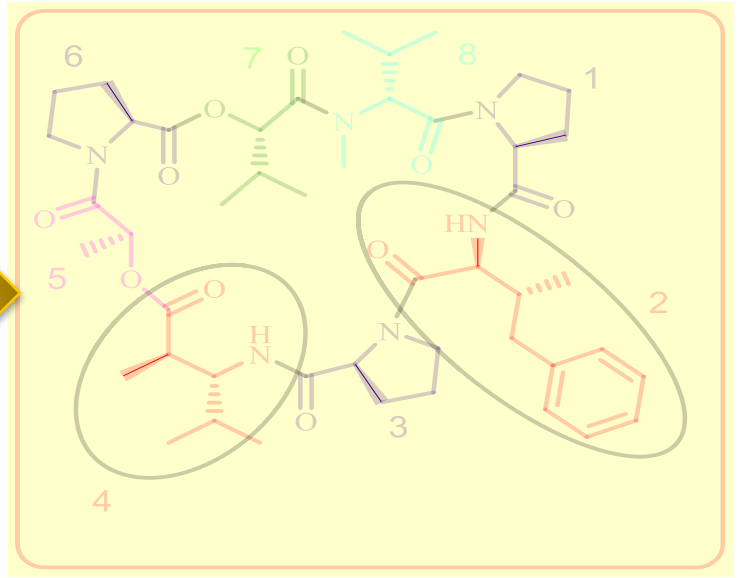
SYNTHESIS OF NOVEL (±)-*CIS-EXO*-NORBORNANE AMINO ACID CONTAINING CYCLIC HEXAPEPTIDE: ANALOGUE OF DOLASTATIN 16



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sciforum-006262

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INTRODUCTION

➢**Cancer** is a major public health burden in both developed and developing countries. Plant derived agents are being used for the treatment of cancer.

➢Several anticancer agents including **taxol, vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, and etoposide derived from epipodophyllotoxin are in clinical use all over the world.**

➢In recent past, the improvement in the technology of deep-sea collection and aquaculture added to the growing recognition of the tremendous biodiversity present in the marine world, and has contributed to the growing interest of exploring the oceans as a potential source of new anticancer candidates.

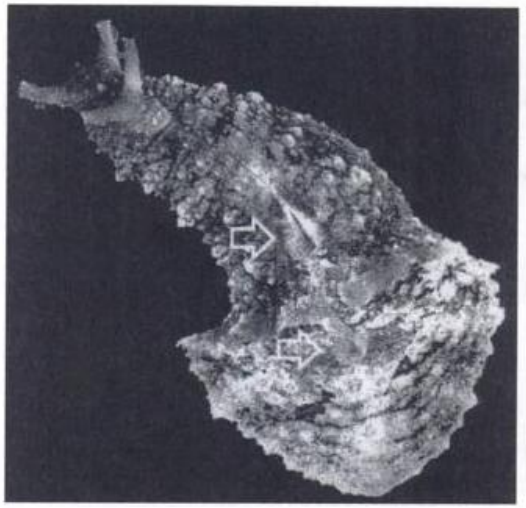
➢This is reflected in the number of marine-derived compounds undergoing preclinical and early clinical development.

➢The dolastatins are a class of peptides that were originally derived from a mollusk from the Indian Ocean, the sea hare ***Dolabella auricularia*.**

➢ These peptides have cytotoxic activity and of the various compounds of this class, Dolastatin 10 and Dolastatin 15, have received the greatest clinical interest.

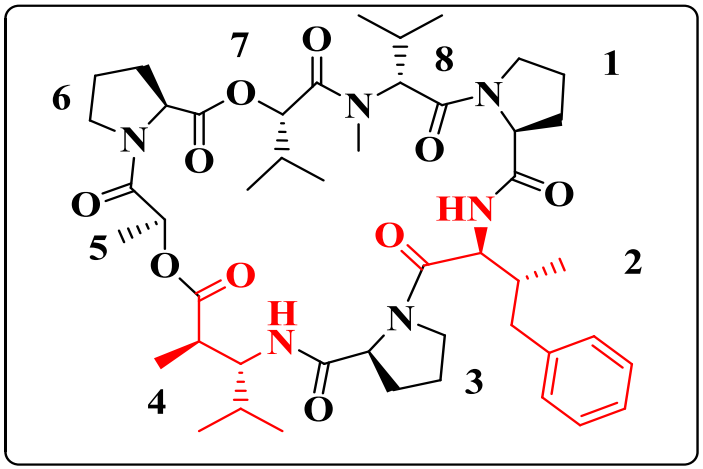
➢ Dolastatin 10 has entered Phase I and Phase II trials, after showing significant anti-tumor activity in preclinical models.

➢ Its mechanism of action involves **inhibition of microtubule assembly**, which causes cell-cycle arrest in metaphase



Dolabella auricularia - a marine shell-less mollusc

LITERATURE REVIEW



DOLASTATIN 16

1,3,6 – Proline

2 - Dolaphenvaline

4 - Dolamethyleuine

} Unusual Aminoacids

5 - (S)- Lactic acid

7 - 2-hydroxy-3-methyl butanoic acid

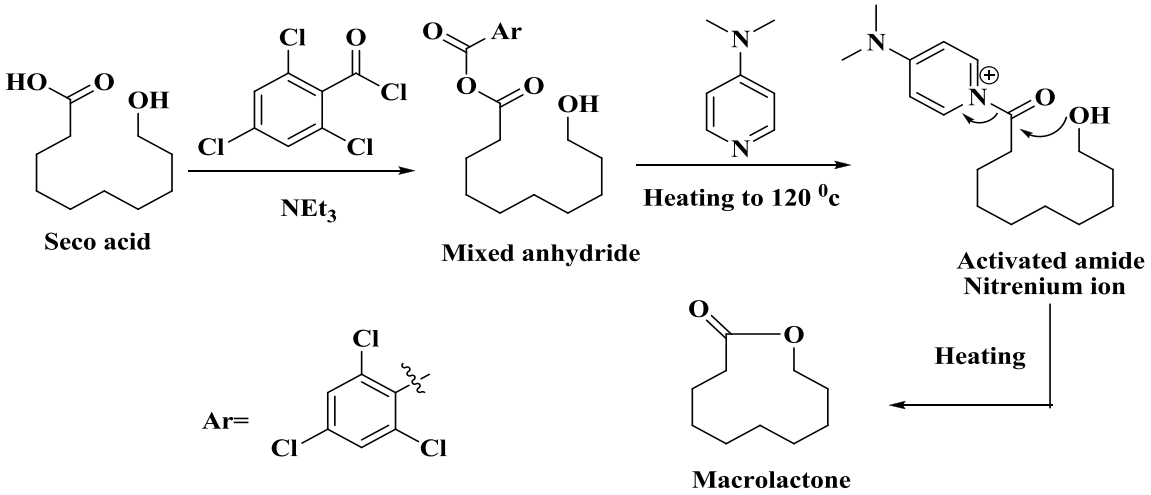
8 - (3-methyl-(2-methyl amino)butanoic acid

BASED ON LITERATURE :

- RENAL CARCINOMA: 1EAX, 2GV6, 2GV7
- GLIOMA: 3BZ3
- LUNG CARCINOMA: 3E5A, 3HA6

YAMAGUCHI MACROLACTONIZATION

In 1979, Yamaguchi *et al.* , first reported a process for mild esterification method using carboxylic 2,4,6-trichlorobenzoic anhydrides in the presence 4-N,N[1]dimethyl aminopyridine for the preparation of large ring lactones from the open chain hydroxyl acids.



In this, the lactonization is carried out by activating the carboxylic group by an acid chloride (2,4,6, trichloro benzoyl chloride) to give its corresponding mixed anhydride, this mixed anhydride is further activated to amide nitrenium ion, which exchange with alcohol moiety to give lactone.

OBJECTIVE OF THE WORK

The dolastatins-16 is having high potency as anti cancer agent and presently it is in phase III clinical trials.

1.Extensive *In silico* SAR study of Dolastatin-16 by using MOE (Molecular Operating Environment) and Glide Docking software in Maestro 9.3

2.Design of more potent cyclic peptide analogues based on Dolastatin-16 structure by using molecular docking study.

3.Total synthesis of designed analogues

4.Biological evaluation of synthesized analogues against cancer cells.

- WORK DONE

✓MOLECULAR MODELLING STUDIES

✓SYNTHESIS

➢These proteins were validated by docking with various dolastatin series like dolastatin 10, dolastatin 11, dolastatin 12, dolastatin 13, dolastatin 14, dolastatin 15 and dolastatin16.

➢**Target preparation**: Crystal structure of the target proteins from the PDB Site (PDB Codes: 1EAX, 2GV6, 2GV7, 3BZ3, 3E5A, 3HA6). The water molecules around 5Å were retained. The protein was protonated and the incomplete residues were mutated with the amino acid templates inbuilt in the software.

➢**Preparation of ligands**: 2D molecule drawer

Energy minimised and Ligprep was performed

➢**Docking:** Receptor grid generation where ligands with length of 20 Å were docked with other defaults settings. Then in ligand docking XP (extra precision) was used.

