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DNA-Templated Combinatorial Assembly of Small Molecule Fragments Amenable to Selection/Amplification Cycles.

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Electronic Supplementary Information

DNA-templated combinatorial libraries. A library of DNA containing all permutation of codons complementary to the PNA libraries flanked by 20-mer primer was obtained by split and pool synthesis from commercial supplier on 1 μmol scale (Microsynth AG, Switzerland) and purified by PAGE. The DNA-templated combinatorial library of drug fragments was prepared by mixing the PNA libraries (N-terminus library and C-terminus library) with the DNA library as well as sequences complementary to the primers on the DNA library (P5' block 5'-ACGAGAGGCTCACAACAGGC-3' and P3' block, 5'-GGATAGACAATAACGACGAC-3'). All the component were mixed at equal molar ratio to obtain a final concentration of 600 nM in 50 mM, Tris $-SO_4$ buffer, pH 8.7 or alternatively PBS buffer, NaCl, 0.2 M, in a final volume of 100 μL. The mixture was heat denatured (10 min at 95 °C) and then allowed to cool to room temperature over the course of one hour.

Screening of carbonic anhydrase. Carbonic anhydrase (CA) (Sigma) was dissolved in PBS at 2 mg/ml and immobilized on carboxylic acid activated magnetic beads (Dynal Invitrogen) following the manufacturer recommendations. 10 μL of beads immobilized CA were used at each round of selection. The DNA/PNA hybrids solution (600 nM, 10 μL) was incubated with the target immobilized protein slurry (10 μL) in an Eppendorf tube for 30 min at RT with gentle agitation. After incubation the tube was placed on a magnetic stand for 2 minutes to pellet the beads along the wall and the supernatant containing the non bound molecules was pipetted off and discarded. The beads were washed 10 times with PBS-Tween 20, 0.1% (PBS-T) 100 μL, to eliminate PNA/DNA hybrids non specifically interacting with the target. After 10 washes, the magnetic beads were resuspended in 50 μL of distilled water and heated at 94°C for 10 min. The beads were magnetically pelletted and the supernatant was recovered in a new tube, diluted 100 fold and 1 μL of this dilution was used for the PCR reaction. The same procedure was carried out in parallel with BSA functionalized magnetic beads to identify specific selection corresponding to carbonic anhydrase by comparison of the selected compounds. PCR amplification was carried out as follows: 2μM primer P3' Cy3-5'-GGATAGACAATAACGACGAC-3', 2μM primer P5' Biot-5'-GCCTGTTGTGAGCCTCTCGT-3', 0.2 units of AmpliTaq Gold (Applied Biosystems), 1.5 mM $MgCl_2$, Amplitaq Gold Buffer II, one first incubation at 95°C for 10 min, 95°C during 1 min, 52°C for 30s, 68°C for 30 s (25 cycles) and a final elongation for 10 min at 72°C. The PCR reactions were analyzed on 3.5% agarose electrophoresis to confirm the amplification of a specific 68 nucleotides product, stained with ethidium bromide and visualized by UV transilumination. 100 μl of PCR reaction from each round of selection were purified using Quiaquick PCR purification kit (Qiagen). The purified PCR product was immobilized on Dynal Streptavidine magnetic beads, and single strand DNA was prepared following a known protocol.(34) The Cy3 labeled DNA strand (ss-FDNA) was conserved at -20°C until hybridization on microarray slides while the immobilized biotinylated strand (template strand) was rehybridized with a molar excess of the PNA libraries and the complementary sequence to the primers (600 nM, 200 μl) during 30 min at 50°C. The non hybridized PNA molecules were washed out of the magnetic beads by five successive washes with 100 μl PBS-T at room temperature. This DNA-PNA adducts corresponding to the selected library members were eluted from the magnetic beads by heating the beads at 95°C (10 μl) for ten minutes on PBS buffer, 5 mM biotin. The eluted DNA/PNA hybrids were engaged in further rounds of selection with immobilized carbonic anhydrase following the same protocol as described above.

The Cy3 labled DNA strands corresponding to four rounds of selection were hybridized to custom array containing the complementary sequences at 35 °C as previously described.(23) The results from the fourth round of sequenceing were also sequenced using TOPO TA cloning systems (Invitrogen ^{TM}) following the manufacturer's protocol and sequenced by GATC Biotech AG sequencing services

Affinity of covalent adducts. The affinities were measured using a microcalorimetric ITC 200 (Microcal). A 500 µM solution of ligand in Tris-SO4, 50 mM buffer, pH 8.5, 2% DMSO was titrated with a 25 µM solution of Carbonic anhydrase (Sigma) in the same buffer at 25 °C. The isothermal titration curves obtained were treated and fitted using Origin 7 software (OriginLab).

General Techniques for chemical synthesis.

All reactions were carried out under a nitrogen atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. Anhydrous solvents were obtained by passing them through commercially available alumina column (Innovative Technology, Inc., ® VA). NovaPEG Rink Amide Resin resin was purchased from Novabiochem®, and was swollen in DCM before each reaction. Solid phase reactions were carried in SPE tubes fitted with a frit and a tap. Automated solid phase synthesis was carried out on an Intavis Multipep instrument (http://www.intavis.com/en/Automated_Peptide_Synthesis/MultiPep_RS/index.php).
LC-MS were recorded using an HP 1100 series or Thermo Electron Corporation HPLC with a Thermo Finnigan Surveyor MSQ Mass Spectrometer System. A Thermo Scientific column (50 x 2.1 mm) was used. MALDI spectra were measured using a Bruker Daltonics AutoflexII TOF/TOF spectrometer. Polymer-bound intermediates were characterized by LC-MS and/or MALDI (2,5-Dihydroxybenzoic acid or a-Cyano-4-hydroxycinnamic acid matrix and desorbed with laser between 35-55%) following a cleavage from the resin. Cleavages were carried out on 0.1-0.3 mg of dry resin with 20 µL of TFA for 20 min at room temperature. The TFA solution was either evaporated or added to 200 µL of Et_2O and centrifuged at 18000g for 5 min to pellet the precipitated compound. The resulting white pellet was then washed with Et_2O (200 µL) and redisolved in 1:1 MeCN:H_2O (40µL) for analysis. The mix and split synthesis was performed according to previously established protocols (F. Debaene, J. DaSilva, Z. Pianowski, F. Duran, N. Winssinger *Tetrahedron* **2007**, *63*, 6577-6586).

General procedures for the synthesis of PNA-encoded libraries

Procedure 1. General procedure for capping the resin. To 100 mg of NovaPEG Rink amide resin were added 2.0 mL of capping mixture (9.2 mL of acetic anhydride and 13 mL of 2,6 lutidine in 188 mL of DMF), and the resin was shaken for 15 min. Subsequently, the resin was washed with 6 x 2 mL of DMF and 6 x 2 mL of CH_2Cl_2.

Procedure 2. General procedure for capping in Intavis AG Multipep RS Synthesizer. To 10 mg of NovaPEG Rink amide resin were added 100 µL of capping mixture (9.2 mL of acetic anhydride and 13 mL of 2,6 lutidine in 188 mL of DMF). After 5 min, the resin was washed with 2 x 250 µL of DMF.

Procedure 3. General procedure for Fmoc deprotection. To 100 mg of NovaPEG Rink amide resin were added 2.0 mL of 20% piperidine solution in DMF, and the resin was shaken for 5 min. Subsequently, the resin was washed with 6 x 2 mL of DMF and 6 x 2 mL of CH_2Cl_2, and the deprotection sequence was repeated a second time.

Procedure 4. General procedure for Fmoc deprotection in Intavis AG Multipep RS Synthesizer. To 10 mg of NovaPEG Rink amide resin were added 100 µL of 20% piperidine solution in DMF. After 2 min, the resin was washed with 250 µL DMF and the sequence was repeated a second time. Finally, the resin was washed with 5 x 250 µL of DMF and 3 x 250 µL of CH_2Cl_2.

Procedure 5. First Lysine coupling on resin, loading reduction. To a solution of 56.2 mg (0.09 mmol, 1.0 equiv, 0.2 mmol/g loading) of Fmoc-Lys(Mtt)-OH in 7.0 mL of NMP were added 68.9 mg (0.45 mmol, 5 equiv) of HOBt followed by 210 µL of DIC. The mixture was stirred for 5 min at room temperature, and then added to 450 mg of NovaPEG Rink amide resin. The reaction mixture was shaken for 16 hours and subsequently the resin was washed with 6 x 10 mL of DMF and 6 x 10 mL of CH_2Cl_2.

Procedure 6. PEG- spacer coupling. To a solution of Fmoc protected-PEG spacer (4.0 equiv) in NMP (60 mM) were added (3.5 equiv) of HCTU (0.5M) in NMP followed by base solution [DIPEA 1.2 M (0.25 mmol, 4 equiv) and 2,6 lutidine 1.8M (0.38 mmol, 6.0 equiv) in NMP]. The mixture was stirred for 5 min at room temperature, and then added to the pre-swollen resin. The reaction mixture was shaken for 16 hours and subsequently the resin was washed with 6 x DMF and 6 x CH_2Cl_2. Finally, the resin was capped (***procedure 1***) and Fmoc was deprotected (***procedure 3***).