Molecular Docking Studies of Various Dolastatins:

Results obtained by docking series of dolastatins against proteins using glide 9.3

Proteins → / Dolastatins ↓	1EAX		2GV6		2GV7		3BZ3		3E5A		3HA6	
	G-score	HB score	G-score	HB score	G-score	HB score	G-score	HB score	G-score	HB score	G-score	HB score
Dolastatin 10	-5.20	-1.02	-1.0	-0.04	-4.68	-0.02	**-5.51**	**-2.13**	-2.05	-0.02	-4.21	-0.96
Dolastatin 11	-3.96	-0.94	-4.44	-1.33	-5.03	-0.94	-2.24	-0.87	-3.01	-1.44	-2.24	-0.55
Dolastatin 13	**-9.01**	**-2.82**	-3.85	-1.64	**-7.97**	**-2.68**	-2.14	-3.01	-2.11	-0.14	-6.47	-1.43
Dolastatin 14	-3.34	-1.25	-5.13	-1.33	-2.17	0	-1.41	-0.03	-1.73	0.00	-4.19	-0.93
Dolastatin 15	-3.08	0.00	-1.89	-0.7	-3.86	-0.14	-3.36	-2.45	-2.71	-0.61	**-9.1**	**-0.95**
Dolastatin 16	-3.53	-1.16	**-6.59**	**-1.28**	**-5.12**	**-0.6**	**-4.11**	**-0.70**	**-5.71**	**-0.94**	**-5.34**	**-1.12**

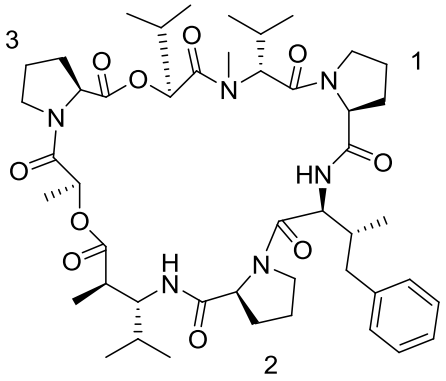
•Each moiety in Dolastatin 16 was **removed** and **cyclized** .

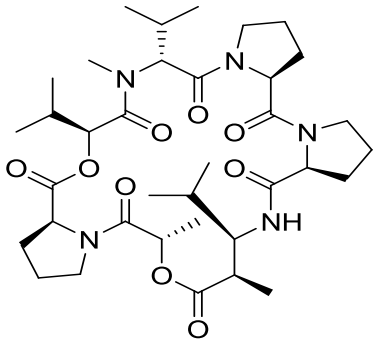
✓Structures were drawn in 2D sketcher

✓ Macromodel energy minimization

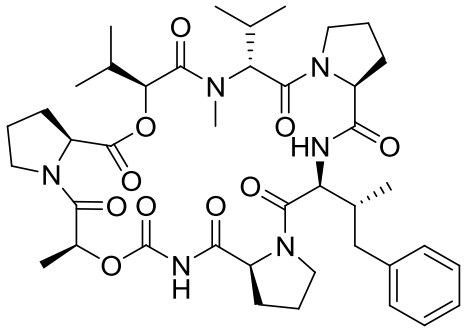
✓ Lig prep was performed

✓These conformers docked into these 6 proteins.

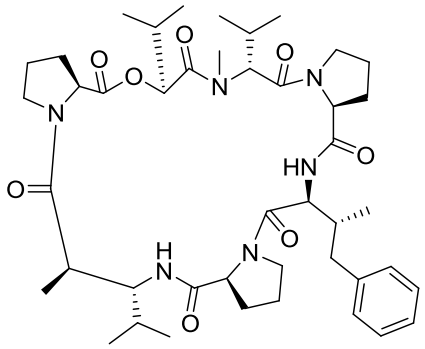




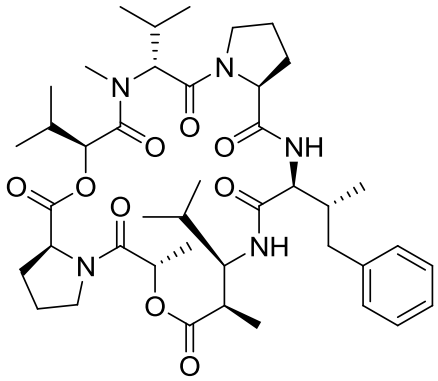
RM 1(Dolaphenylvaline removed)



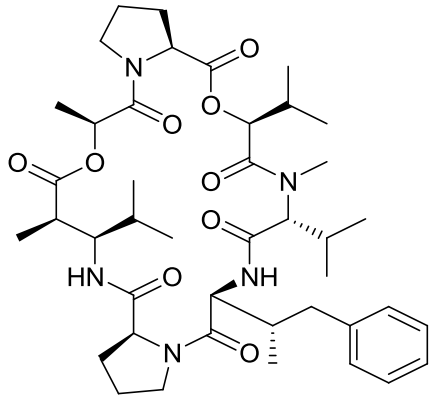
RM 2 (Dolamethyleuine removed)



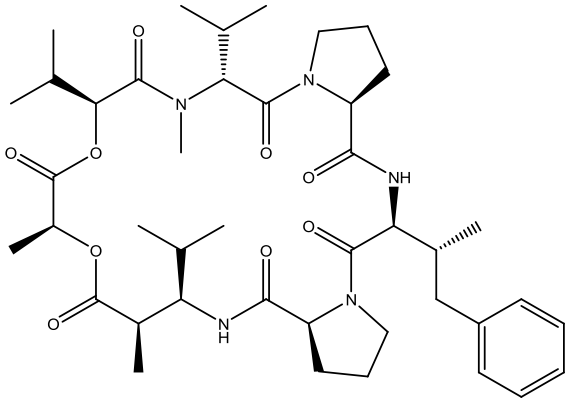
RM 3 (Lactic acid removed)



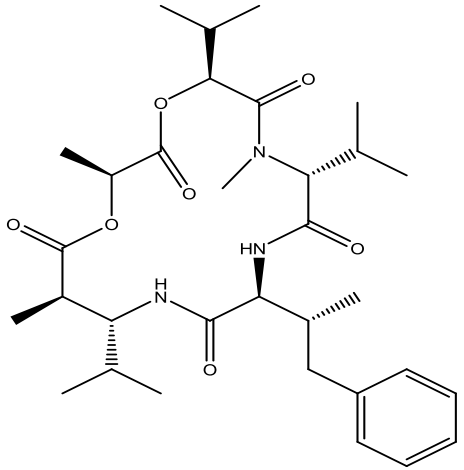
RM 4 (1st proline removed)



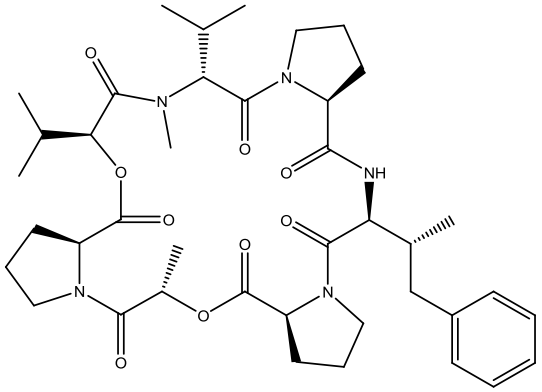
RM 5 (2nd proline removed)



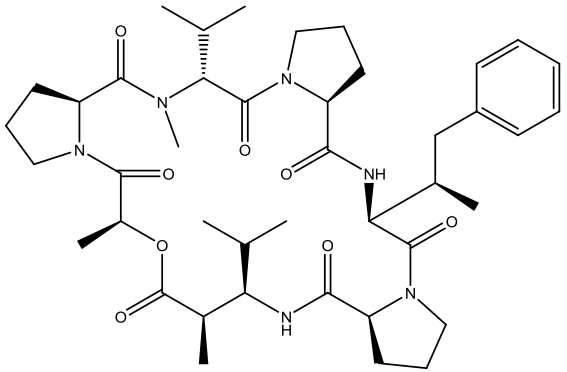
RM 6 (3rd proline removed)



RM 7 (three prolines removed)



RM 8 (2-hydroxy-3-methyl butanoic acid removed)



RM -9 (3-methyl-(2-methyl amino)butanoic acid removed)

Table 1: Docking scores in MOE when amino acids removed

S.NO.	Analogues	1EAX	2GV6	2GV7	3BZ3	3E5A	3HA6
1	Dolastatin 16	-3.53	-6.59	-5.12	-4.11	-5.71	-5.34
2	**RM 1**(Dolaphenvaline removed)	-2.62	-4.51	-4.45	-0.73	-2.63	-1.92
3	**RM 2(**Dolamethyleuine removed)	**-3.57**	**-6.6**	**-6.10**	**-4.23**	-5.01	-4.9
4	**RM 3** (Lactic acid removed)	**-3.95**	-6.23	**-6.20**	**-4.79**	**-6.62**	-4.1
5	**RM 4** (1^{st} proline removed)	-2.76	-5.51	-5.13	-2.31	-2.60	-2.36
6	**RM 5** (2^{nd} proline removed)	-2.68	-4.79	-4.94	-3.59	-6.39	-2.99
7	**RM 6** (3^{rd} proline removed)	**-3.78**	**-6.88**	**-6.37**	**-4.56**	-4.95	**-6.2**
8	**RM 7** (three prolines removed)	**-4.49**	**-7.01**	**-6.72**	**-4.72**	**-6.7**	**-6.4**
9	**RM 8** (2-hydroxy 3-methyl butanoic acid removed)	**-4.06**	-6.59	**-6.90**	**-4.15**	**-6.64**	**-6.25**
10	**RM 9** (3-methyl-2-methyl amino butanoic acid removed)	**-3.57**	-6.45	-4.95	**-4.48**	-4.21	-5.01

Table 2: Docking scores in glide when amino acids removed

S.NO.	ANALOGUES	1EAX	2GV6	2GV7	3BZ3	3E5A	3HA6
1	Dolastatin 16	-2.59	-12.47	-12.53	-3.04	-11.56	-11.44
2	RM 1(Dolaphenvaline removed)	3.1	-11.65	-11.22	-2.4	-9.57	-10.15
3	RM 2(Dolamethyleuine removed)	**-3.23**	**-13.81**	**-14.37**	**-9.7**	-9.49	-11.09
4	RM 3 (Lactic acid removed)	**-11.83**	-10.85	**-12.94**	**-8.19**	**-12.88**	-10.73
5	RM 4 (1^{st} proline removed)	-0.35	-11.87	-11.79	-2.75	-8.78	-9.19
6	RM 5 (2^{nd} proline removed)	6.62	-11.03	-10.67	-2.54	-8.17	-8.12
7	RM 6 (3^{rd} proline removed)	**-2.69**	**-12.71**	**-12.61**	**-9**	-10.82	**-13.74**
8	RM 7 (three prolines removed)	**-11.95**	**-12.6**	**-12.61**	**-10**	**-12.4**	**-11.51**
9	RM 8 (2-hydroxy 3-methyl butanoic acid removed)	**-3.08**	-10.81	**-13.21**	**-7.75**	**-12.77**	**-12.68**
10	RM 9 (3-methyl-2-methyl amino butanoic acid removed)	**-6.1**	-11.17	-10.92	**-9.52**	-8.21	-8.79