Procedure 7. General procedure for amino acid coupling to resin in Intavis AG Multipep RS Synthesizer To a solution of 8.0 µmol (4.0 equiv) of Fmoc protected aminoacid in 40 µL of NMP were added 14 µL (7.0 µmol, 3.5 equiv) of HCTU 0.5 M in NMP, followed by 6.7 µL of base solution [DIPEA 1.2 M (0.008 mmol, 4.0 equiv) and 2,6 lutidine 1.8 M (0.012 mmol, 6.0 equiv) in NMP]. The mixture was then added to 10 mg (2.0 µmol, 1.0 equiv) of the corresponding resin. After 20 min the resin was filtered and washed with DMF and the sequence was repeated. The resin was then filtered and washed with 6 x 250 µL of DMF and 6 x 250 µL of CH_2Cl_2. Finally, the resin was capped (***procedure 2***) and Fmoc was deprotected according to ***procedure 4***.

Procedure 8. General procedure for the azide generation. To a solution of 294 mg (1.4 mmol, 11 equiv) imidazole-1-sulfonyl azide hydrochloride (E.D. Goddard-Borger, R.V. Stick Org. Lett. 2007, 9(19), 3797-3800) in 12.6 mL of MeOH were added successively 305 mg (2.2 mmol, 18 equiv) K_2CO_3 and 8.0 mg (0.05 mmol, 0.35 equiv) anhydrous $CuSO_4$ and the resulting solution was sonicated for 20 min. To 10 mg (2.0 µmol, 1.0 equiv) of the corresponding resin were added 250 µL of the previously prepared

solution. After 16 hours, the resin was washed with 6 x 250 μL of sodium diethyl dithiocarbamate 0.02 M in DMF, 6 x 250 μL of DMF, 6 x MeOH and 6 x CH_2Cl_2, and the sequence was repeated again.

Procedure 9. Mtt deprotection on NovaPEG resin in Intavis AG Multipep RS Synthesizer. To 10 mg of resin (0.2 mmol, 1.0 equiv) were added 250 μL of 50% hexafluoroisopropanol in DCE. After, the resin was washed with 2 x 250 μL CH_2Cl_2 and 4 x 250 μL of DMF. This procedure was repeated 9 times during 1 hour.

Procedure 10*. General procedure for PNA synthesis on NovaPEG resin in Intavis AG Multipep RS Synthesizer.** To a solution of 8.0 μmol (4.0 equiv) of the corresponding Fmoc or Mtt -protected PNA monomer (the nucleobases are Boc protected - S. Pothukanuri, Z. Pianowski, N. Winssinger, *Eur. J. Org. Chem.*, **2008**, *18*, 3141-48) in 40 μL of NMP were added 14 μL (7.0 μmol, 3.5 equiv) of HCTU 0.5M in NMP, followed by 6.7 μL of base solution [DIPEA 1.2 M (0.008 mmol, 4.0 equiv) and 2,6 lutidine 1.8M (0.012 mmol, 6.0 equiv) in NMP]. The mixture was then added to 10 mg of the corresponding resin After 20 min the resin was filtered and washed with DMF and the sequence was repeated, then, the resin was washed with 6 x 100 μL of DMF and 6 x 100 μL of CH_2Cl_2. Finally, the resin was capped (procedure 2)*** and Fmoc was deprotected according to ***procedure 4***.

Procedure 11. General procedure for cleavage of compounds from the resin. The resin was treated with TFA for 4 hours. The TFA solution was precipitated in Et_2O (10 times TFA volume) and centrifuged to recover the product as a pellet. The precipitate was re-dissolved in H_2O (500 μL for crude cleaved from 10 mg of resin) then lyophilized.

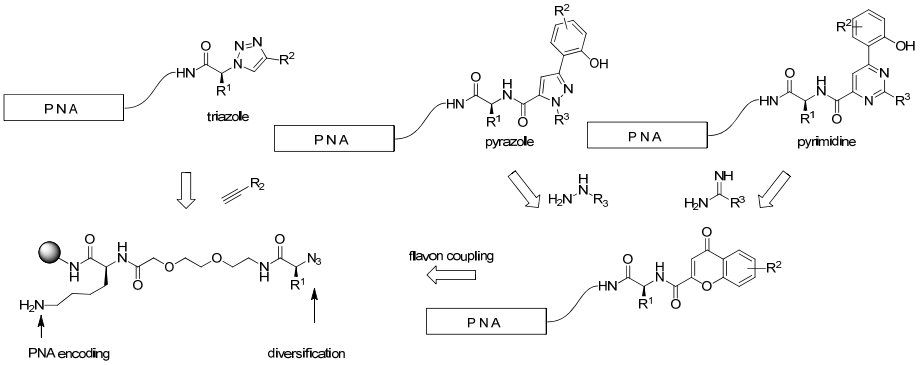
Procedure 12. General procedure for Click cycloaddition reaction on NovaPEG resin in Intavis AG Multipep RS Synthesizer. To the corresponding resin (11.5 mg) were added successively 173 μL (0.0173 mmol, 7.5 equiv) of alkyne 0.1M in NMP, 17.3 μL (17.2 μmol, 7.5 equiv) of sodium ascorbate 198 mg/mL in H_2O, 4.4 μL (0.57 μmol, 0.25 equiv) of copper sulfate 21.4 mg/mL in H_2O and 44 μL (1.1 mmol, 0.5 equiv of TBTA). After 16 hours, the resin was washed with 6 x 250 μL of sodium diethyl dithiocarbamate 0.02 M in DMF, 6 x 250 μL of DMF, 6 x MeOH and 6 x CH_2Cl_2 (V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem. Int. Ed. Engl.*, **2002**, 41, 2596-2599; C. W. Tornoe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057-3064; N.G. Angelo, P.S. Arora, J. Org. Chem. 2007, 72, 7963-7967)

Procedure 13. General procedure for the loading of the flavones. The resin was swollen in CH_2Cl_2 for 20 min and treated with a premixed (30 min) solution of the corresponding flavon (5.0 equiv), HOBt (5.0 equiv), DIC (15 equiv) in NMP during 5 hours. Then the resin was filtered and washed with DMF and CH_2Cl_2.

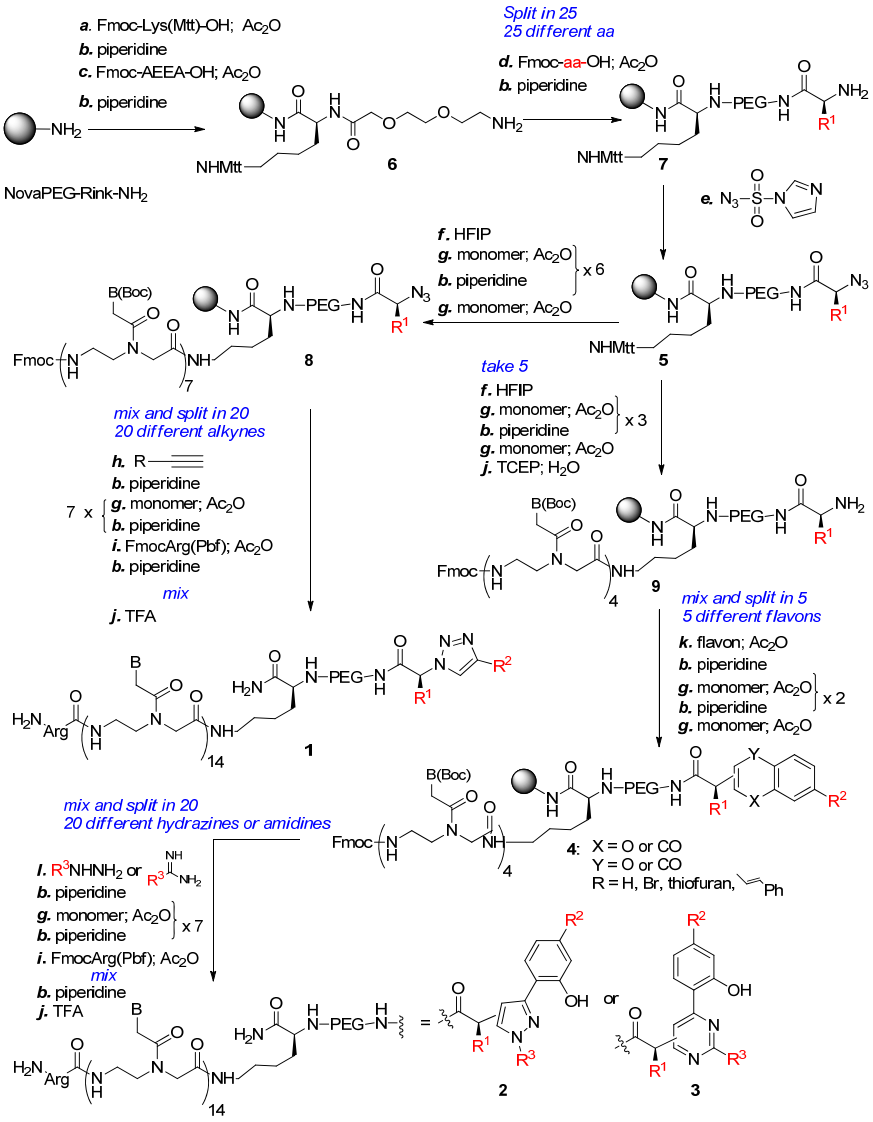
Procedure 14. General procedure for the reactions with hydrazine or amidinium salt. The resin (1.0 equiv) was treated with a premixed solution of the corresponding hydrazine or amidinium salt (3.0 equiv) and an aqueous solution of $NaHCO_3$ (3M, 4.0 equiv) in *n*BuOH (0.5 mL), and then was shaken at 50 ^oC for 18 hours. The mixture was filtered and washed sequentially with DMF and CH_2Cl_2.

Procedure 15. General procedure for the reduccion of the azide. The resin (10 mg, 2 μmol, 1.0 equiv) was treated with a 1M solution of PMe_3 (3.0 equiv, THF) in THF (200 μL) for 5 min. After, H_2O (200 μL) was added and the reaction was shaken for 8 hours. The resin was washed with THF, H_2O and finally with DMF and CH_2Cl_2.

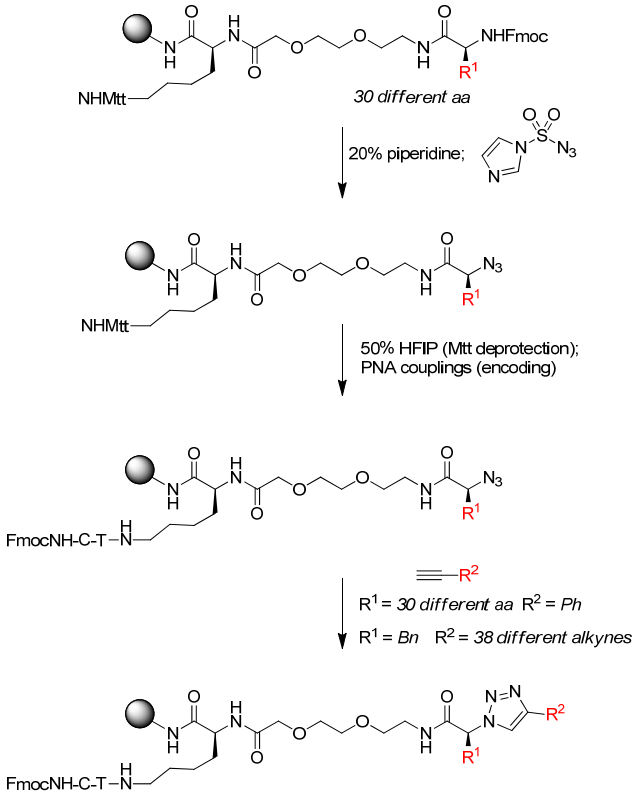
PNA-encoded heterocycles libraries, general scheme.



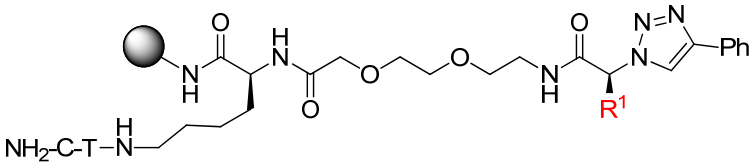
PNA-encoded heterocycles libraries, detail synthesis.



1.- Validation of the triazole library synthesis.



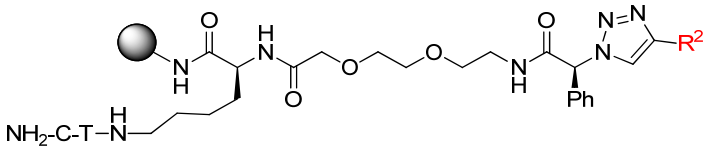
Click reaction validation, 30 aa reacted with Phenylacetylene followed by Fmoc deprotection.



NovaPEG Rink amine resin (500 mg) was loaded with FmocLys(Mtt)OH (***procedure 5)*** followed by capping of the un-reacted amine groups ***(procedure 1)***. Fmoc deprotection (***procedure 3)***, loading of the spacer (***procedure 6***) and second Fmoc deprotection (***procedure 3***) yielded a resin that was split on to 30 different columns (10 mg of resin per column) for the loading of 30 different aminoacids in the Multipep Synthesizer (***procedure 7*** followed by ***procedure 4***). Then, following ***procedure 8*** the Fmoc deprotected amino acids were oxidized to the corresponding azides. The orthogonal Mtt protecting group on the side chain of the Lys was then deprotected using the protocol described in ***procedure 9*** and monomers T and C were loaded onto all the different resins following ***procedure 10.*** Click reaction with phenyl acetylene following ***procedure 12,*** Fmoc deprotection ***(procedure 4***) and cleavage of the compounds from the resin ***(procedure 11)*** allowed the analysis of the different aminoacids by Maldi. Asp, L-2-amino-3-(dimethylamino)-propionic acid, Leu and Pro did not give 100% conversions and were discharge as building blocks for the library synthesis.

Entry.	Aminoacid used	m/z ($M+Na^+$) calculated	m/z ($M+Na^+$) found
1	2S,3S)-2-amino-3-methoxybutanoic acid	1175.23	1174.39
2	(S)-2-amino-3-methoxypropionic acid	1161.23	1160.48
3	Alanine (Ala)	1131.19	1130.48
4	allo-Thr	1161.19	1160.55
5	Arg	1194.30 ($M+H^+$)	1193.56 ($M+H^+$)
6	Asn	1174.19	1173.51
7	Asp	1175.22	X
8	D-Phe	1207.27	1206.46
9	Gln	1188.22	1187.48
10	Glu	1189.22	1188.62
11	Gly	1117.14	1116.50
12	His	1197.23	1196.49
13	Homoser	1161.19	1160.51
14	L-2-amino-3-(dimethylamino)-propionic acid	1174.23	X
15	L-4-Pyridylalanine	1208.23	1207.50
16	L-allylglycine	1135.22 ($M+H^+$)	1134.38 ($M+H^+$)
17	L-beta-t-butylalanine	1187.27	1186.53
18	L-cyclopropylglycine	1157.20	1156.45
19	Leu	1173.25	X
20	Ile	1173.25	1172.47
21	L-norTyr	1209.24	1208.46
22	Lys	1189.21	1187.51
23	Nle	1173.23	1172.46
24	Phe	1207.26	1206.53
25	Pro	1157.20	X
26	Ser	1147.16	1146.41
27	Thr	1161.22	1160.61
28	Trp	1247.29	1245.52
29	Tyr	1223.26	1222.47
30	Val	1159.23	1158.50

Click reaction validation, phenylalaline azide resin reacted with 38 different alkynes followed by Fmoc deprotection

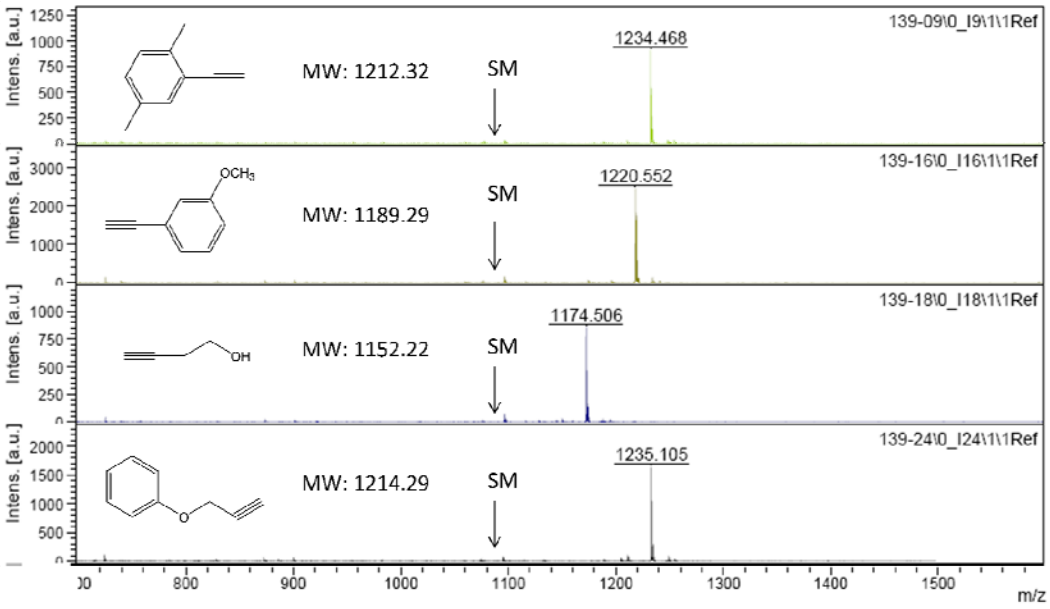


The corresponding resin loaded with phenylalanine was reacted with 38 different alkynes following ***procedure 12,*** Fmoc deprotection ***(procedure 4***) and cleavage of the compounds from the resin ***(procedure 11)*** allowed the analysis by Maldi of the cycloaddition reaction with the different alkynes. All of them with the exception of Propargylamine hydrochloride worked with total conversion.