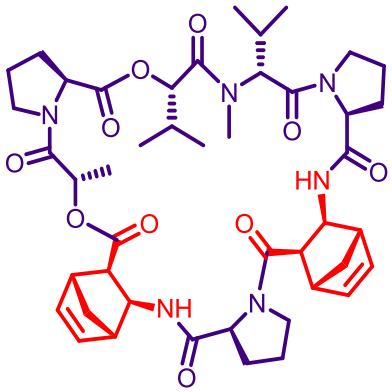
❖The further study was carried by replacing the unusual aminoacids that are dolaphenvaline and dolamethyleuine with

✓ Exo norbornene,

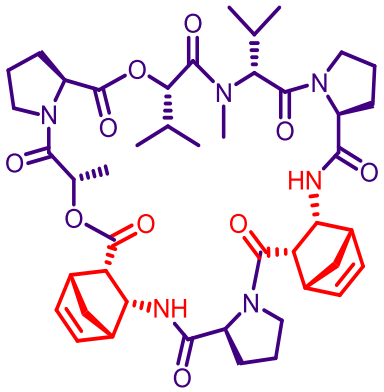
✓Endo norbornene amino acid (NBA) and

✓ Sugar Amino Acid (SAA) derived from D-glucose.

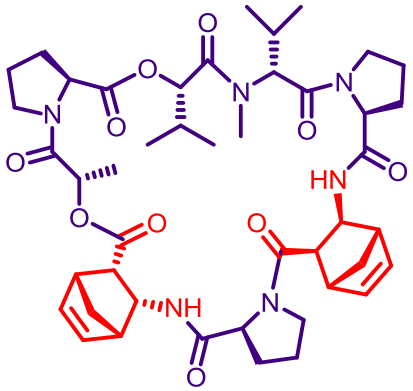
The structure of these analogues are the following:



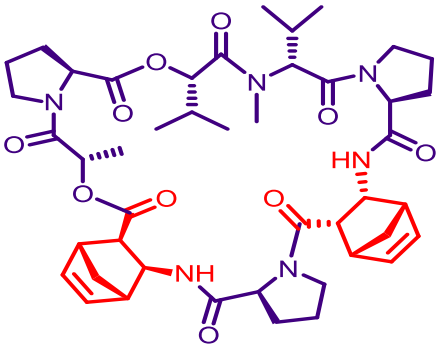
RP 1(both *exo* NBA)



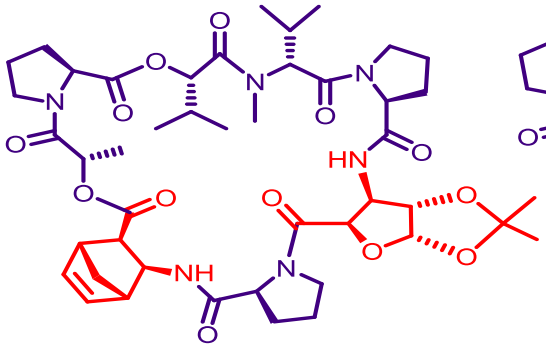
RP 2 (both *endo* NBA)



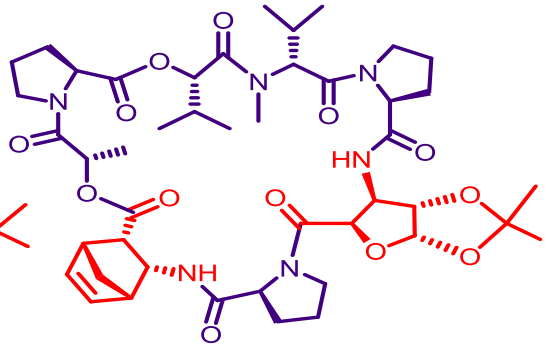
RP 3 (dolaphenvaline replaced with exo ring and dolamethyleuine with endo NBA)



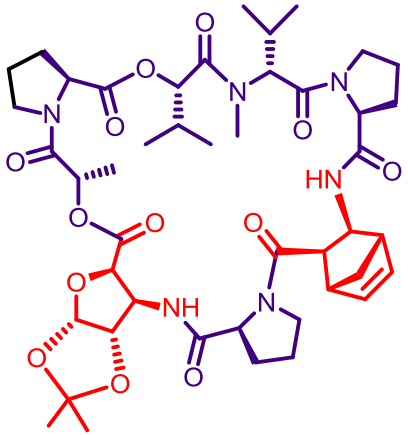
RP 4 (dolaphenvaline replaced with endo ring and dolamethyleuine with exo NBA)



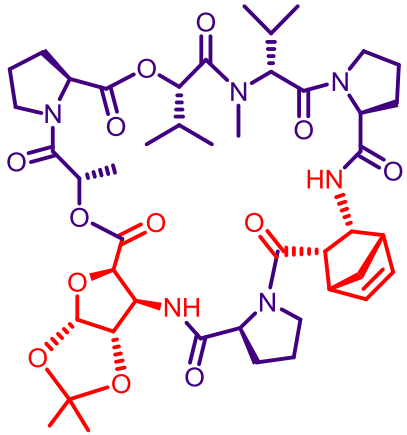
RP 5 (dolaphenvaline replaced with SAA and dolamethyleuine with exo NBA)



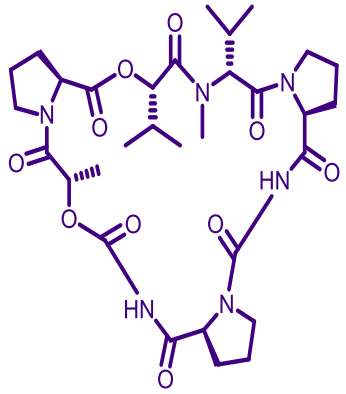
RP 6 (dolaphenvaline replaced with SAA and dolamethyleuine with endo NBA)



RP 7 (dolaphenvaline replaced with exo NBA and dolamethyleuine with SAA)



RP 8 (dolaphenvaline replaced with endo NBA and dolamethyleuine with SAA)



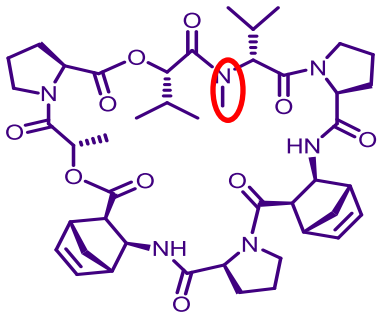
RP 9 (Both unusual amino Removed)

Table 3: Results in glide (maestro 9.3) for the designed analogues:

S.No	Proteins	1EAX		2GV6		2GV7		3BZ3		3E5A		3HA6	
	ANALOGUES	G SCORE	HB	G SCORE	HB	G SCORE	HB	G SCORE	HB	G SCORE	HB	G SCORE	HB
1	**DOLASTATIN 16**	-3.53	-1.16	-6.59	-1.28	-5.12	-0.60	-4.11	-0.70	-5.71	-0.94	-5.34	-1.12
2	**RP 1:** (Both *exo* NBA)	**-3.56**	**-1.95**	**-7.21**	**-1.7**	**-6.72**	**-0.82**	**-5.6**	**-0.90**	**-6.89**	-0.83	**-6.12**	**-1.2**
3	**RP 2:**(Both *endo* NBA)	-1.8	0	-4.93	-0.69	-5.04	-1.13	-0.87	0	-3.96	-1.62	-1.2	-0.7
4	**RP 3**	-2.68	-1.7	-4.69	-0.2	-4.21	-0.21	-0.71	-0.7	-2.63	-0.83	-2.14	-0.5
5	**RP 4**	-0.89	0	-4.74	-0.52	-4.3	-0.7	-2.9	0.0	-2.5	-0.77	-0.5	0.0
6	**RP 5**	-1.05	0.0	**-6.67**	**-1.2**	**-6.7**	**-1.08**	**-7.67**	**-0.77**	**-5.98**	**-1.21**	**-5.78**	**-1.31**
7	**RP 6**	-2.76	0	-4.11	-0.4	-3.56	-1.07	-1.41	-0.64	-2.54	0.0	-1.48	-0.7
8	**RP 7**	**-4.94**	**-1.20**	**-7.43**	-0.63	**-6.08**	**-1.33**	**-5.07**	-0.54	**-6.71**	**-1.03**	**-6.12**	-0.9
9	**RP 8**	-1.38	-0.43	-4.06	0	-4.42	-1.28	-2.14	-1.69	-4.16	-1.89	-2.31	0.0
10	**RP 9**	-3.51	-1.33	-4.05	-1.05	-3.8	-0.7	-2.68	-1.18	-2.75	-1.35	-1.24	-0.4

•We designed the analogues based on the above results:

- Dolamethyleuine and dolaphenvaline replaced with exonorbornane rings
- Lactic acid was removed
- 3rd proline (proline near lactic acid) was removed
- 2-hydroxy-3-methyl butanoic acid was removed



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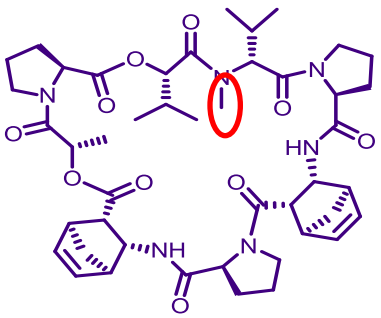

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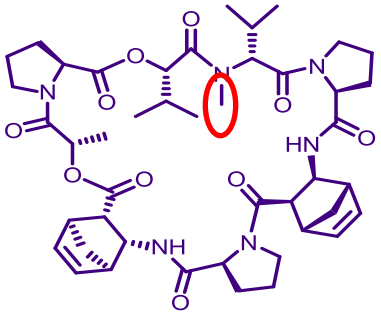