Entry	Alkyne	m/z ($M+Na^+$) calculated	m/z ($M+Na^+$) found
1	4-Ethynylanisole	1236.58	1236.62
2	1-Ethynyl-2-methoxybenzene	1236.82	1236.62
3	2-Ethynylaniline	1221.81	1220.20
4	1-Ethynyl-4-phenoxybenzene	1298.89	1298.59
5	4-Methyl-1-pentyne	1186.80	1186.63
6	4-Ethynylbiphenyl	1282.89	1282.59
7	N-Methyl-N-propargylbenzylamine	1263.89	1263.63
8	3-Ethynylanisole	1236.82	1236.51
9	2-Ethynyl-1,4-dimethylbenzene	1234.85	1234.47
10	3-Cyclohexyl-1-propyne	1226.87	1226.56
11	3-Dimethylamino-1-propyne	1187.79	1187.64
12	N-Methylpropargylamine	1173.77	1172.26
13	2-Methyl-3-butyn-2-ol	1188.78	1188.65
14	Cyclopentylacetylene	1198.81	1198.62
15	2-Ethynyltoluene	1220.82	1220.53
16	3-Ethynyltoluene	1220.82	1220.56
17	4-Ethynylaniline	1221.81	1221.52
18	3-Butyn-1-ol	1174.75	1174.51
19	2-Ethynylbenzyl alcohol	1236.82	1236.53
20	1-Ethynylnaphthalene	1256.85	1256.55
21	4-Ethynyltoluene	1220.82	1220.47
22	Propargylamine hydrochloride	1196.20	X
23	3-Butyn-2-ol	1174.75	1173.23
24	Phenyl propargyl ether	1236.82	1235.11
25	Cyclopropylacetylene	1170.76	1169.23
26	4-Pentyn-1-ol	1188.78	1187.20
27	5-Methyl-1-hexyne	1200.83	1199.26
28	5-Methyl-1-hexyn-3-ol	1216.83	1215.19

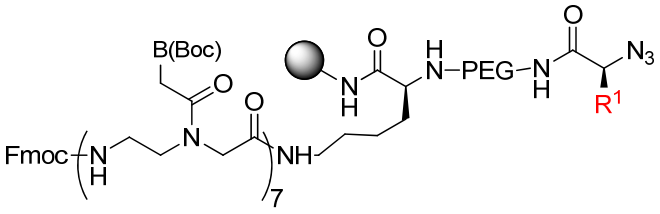
29	N-Propargylphthalimide	1289.84	1288.00
30	1-Pentyn-3-ol	1188.78	1187.23
31	3,4-Dichlorophenylacetylene	1275.68	1273.05
32	3-Hydroxyphenylacetylene	1222.79	1222.56
33	4-(Trifluoromethoxy) phenylacetylene	1290.79	1289.02
34	3-Ethynylaniline	1221.81	1220.15
35	Methyl propargyl ether	1174.75	1174.63
36	1-Ethynylcyclohexene	1210.83	1209.27
37	Phenylacetylene	1206.79	1205.19
38	1-heptyne	1200.83	1199.19

Selected examples from the validation of different alkynes in the cycloaddition reaction.



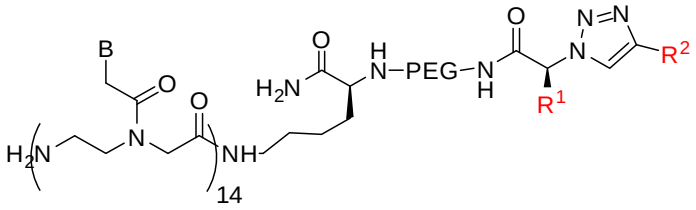
2.- 500 membered Triazole library synthesis

First point of diversity 25 different aminoacids. NovaPEG Rink amine resin (500 mg) was loaded with FmocLys(Mtt)OH (***procedure 5***) followed by capping of the un-reacted amine groups ***(procedure 1)***. Fmoc deprotection (***procedure 3***), loading of the spacer (***procedure 6***) and second Fmoc deprotection (***procedure 3***) yielded a resin that was split on to 25 different columns (10 mg of resin per column) for the loading of 25 different aminoacids in the Multipep Synthesizer (***procedure 7*** followed by ***procedure 4***). Then, following ***procedure 8*** the Fmoc deprotected amino acids were oxidized to the corresponding azides. The orthogonal Mtt protecting group on the side chain of the Lys was then de-protected using the protocol described in ***procedure 9*** and corresponding 7-mers were loaded onto all the different resins following ***procedure 10.***



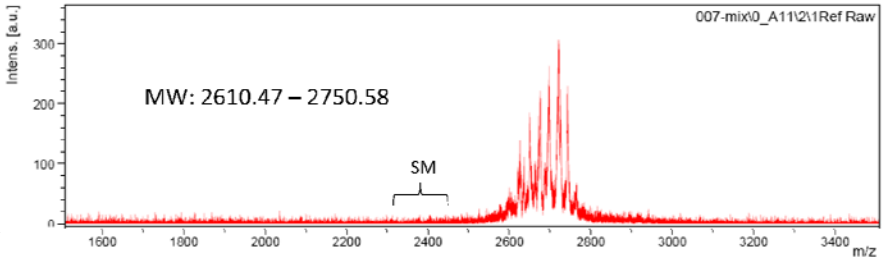
No.	Aminoacid	CODON	m/z calcul.	m/z found
1	2S,3S)-2-amino-3-methoxybutanoic acid	GCCG T*GG	2686.54	2687.04
2	(S)-2-amino-3-methoxypropionic acid	GCCG C*CG	2617.53	2617.26
3	Ala	GCCG G*CA	2611.48	2611.96
4	allo-Thr	GCCG C*GA	2641.50	2642.52
5	Arg	GCCG G*GC	2712.59	2713.13
6	Asn	GGAA T*GG	2733.55	2734.95
7	D-Phe	GGAA C*CG	2711.59	2712.13
8	Gln	GGAA G*CA	2716.55	2716.69
9	Glu	GGAA C*GA	2717.55	2718.02
10	Gly	GGAA G*GC	2661.47	2661.99
11	His	CGGC T*GG	2708.54	2709.62
12	Homoser	CGGC C*CG	2617.49	2618.06
13	L-4-Pyridylalanine	CGGC G*CA	2688.54	2689.13
14	L-beta-t-butylalanine	CGGC C*GA	2667.58	2668.16
15	L-cyclopropylglycine	CGGC G*GC	2653.51	2653.93
16	Leu	AAGG T*GG	2732.58	2733.07
17	Ile	AAGG C*CG	2677.57	2678.61
18	Lys	AAGG G*CA	2717.54	2717.11
19	Nle	AAGG C*GA	2701.56	X

20	Phe	AAGG G*GC	2751.58	2752.05
21	Ser	GAAC T*GG	2666.48	2667.02
22	Thr	GAAC C*CG	2625.54	2625.98
23	Trp	GAAC G*CA	2735.62	2736.02
24	Tyr	GAAC C*GA	2711.59	2712.10
25	Val	GAAC G*GC	2663.55	X



Second point of diversity, 20 different alkynes. The resins from the previous reactions were combined, thoroughly mixed and redistributed into 20 columns. Resin transfers were carried out as slurry in DCM/DMF using pipetting.

Maldi of the 25 different resins mix:

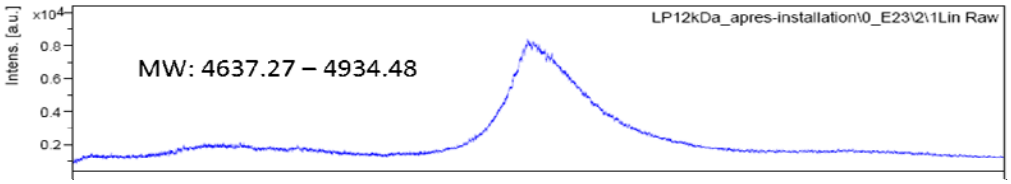


After click cycloaddition reaction with one alkyne per column (***procedure 12***) the second PNA codon (7mer) was introduced following ***procedure 10***. The success of the reactions in each pool was assessed by MALDI showing complete conversions.

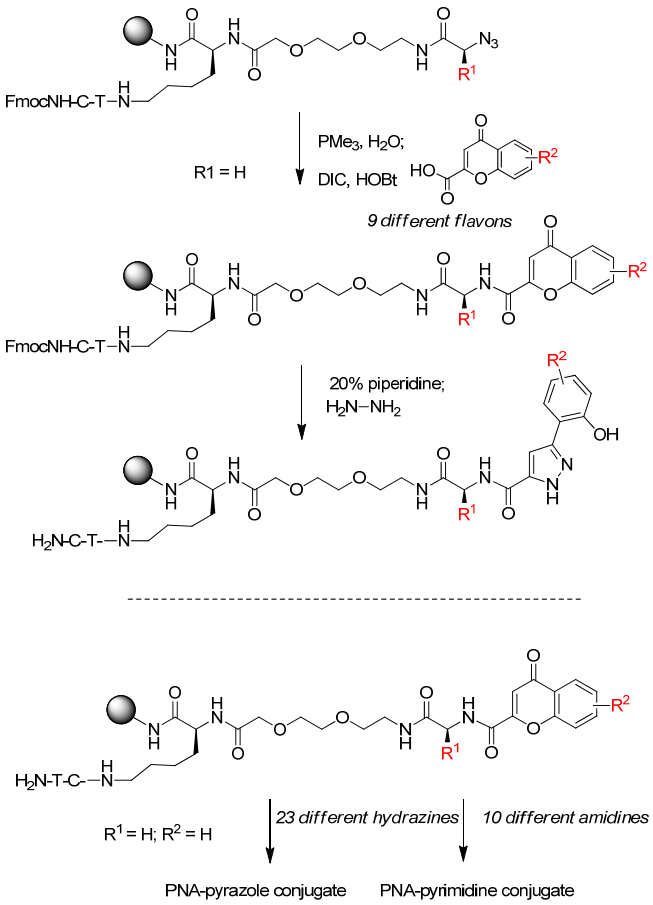
No.	**Alkyne**	**CODON**	**m/z ($M+H^+$) calcul. range**	**m/z ($M+H^+$) found range**
1	4-Ethynylanisole	GTG* CGAA	4717.31-4857.42	4700-4900
2	1-Ethynyl-2-methoxybenzene	GTG* GAGA	4757.32-4897.43	4750-4950
3	2-Ethynylaniline	GTG* CAGG	4718.30-4858.41	4700-4900
4	1-Ethynyl-4-phenoxybenzene	GTG* GACG	4795.38-4935.49	4750-4950
5	N-Methyl-N-propargylbenzylamine	GCA* CGAA	4713.39-4853.50	4700-4900

6	3-Ethynylanisole	GCA* GAGA	4726.33-4866.44	4700-4900
7	3-Cyclohexyl-1-propyne	GCA* CAGG	4692.36-4832.47	4650-4850
8	3-Dimethylamino-1-propyne	GCA* GACG	4653.28-4793.39	4650-4850
9	2-Methyl-3-butyn-2-ol	ACG* CGAA	4638.28-4778.39	4600-4800
10	4-Ethynylaniline	ACG* GAGA	4711.32-4851.43	4700-4900
11	3-Butyn-1-ol	ACG* CAGG	4640.24-4780.35	4600-4800
12	3-Butyn-2-ol	ACG* GACG	4640.24-4780.35	4600-4800
13	Phenyl propargyl ether	CGA* CGAA	4686.32-4826.43	4650-4850
14	Cyclopropylacetylene	CGA* GAGA	4660.27-4800.38	4650-4850
15	4-Pentyn-1-ol	CGA* CAGG	4654.27-4794.38	4650-4850
16	N-Propargylphthalimide	CGA* GACG	4755.33-4895.44	4750-4950
17	3-Hydroxyphenylacetylene	AGC* CGAA	4672.29-4812.40	4650-4850
18	3-Ethynylaniline	AGC* GAGA	4711.32-4851.43	4700-4900
19	Phenylacetylene	AGC* CAGG	4672.28-4812.39	4650-4850
20	1-heptyne	AGC* GACG	4666.32-4806.43	4650-4850

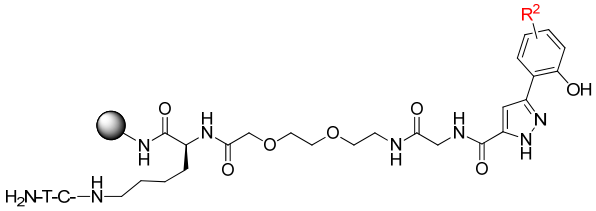
Library final MALDI.



3.- Validation of the pyrimidines and pyrazoles library.

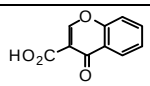
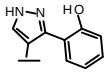
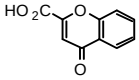
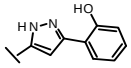
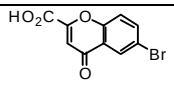
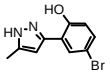
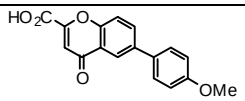
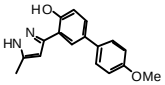
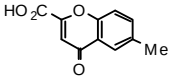
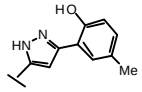
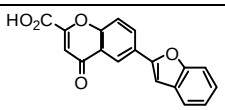
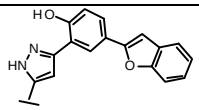
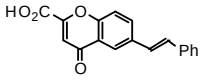
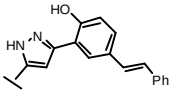
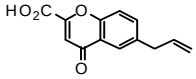
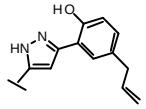
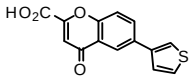
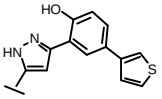


Reaction of 9 different flavones with hydrazine.

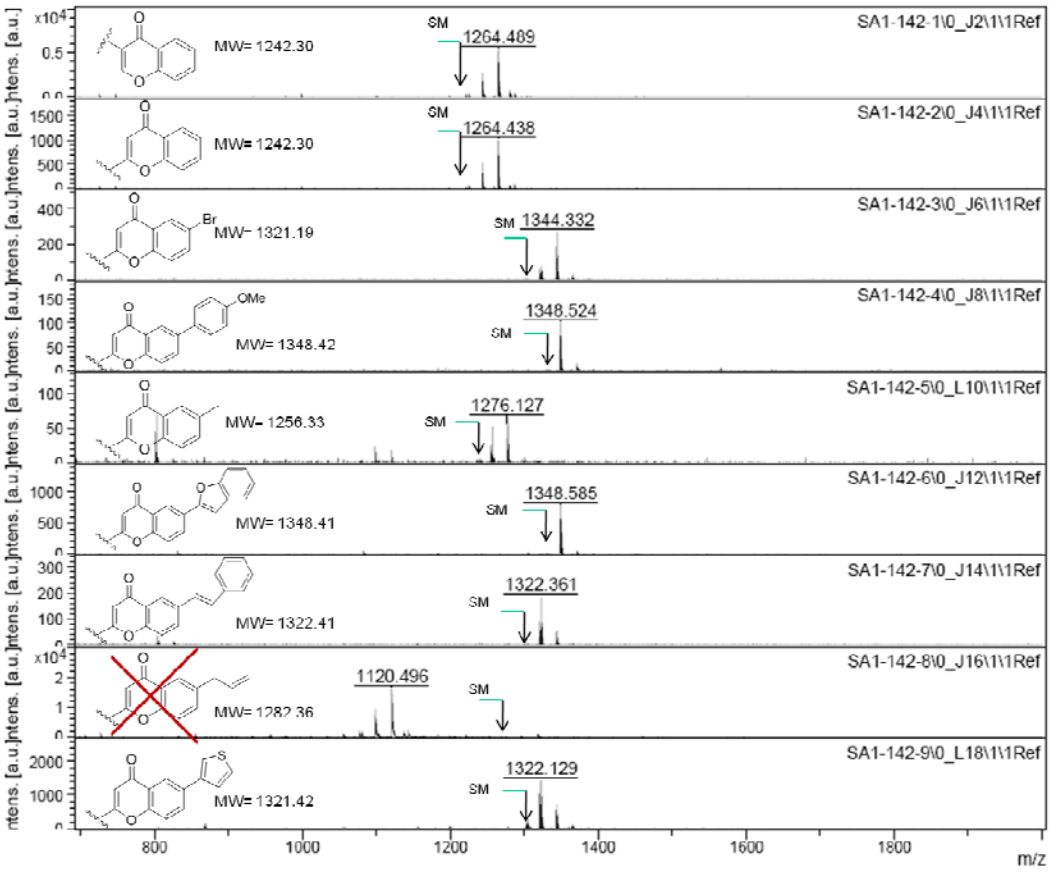


NovaPEG Rink amine resin was loaded with FmocLys(Mtt)OH (***procedure 5***) followed by capping of the un-reacted amine groups ***(procedure 1)***. Fmoc deprotection (***procedure 3)***, loading of the spacer (***procedure 6***) and second Fmoc deprotection (***procedure 3***) yielded a resin that was coupled with alanine (***procedure 7*** followed by ***procedure 4***) Then, following ***procedure 8*** the Fmoc deprotected amino acids were oxidized to the corresponding azides. The orthogonal Mtt protecting group on the side chain of the Lys was then de-protected using the protocol described in ***procedure 9*** and monomers T and C were loaded onto the resin following ***procedure 10.*** The resin was then distributed in five columns in the Multipep Synthesizer and the azides were

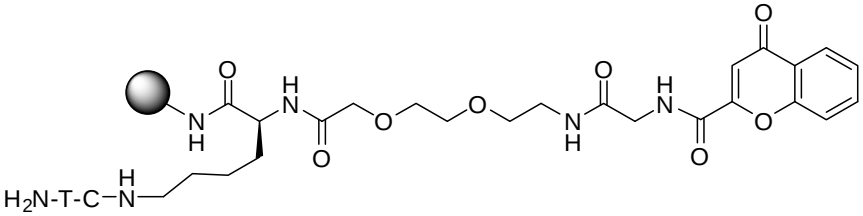
reduced (***procedure 15***). Then the resins were coupled with 9 different flavones following ***procedure 13*** and capped using ***procedure 2***. Finally the resins were reacted with hydrazine following ***procedure 14*** and the compounds were cleaved from the resin ***(procedure 11)*** and analyzed by Maldi. Flavone **5** and **8** did not give clean reaction product with hydrazine.