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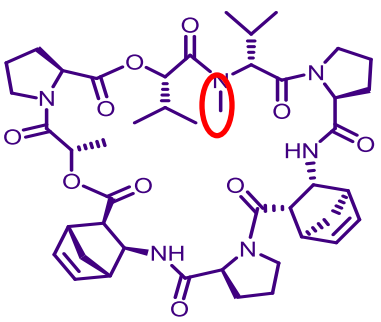

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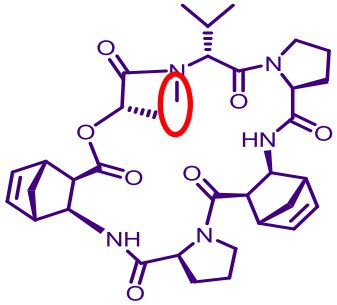

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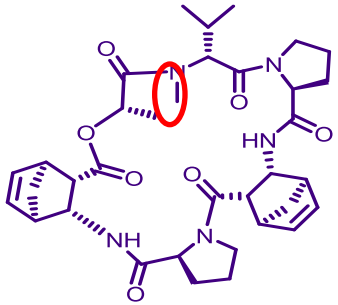

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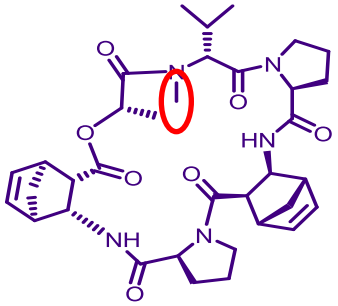

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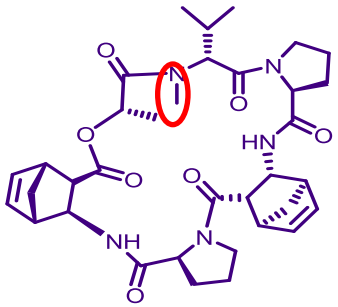

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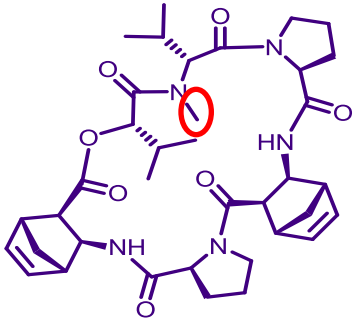

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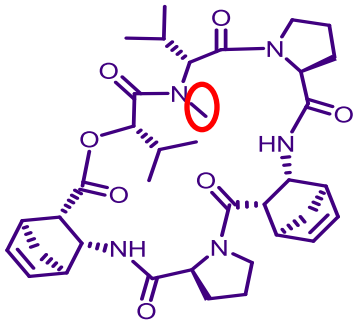

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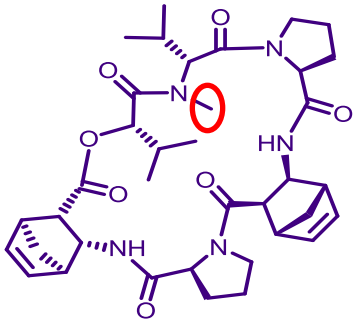

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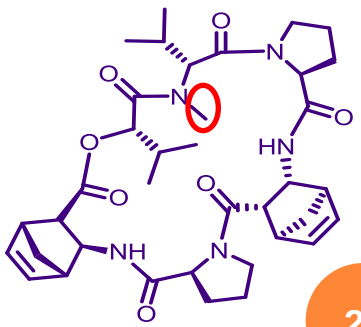
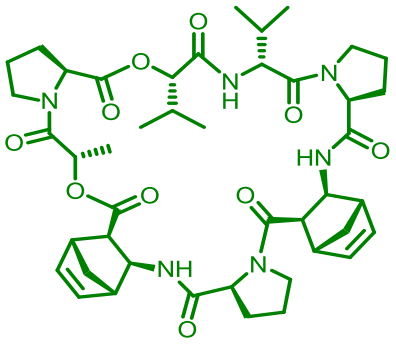


3c

Table 4: PROPOSED MOLECULES WITH METHYL GROUP IN MOE

S.NO.	Compound Code	1EAX	2GV6	2GV7	3BZ3	3E5A	3HA6
1	DOLASTATIN 16	-2.59	-12.47	-12.58	-3.04	-11.56	-11.44
2	1	5.09	-11.61	-12.11	-4.12	-8.52	-11.03
3	1a	7.06	-12.53	**-14.55**	-3.8	-8.17	-9.22
4	1b	0.69	**-13.65**	-12.84	-5.24	-7.57	-10.77
5	1c	4.2	-13.33	-12.07	-8.69	-8.68	**-11.73**
6	2	-2.76	-11.71	-11.96	-8	-10.1	-11.33
7	2a	**-5.26**	-11.51	-11.67	-6.3	-11.24	-9.96
8	2b	-4.31	-12.26	-11.41	-5.86	-10.46	-11.03
9	2c	-4.15	-11.42	-12.06	-8.52	-9.79	-9.04
10	3	1	-11.99	-11.73	**-9.76**	**-11.86**	-9.31
11	3a	-2.82	-11.16	-12.13	-6.55	-9.58	-10.45
12	3b	0.54	-10.68	-12.04	-6.53	-9.45	-11.36
13	3c	1.2	-10.69	-11.53	-5.54	-10.41	-9.67



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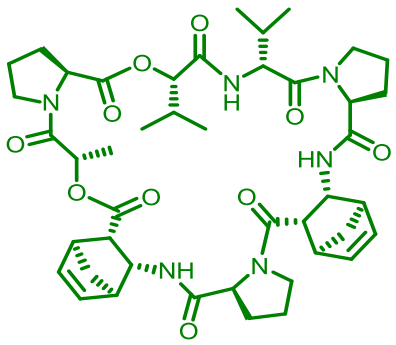

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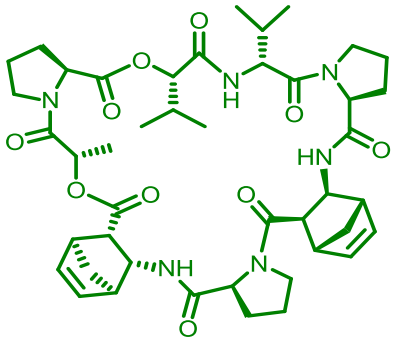

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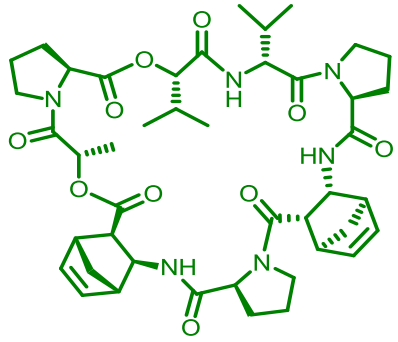

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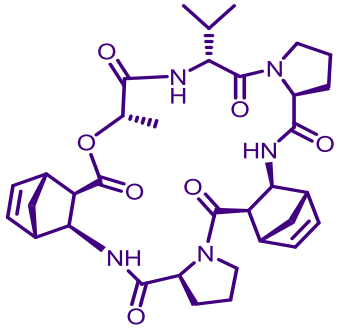

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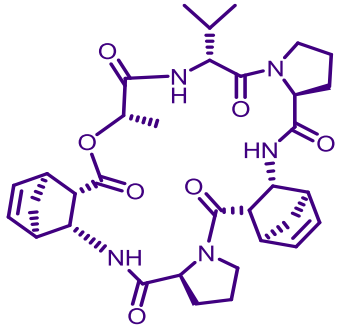

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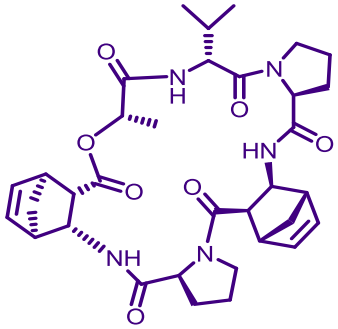

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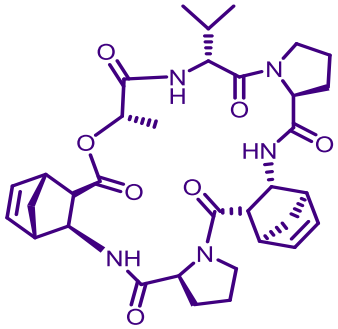

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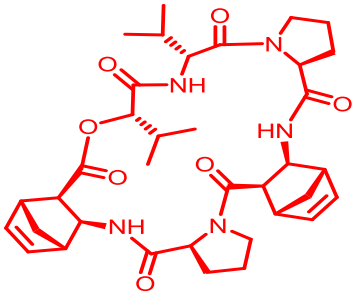

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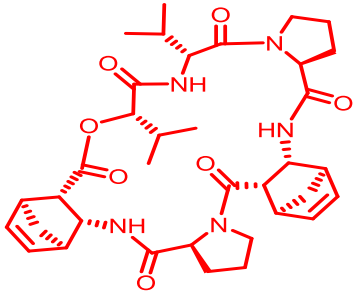

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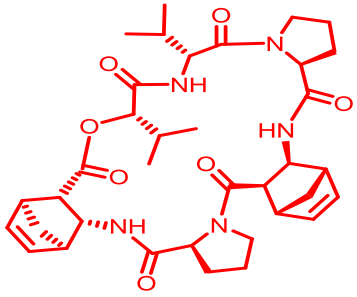