No.	Flavone	Compound	m/z ($M+Na^+$) calcul.	m/z ($M+Na^+$) found
1			1264.30	1264.49
2			1264.30	1264.44
3			1344.19	1344.33
4			1348.42 $M+H^+$	1348.52 $M+H^+$
5			1256.33 $M+H^+$	20% impurity
6			1348.41 $M+H^+$	1348.58 $M+H^+$
7			1322.41 $M+H^+$	1322.36 $M+H^+$
8			1282.36 $M+H^+$	X
9			1321.42 $M+H^+$	1322.13 $M+H^+$

Maldis for the validation of the 9 different flavones with hydrazine.

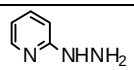
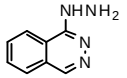
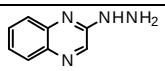
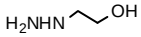
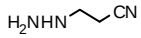
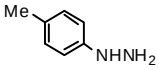
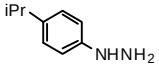
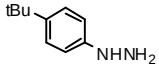
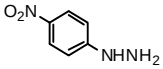
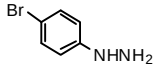
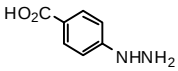
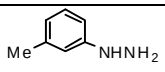
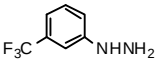
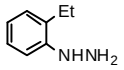
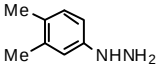
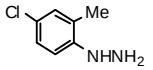


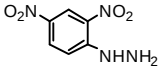
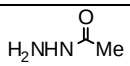
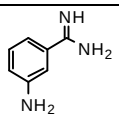
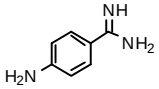
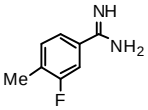
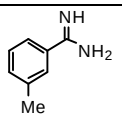
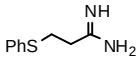
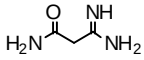
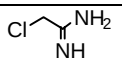
Reaction of flavone 1 with 23 hydrazines and 10 amidinium salts.



NovaPEG Rink amine resin was loaded with FmocLys(Mtt)OH (***procedure 5***) followed by capping of the un-reacted amine groups ***(procedure 1)***. Fmoc deprotection (***procedure 3)***, loading of the spacer (***procedure 6***) and second Fmoc deprotection (***procedure 3***) yielded a resin that was coupled with alanine (***procedure 7*** followed by ***procedure 4***) Then, following ***procedure 8*** the Fmoc deprotected amino acids were oxidized to the corresponding azides. The orthogonal Mtt protecting group on the side chain of the Lys was then deprotected using the protocol described in ***procedure 9*** and monomers C and T were loaded onto the resin following ***procedure 10.*** After azide reduction (***procedure 15***). flavon **1** was coupled to the resin followed by a capping protocol (***procedure 1***). The resin was then distributed onto 33 columns in the Multipep Synthesizer swollen in CH_2Cl_2 for 20 min and then reacted with 23 different hydrazines

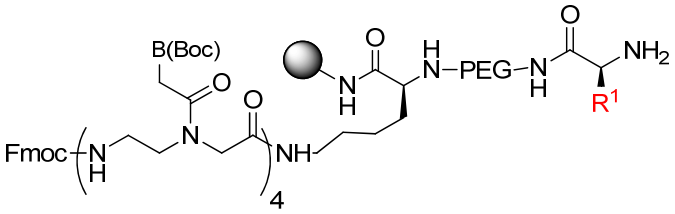
and 10 amidinium salts following ***procedure 14***. The compounds were cleaved from the resin ***(procedure 11)*** and analyzed by LC-MS.

Entry	Hydrazines or amidinium salts	m/z ($M+H^+$) calcul.	m/z ($M+H^+$) found
1	N_2H_4	204.32	205.21
2	$MeNHNH_2$	218.44	219.24
3	$PhNHNH_2$	280.14	281.22
4	$BnNHNH_2$	294.14	296.33
5	NH_2OH	205.99	206.33
6	tBuNHNH$_2$	260.34	261.25
7		281.35	282.35
8		332.18	333.44
9		332.18	333.33
10		248.10	249.30
11		255.66	256.19
12		313.44	314.55
13		358.68	359.76
14		372.14	373.17
15		361.60	362.77
16		395.50	396.20
17		324.15	325.55
18		313.44	314.43
19		348.36	349.22
20		321.34	322.24
21		344.66	345.66
22		320.45	321.24

23		370.14	371.22
24		246.18	247.77
25		266.50	267.44
26		328.61	329.61
27		380.09	381.90
28		380.09	381.44
29		360.63	361.74
30		342.86	343.76
31		374.71	375.22
32		273.13	274.33
33		276.59	X

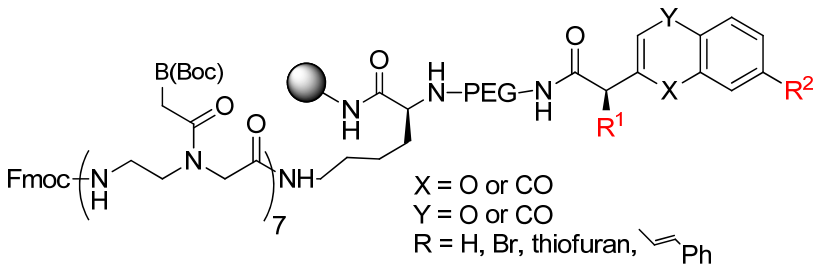
4.- 500 membered pyrimidines and pyrazoles library synthesis

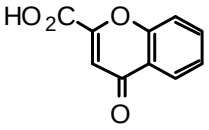
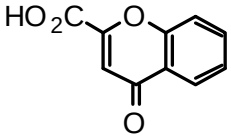
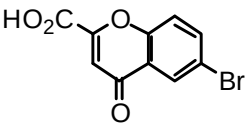
First point of diversity, 5 different aminoacids. NovaPEG Rink amine resin (250 mg) was loaded with FmocLys(Mtt)OH (***procedure***) followed by capping of the un-reacted amine groups ***(procedure 1)***. Fmoc deprotection (***procedure 3)***, loading of the spacer (***procedure 6***) and second Fmoc deprotection (***procedure 3***) yielded a resin that was split on to 5 different columns (50 mg of resin per column) for the loading of 5 different amino acids in the Multipep Synthesizer (***procedure 7*** followed by ***procedure 4***). Then, following ***procedure 8*** the Fmoc deprotected amino acids were oxidized to the corresponding azides. The orthogonal Mtt protecting group on the side chain of the Lys was then de-protected using the protocol described in ***procedure 9*** and corresponding 4-mers were loaded onto all the five different resins following ***procedure 10.*** Maldi analysis of an analytical cleavage from each resin confirmed the completion of each sequence.

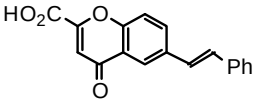
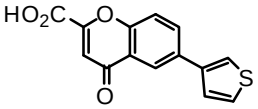


R^1= Phe (GCCG), Val (GGAA), Leu (CGGC), Ala (AAGG), Ile (GAAC)

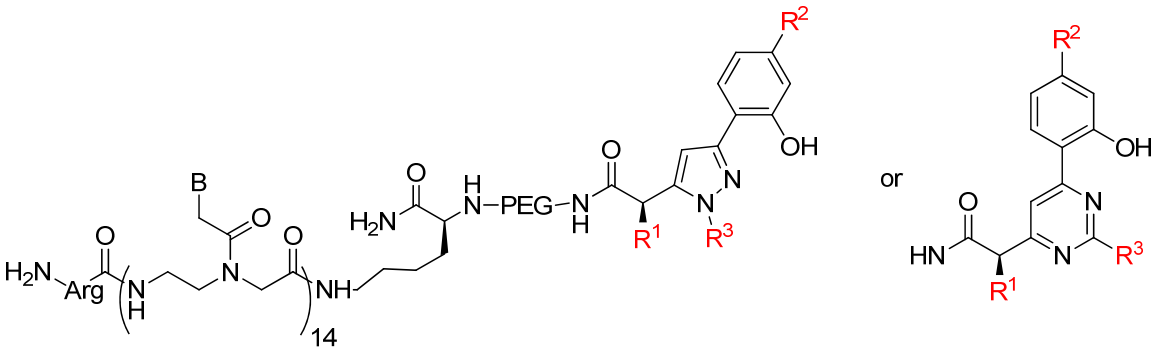
Second point of diversity, 5 different flavones. The 5 resins prepared in the previous step were suspended in 0.5 mL CH_2Cl_2 and mixed together in a SPE tube. The resin was shaken for 30 minutes, washed with 2 x CH_2Cl_2 and then the resin was distributed in 5 different columns in the Multipep Synthesizer. Then the azide group was reduced following ***procedure 15*** and each of the resins was coupled to a different flavon (***procedure 13***) and capped (***procedure 2***). PNA encoding according to ***procedure 10*** was then carried out.



Entry	Flavon	CODON	m/z ($M+Na^+$) calcul.	m/z ($M+Na^+$) found
1		T*GG	Phe: 2661.53 Val: 2691.43 Leu: 2693.42 Ala: 2691.44 Ile: 2691.43	Phe: 2661.92 Val: 2692.99 Leu: 2694.42 Ala: 2692.99 Ile: 2692.99
		C*CG	Phe: 2661.51 Val: 2661.43 Leu: 2774.41 Ala: 2680.43 Ile: 2762.43	Phe: 2661.92 Val: 2662.09 Leu: 2776.42 Ala: 2681.43 Ile: 2763.41
3		G*CA	Phe: 2930.42 Val: 2811.34 Leu: : 2877.32 Ala: 2883.34 Ile: 2885.34	Phe: 2931.93 Val: 2813.35 Leu: 2878.32 Ala: 2883.24 Ile: 2886.35

4		C*GA	Phe: 2834.62 Val: 2874.64 Leu: 2890.62 Ala: 2906.54 Ile: 2908.64	Phe: 2832.56 Val: 2874.54 Leu: 2891.45 Ala: 2905.54 Ile: 2909.54
5		G*GC	Phe: 2930.617 Val: 2930.5394 Leu: 2896.517 Ala: 2892.539 Ile: 2894.6332	Phe: 2932.312 Val: 2931.235 Leu: 2897.613 Ala: 2892.543 Ile: 2895.654

Third point of diversity, 20 different hydrazines or amidinium salts.

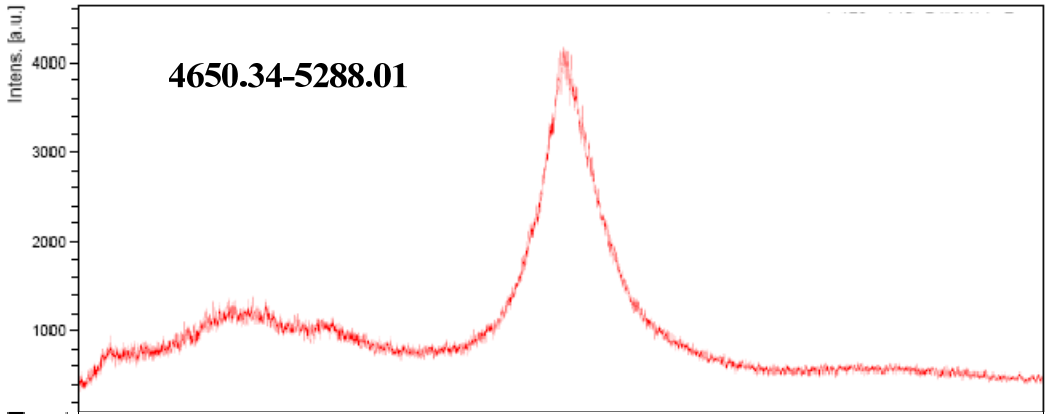


The five resin pools were mixed in a 12 mL tube, swollen with CH_2Cl_2 (20 min) and split in 20 columns (12.5 mg) in the Multipep Synthesizer. The corresponding amidine or hydrazine was coupled following ***procedure 14*** and after Fmoc deprotection (***procedure 4***) the corresponding 7mers were coupled following ***procedure 10***. Final Fmoc deportection (***procedure 4***) and cleavage of the compounds from the resin (***procedure 11***) gave 20 different pools. Each pool was analyzed by MALDI to confirm the completion of the reaction sequence.

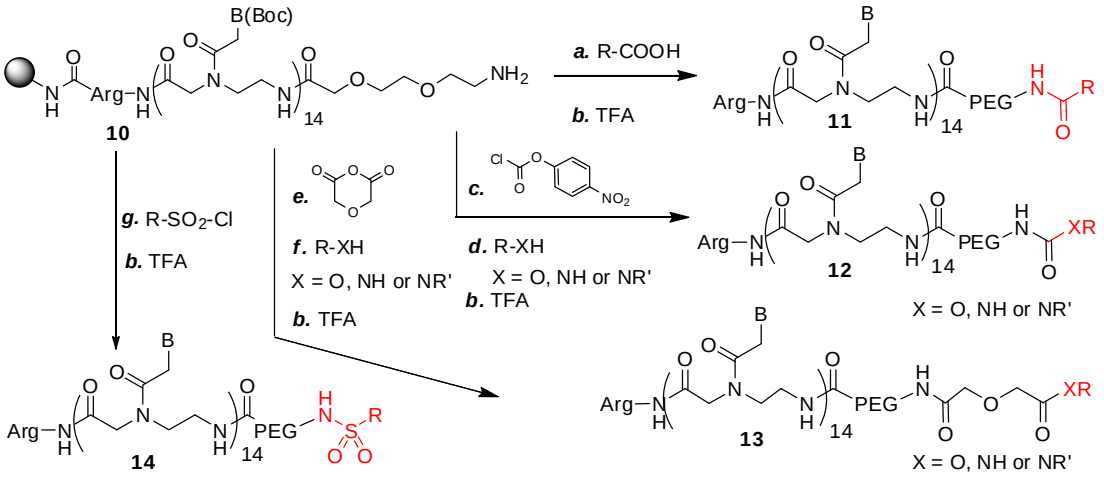
Entry	Hydrazine/ammidine	CODON	m/z ($M+Na^+$) calcul. range	m/z ($M+Na^+$) found
1	Hydrazine	GTGCGAA	4984.37-5140.50	4998.5-5249.17
2	Methylhydrazine	GTGGAGA	5038.39-5198.63	5050.34-5200.43
3	Hydroxilamine	GTGCAGG	5001.35-5161.59	5010.23-5176.33
4	2-Hydrozinopyridine	GTGGACG	5099.39-5257.63	5100.22-5260.13

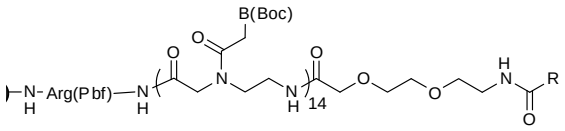
5	Hydralazine	GCAAGGC	5097.41-5257.64	5099.22-5288.01
6	p-Tolylhydrazine	GCACGAA	5043.42-5203.66	5050.22-5205.11
7	4-tButylhydrazine	GCAGAGA	5131.47-5291.70	5129.43-5300.21
8	4-Nitrophenylhydrazine	GCACAGG	5090.38-5250.62	5100.23-5260.33
9	4-Hydrazinobenzoic acid	ACGGACG	5089.39-5249.62	5090.22-5250.99
10	3-(Trifluoromethyl)phenylhydrazine	ACGAGGC	5113.39-5273.62	5110.88-5285.44
11	2-Ethylphenylhydrazine	ACGCGAA	5057.14-5217.37	5060.33-5216.44
12	3,4-Dimethylphenylhydrazine	ACGGAGA	5097.45-5257.68	5100.22-5260.44
13	4-Chloro-*o*-tolylhydrazine	CGACAGG	5094.07-5254.30	5098.33-5253.99
14	Acetamidine	CGAGACG	4995.54-5155.77	4998.33-5160.33
15	Benzamidine	CGAAGGC	5057.40-5217.63	5060.33-5210.44
16	3-Aminobenzamidine	CGACGAA	5056.63-5216.87	5070.33-5210.33
17	4-Aminobenzamidine	AGCGAGA	5096.64-5256.88	5098.22-5254.66
18	2-(Phenylthio)ethanimidamide	AGCCAGG	5071.41-5231.65	5077.22-5230.55
19	3-methylbenzamidine	AGCGACG	5038.39-5198.62	5035.44-5199.22
20	Malonamidine	AGCAGGC	5043.22-5100.23	5042.33-5102.33

Library final MALDI.

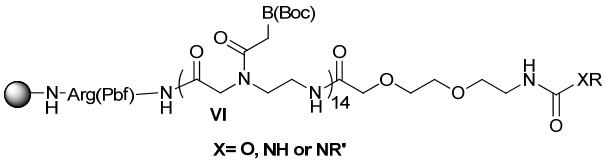


5. 125 membered N-terminus natural product/active compound library synthesis PNA prepared according to ***procedure 10*** were derivatized with commercially available pharmacophores by coupling of an acid, sulfonyl chloride, alcohol or amine.