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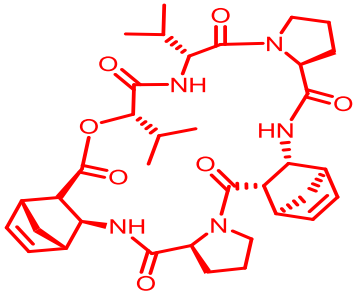
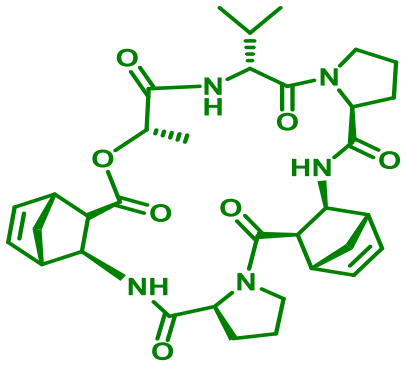


3c

Table 5: PROPOSED MOLECULES DOCKING SCORES IN GLIDE 9.3

S.NO.	ANALOGUES	1EAX		2GV6		2GV7		3BZ3		3E5A		3HA6	
		G	HB	G	HB	G	HB	G	HB	G	HB	G	HB
1	DOLASTATIN 16	-3.53	-1.16	-6.59	-1.28	-5.12	-0.6	-4.11	-0.7	-5.71	-0.94	-5.34	-1.12
2	16 METHYL REMOVED	-3.47	-0.7	-5.34	-0.2	-5.09	-0.7	-2.78	-1.03	-4.58	-1.13	-4.34	-2.23
3	1	-3.89	-0.1	**-8.06**	**-0.7**	-5.49	-1.22	-4.33	-1.42	-6.46	-1.54	-5.32	-1.04
4	1a	-4.35	-0.37	-6.89	-1.1	-5.15	-1.29	**-6.8**	**-1.10**	-5.77	-0.73	**-6.4**	**-0.82**
5	1b	-3.98	0	-7.14	-0.9	-5.96	-1.15	-4.45	-0.81	-5.9	-0.64	-5.49	-1.3
6	1c	-4.32	-0.73	-7.17	-0.69	-5.87	0	-4.31	-0.53	-6.69	-0.92	-5.76	-1.48
7	2	-4.25	-0.7	-6.83	-0.76	-5.75	-1.14	-4.47	-1.33	-6.42	-0.67	-5.44	-0.7
8	2a	-3.77	-0.7	-7.03	-0.7	**-7.34**	**-1.2**	-4.87	-1.06	-6.17	-0.67	-6.25	-1.33
9	2b	-4.36	-1.32	-7.31	-1.33	-6.68	-1.33	-4.45	-0.9	**-6.41**	**-1.08**	-6.34	-1.26
10	2c	-3.9	-0.7	-6.56	-0.7	-6.64	-1.17	-4.5	-1.04	-6.03	-1.29	-5.5	0
11	3	-3.91	-0.77	-6.98	-1.29	-6.56	-0.67	-4.37	-1.02	-6.19	-1.78	-6.16	-0.83
12	3a	**-5.67**	**-1.22**	-7.01	-1.33	-5.52	-1.15	-5.1	-0.71	-6.53	-1.2	-6.01	-0.7
13	3b	-3.61	-1.13	-6.98	-1.01	-5.94	-1.79	-4.47	-0.89	-6.13	-0.79	-6.08	-0.85
14	3c	-3.76	-0.04	-7.02	-1.29	-6.1	-0.78	-4.73	-0.66	-6.66	-1.46	-6.21	-0.62



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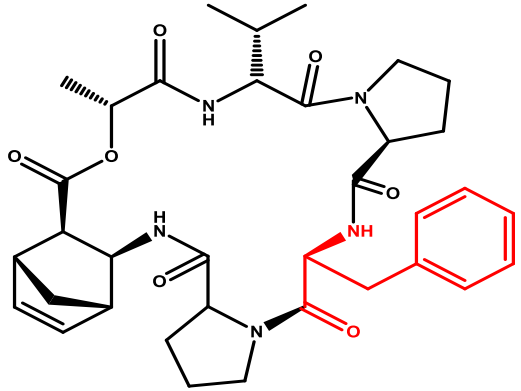

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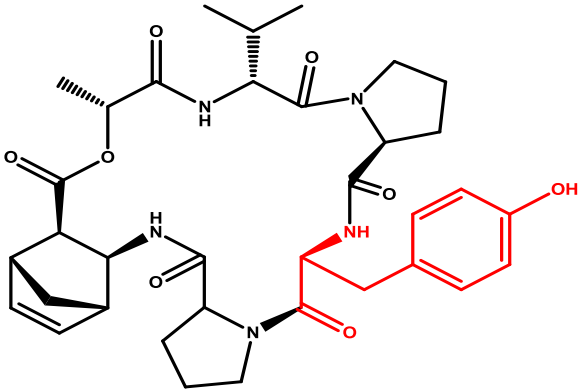

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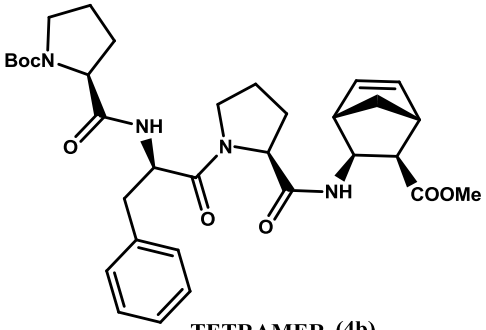
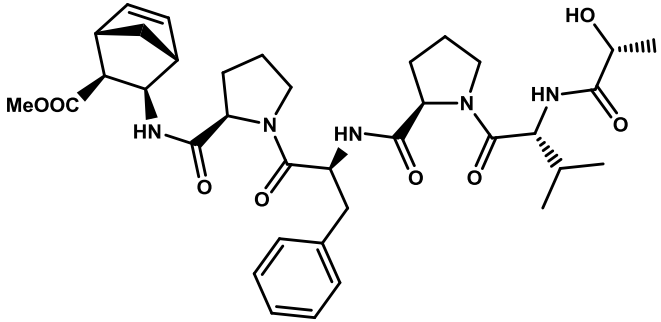


TETRAMER (4b)



HEXAMER (4c)

Table 6: PROPOSED MOLECULES IN GLIDE

S.No	PROTEINS	1EAX		2GV6		2GV7		3BZ3		3E5A		3HA6	
	ANALOGUE	G	HB	G	HB	G	HB	G	HB	G	HB	G	HB
1	Dolastatin 16	-3.53	-1.16	-6.59	-1.28	-5.12	-0.6	-4.11	-0.70	-5.71	-0.94	-5.34	-1.12
2	**4**(Phenylalanine lactone)	-5.08	-1.05	-8.46	-1.08	-7.29	-0.88	-7.39	-1.21	-7.15	-1.92	-6.96	-1.56
3	**4a**(Para hydroxyphenylalanine lactone)	-3.91	-0.32	-6.28	-0.45	-6.27	-0.34	-4.05	-1.12	-5.47	-0.14	-5.87	-0.63
4	**4b**(Tetramer)	-5.31	-1.07	-6.75	-1.41	-6.64	-0.04	-5.46	-0.82	-6.12	-1.16	-5.94	-1.36
5	**4c**(Hexamer)	-8.21	-2.32	-7.23	-1.03	-6.58	-0.56	-6.27	-1.13	-6.94	-1.04	-6.12	-1.43

HYDROGEN BONDING INTERACTIONS WITH AMINOACIDS

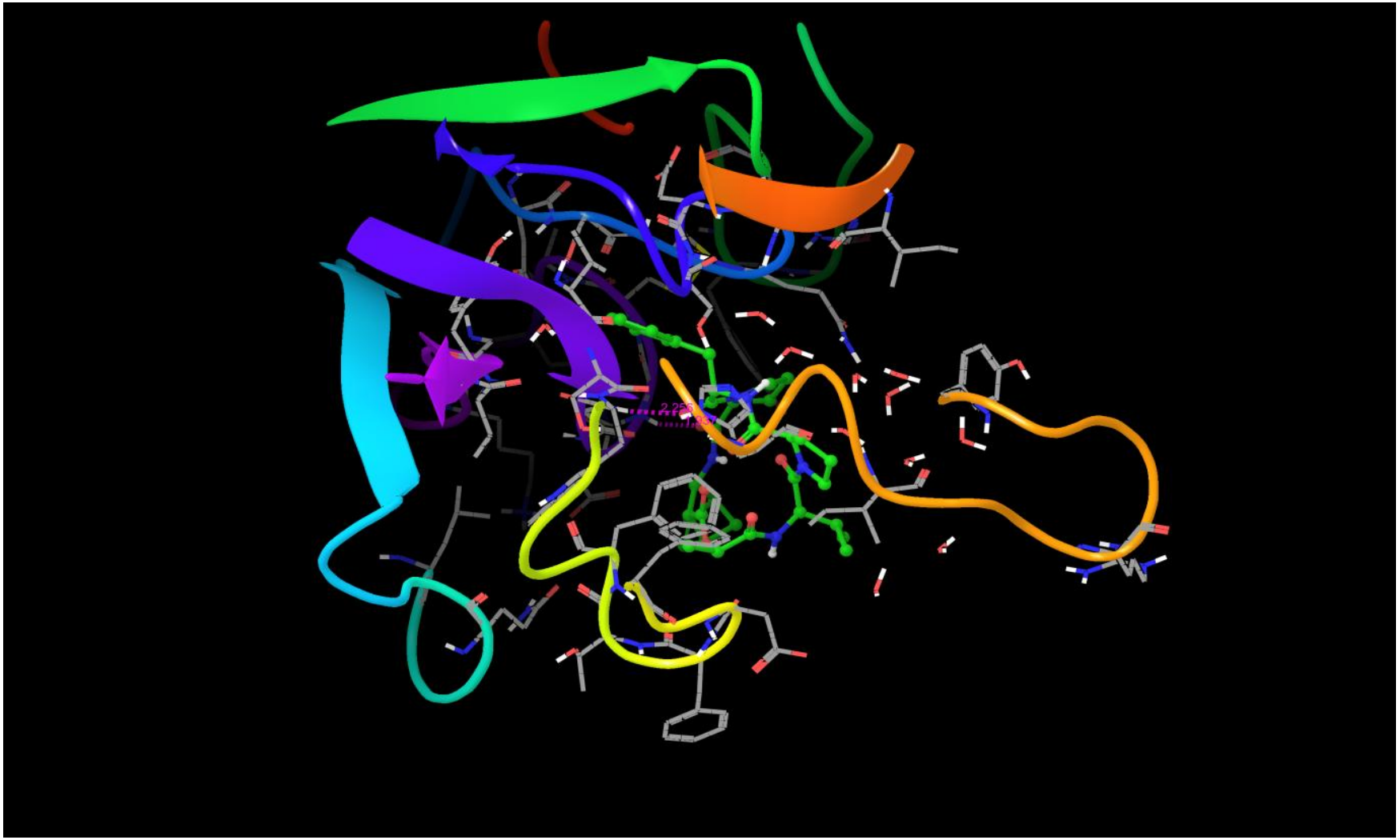
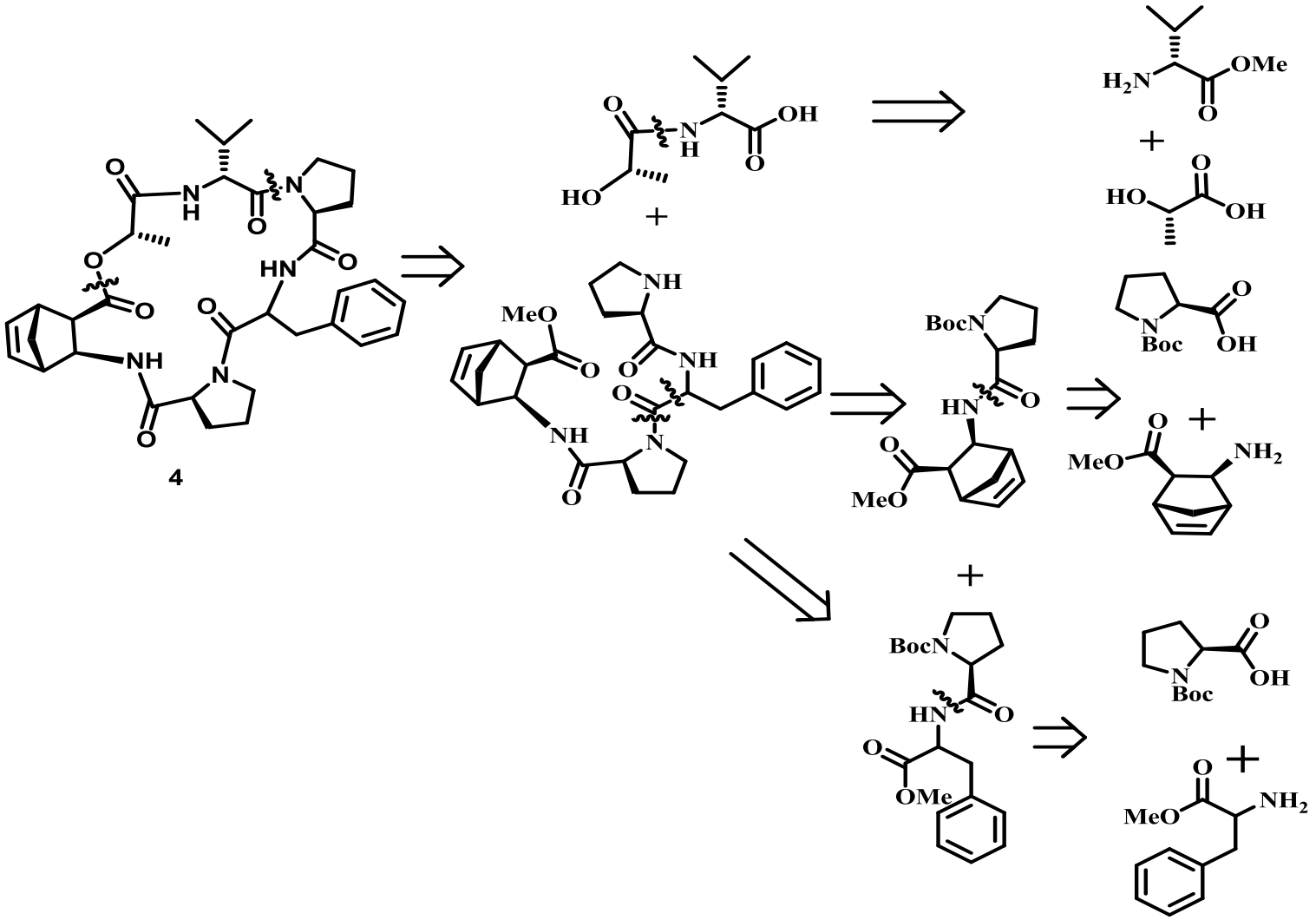


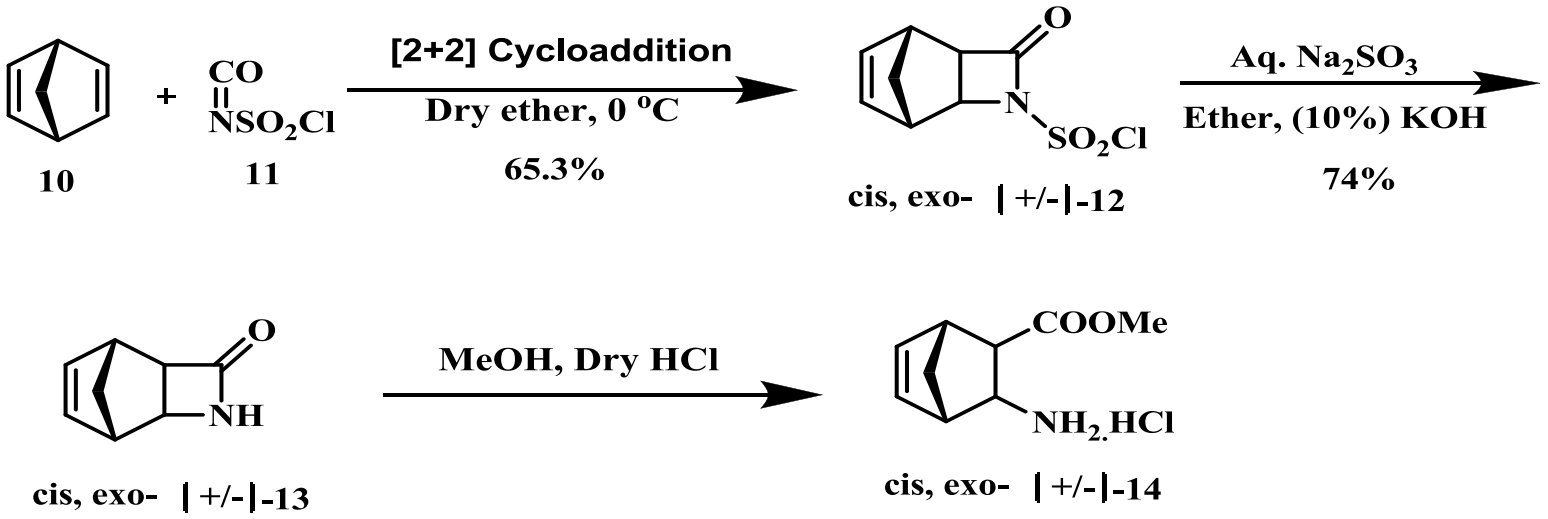
Figure 10: **4** in 2GV6 protein, interaction with **GLY 216** and **GLY 219**

SYNTHESIS

RETROSYNTHETIC DESIGN

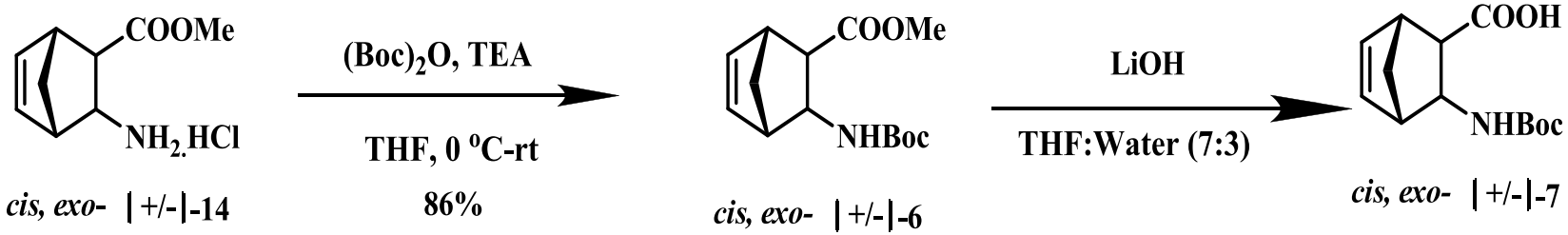


SYNTHESIS OF MONOMERS



10 + 11 → cis, exo- |+/-|-12 → cis, exo- |+/-|-13 → cis, exo- |+/-|-14

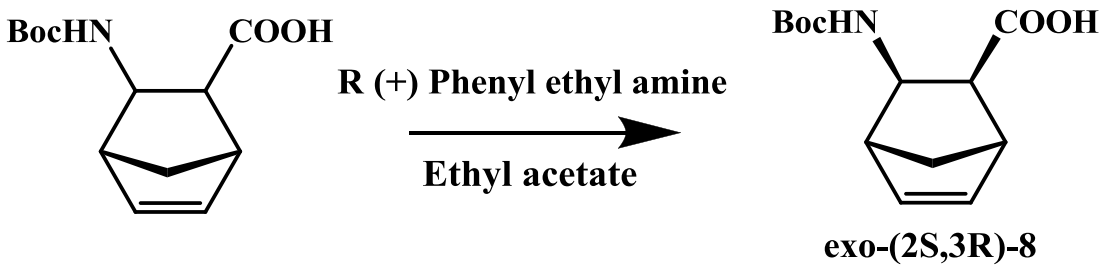
Scheme 1



cis, exo- |+/-|-14 → *cis, exo-* |+/-|-6 → *cis, exo-* |+/-|-7

Scheme 2

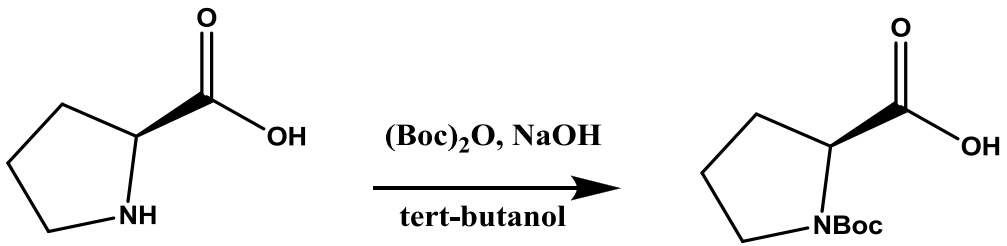
RESOLUTION OF MONOMER



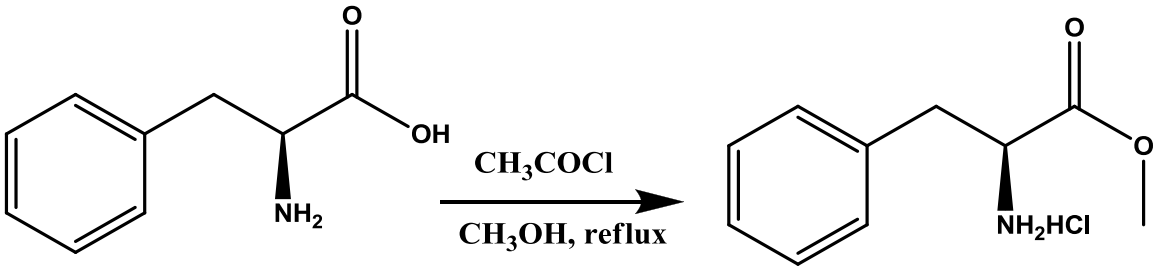
Scheme 3

The chiral monomer **8** is confirmed by optical rotation of this compound is $[\alpha]_D^{25} = +91.8$ in MeOH

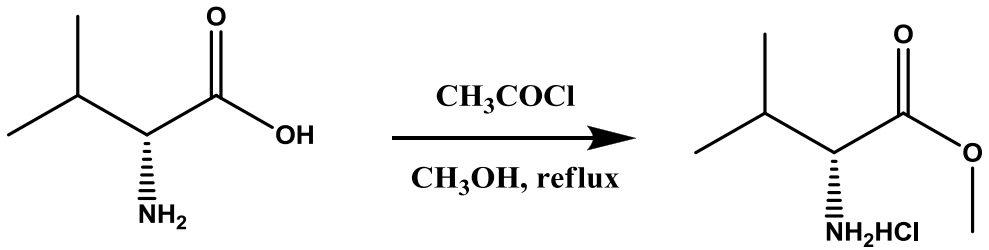
(S)-1, 2-Pyrrolidinedicarboxylic acid 1-(1,1-dimethylethyl) ester



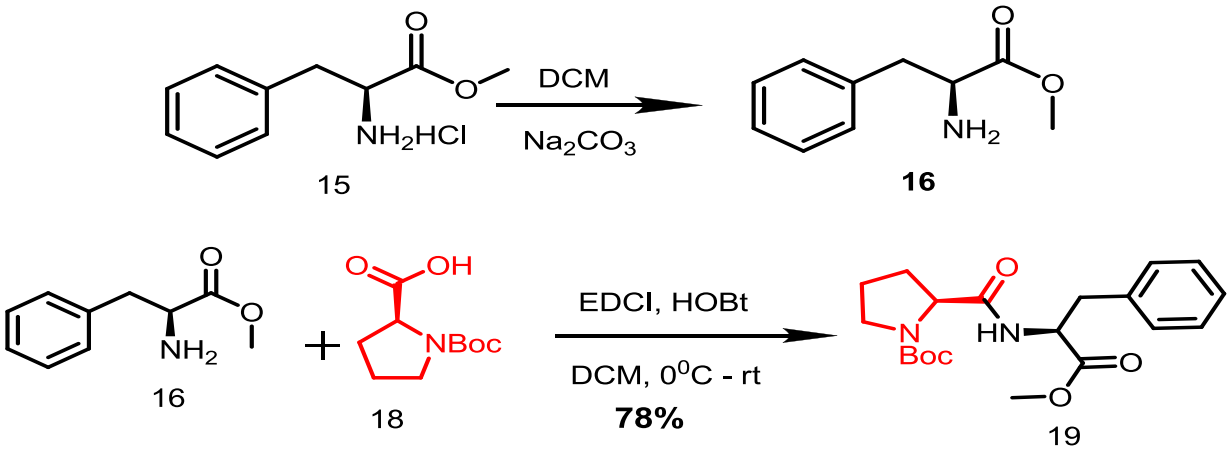
Synthesis of (S)-methyl 2-amino-3-phenyl propanoate hydrochloride:



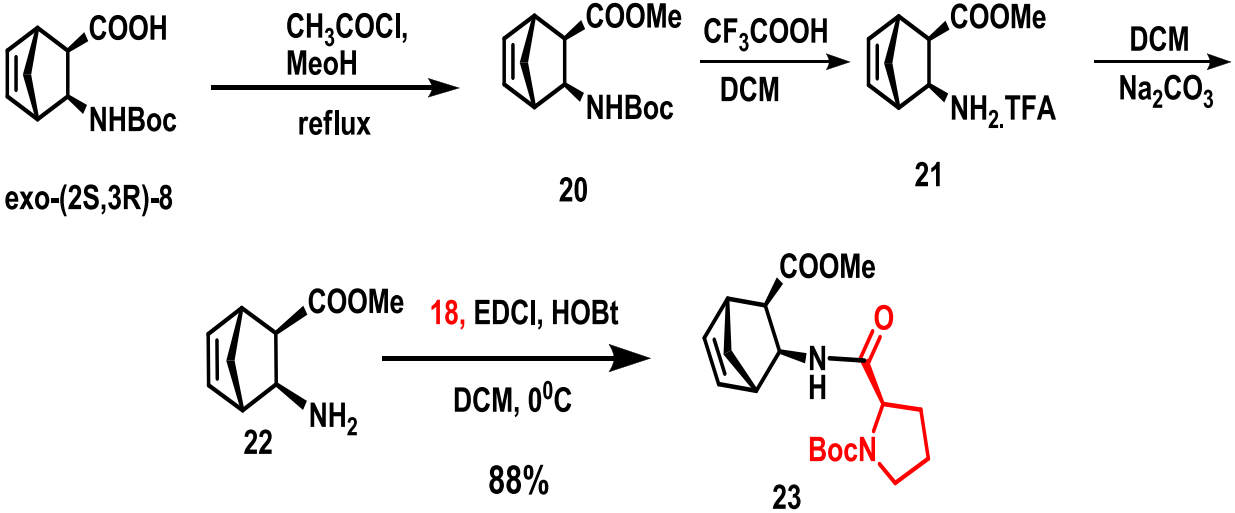
Synthesis of (R)-methyl 2-amino-3-methyl butanoate hydrochloride:



SYNTHESIS OF DIMERS

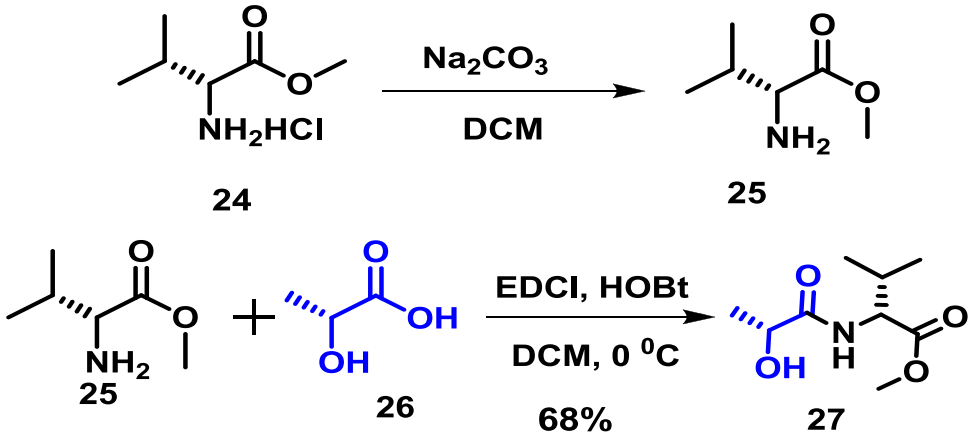


Scheme 4



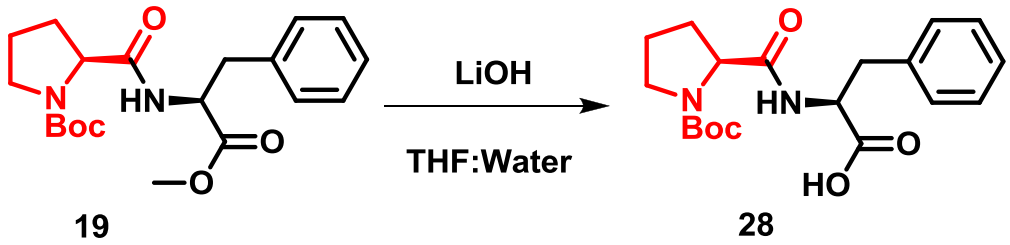
Scheme 5

SYNTHESIS OF DIMERS

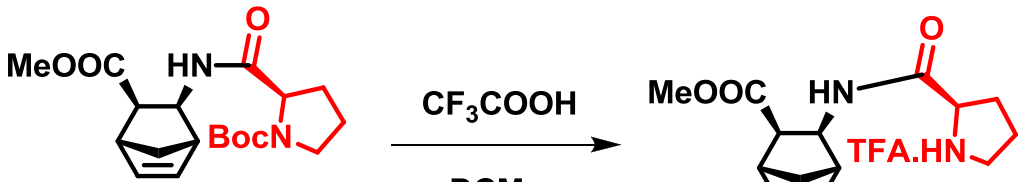


Scheme 6

SYNTHESIS OF TETRAMER

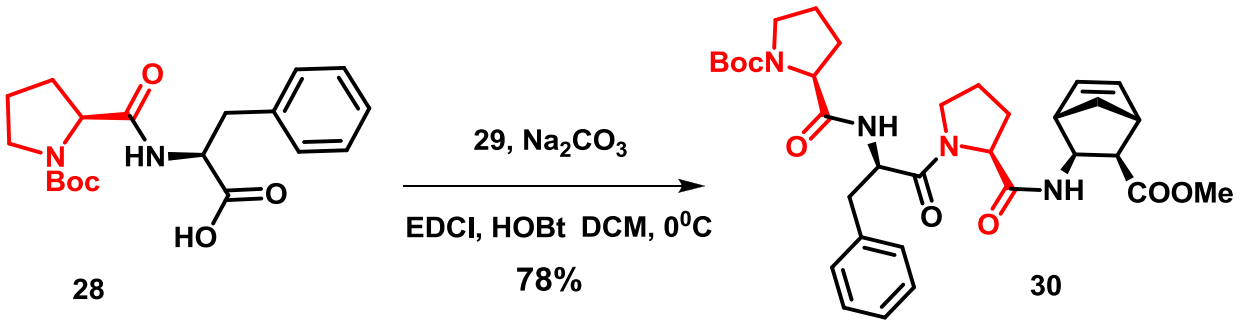


19 28



23 29

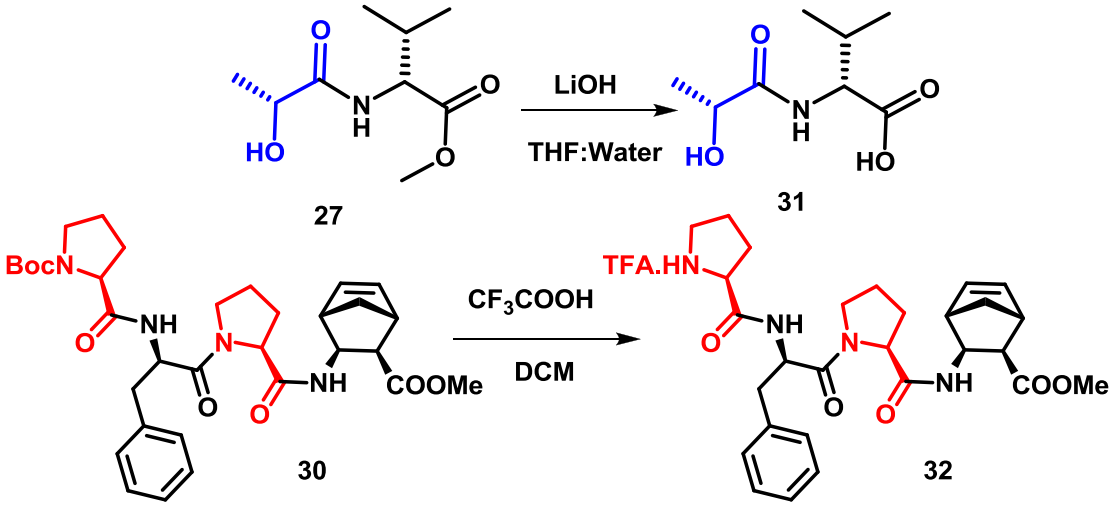
Scheme 7



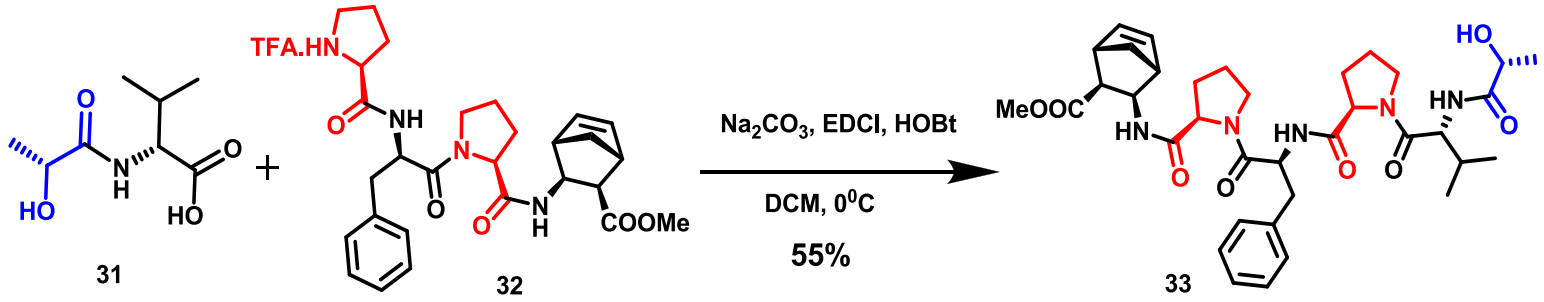
28 30

Scheme 8

SYNTHESIS OF HEXAMER

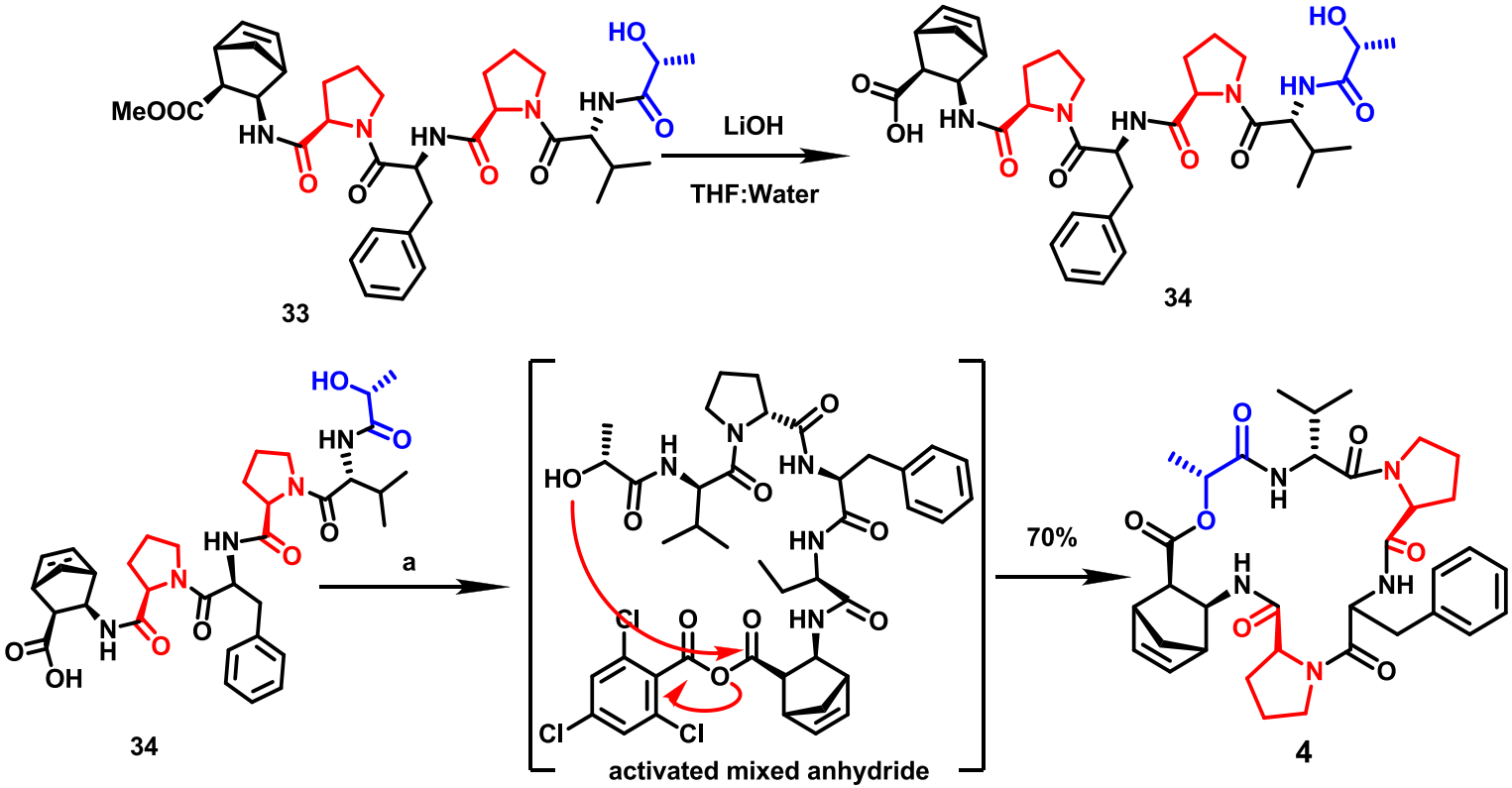


Scheme 9



Scheme 10

YAMAGUCHI MACROLACTONIZATION



Scheme 11

CONCLUSION

The extensive SAR study of Dolastatin 16 is done by using molecular modeling softwares Glide 9.3 and MOE (Molecular Operating Environment) docking tools.

It is concluded that unusual amino acids **NBAA and SAA** can be used to replace the amino acids dolaphenvaline and dolamethyleuine in Dolastatin 16.

Furthermore, it is observed that dolaphenvaline (aromatic amino acid) can be replaced with **L-phenyl alanine** and dolamethyleuine can be replaced with **NBAA** (unusual β amino acid).

Based on the computational results, designed analogue **4** was selected for the synthesis and biological evaluation.

Monomers, dimers, tetramer, hexamer and lactone are synthesized by using appropriate reagents and reaction conditions to get the good yields.

Spectral characterization of dimers, tetramer, hexamer and macrolide is performed using ^{1}H NMR, mass, and IR.

The synthesized analogue and its intermediates were submitted for the biological evaluation against cancer cell and results are awaited.

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Thank You