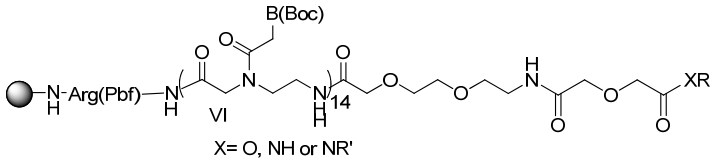




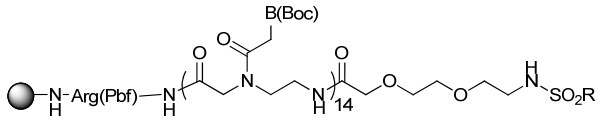
Carboxylic acid coupling. The corresponding carboxylic acid (0.01 mmol, 5.0 equiv) was dissolved in 200µL of NMP and HOBt (1.5 mg, 0.01 mmol, 5.0 equiv) followed by diisopropylcarbodiimide (4.7 µL, 0.03 mmol, 15.0 equiv) were added. The mixture was stirred for 15 min and then added to the corresponding resin. The reaction was shaken for 8 hours at r.t. Finally, the resin was washed with DMF and CH_2Cl_2.



Alcohol/amine coupling via chloroformate activation 4-nitrophenyl chloroformate (3.2 mg, 0.016 mmol, 8.0 equiv) and 2,6 lutidine (3.8 µL, 0.032 mmol, 16.0 equiv) were dissolved in 200 µL of 1,2-dichloroethane (solution A). 4-DMAP (2 mg, 0.016 mmol, 8 equiv) was dissolved in 28 µL of 1,2-dichloroethane (solution B). Solution A, followed by solution B were added to the corresponding resin and the reaction was shaken for 16h at r.t. The resin was subsequently washed with 1,2-dichloroethane, and the activation procedure was repeated a second time. Finally, the resin was washed with DMF and CH_2Cl_2. The alcohol or amine (0.07 mmol, 35.0 equiv), followed by DIPEA (23.1 µL, 0.14 mmol, 70.0 equiv – only for amine hydrochlorides) and DMAP (24.4 mg, 0.20 mmol, 100.0 equiv) were dissolved in 280 µL 1,2-dichloroethane. Then, the solution was added to the corresponding resin, and the reaction was shaken for 16 hours at 50°C. Finally, the resin was washed with DMF and CH_2Cl_2.

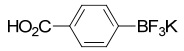
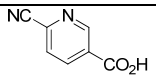
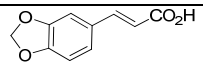
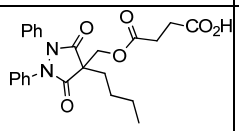
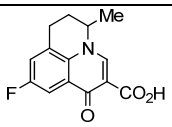
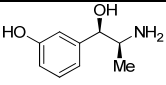
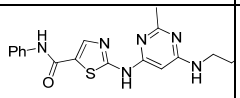
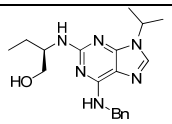
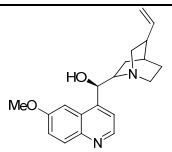
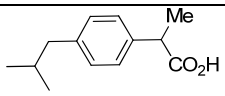
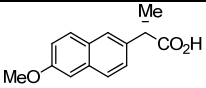
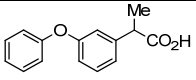
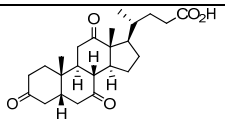
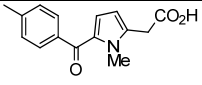


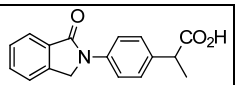
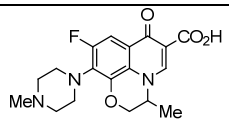
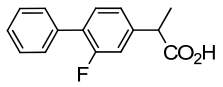
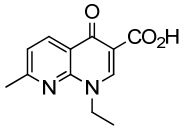
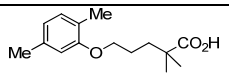
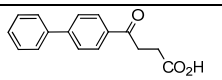
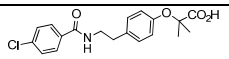
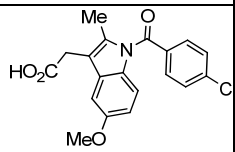
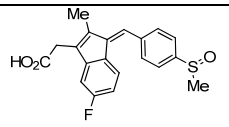
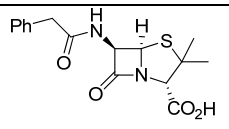
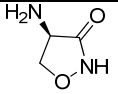
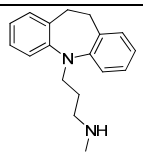
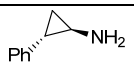
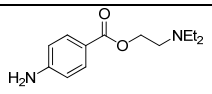
Alcohol/amine coupling via diglycolic anhydride Diglycolic anhydride (2.3 mg, 0.02 mmol, 10.0 equiv) and 2,6 lutidine (4.7 µL, 0.04 mmol, 20.0 equiv) were dissolved in 200 µL NMP. The solution was added to the resin and the reaction was shaken for 16 h at r.t. Finally, the resin was washed with DMF and CH_2Cl_2. BOP (7.1 mg, 0.016 mmol, 8.0 equiv), HOBt (2.5 mg, 0.016 mmol, 8.0 equiv) and DIPEA (4.2 µL, 0.024 mmol, 12.0 equiv) were dissolved in 100 µL NMP. The solution was added to the corresponding resin and the reaction was shaken for 20 min. Amine or alcohol (0.02 mmol, 10.0 equiv), followed by DIPEA (6.7 µL, 0.04 mmol, 20.0 equiv – only for amine hydrochlorides) and DMAP (4.9 mg, 0.040 mmol, 20 equiv) were dissolved in 100 µL NMP. The solution was subsequently added to the resin, after removing the activation solution, and the reaction was shaken for 16h at 35°C. Finally, the resin was washed with DMF and CH_2Cl_2.

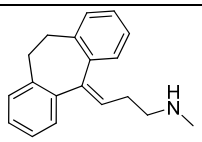
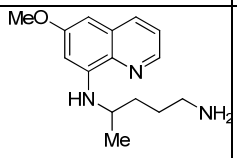
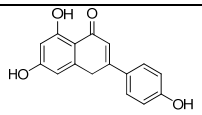
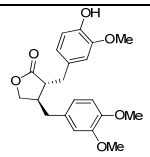
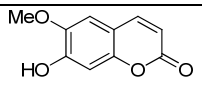
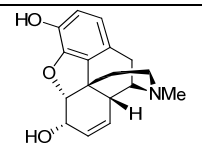
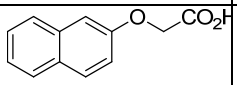
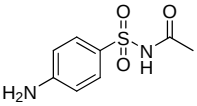
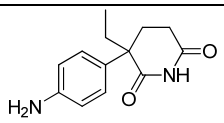
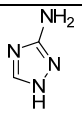
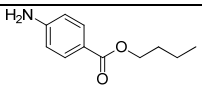
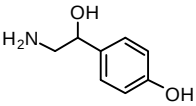
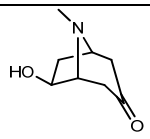


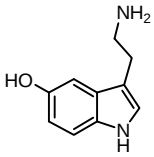
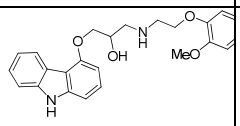
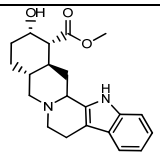
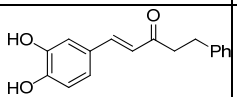
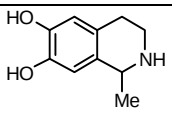
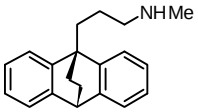
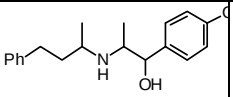
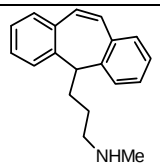
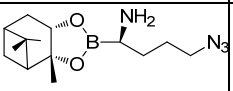
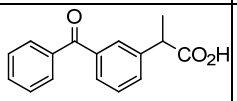
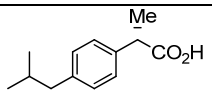
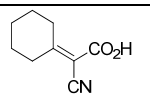
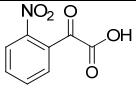
Sulfonyl chlorides coupling The resins were treated with a solution of the corresponding sulfonyl chloride (0.02 mmol, 10.0 equiv) and DIPEA (0.04 mmol, 20.0

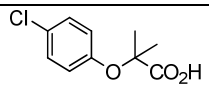
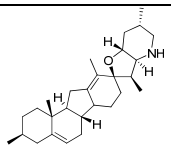
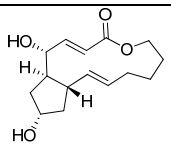
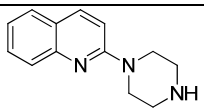
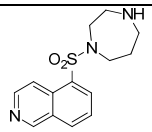
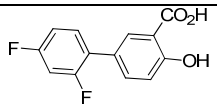
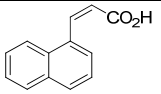
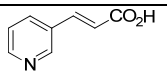
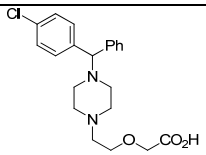
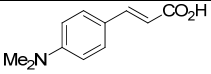
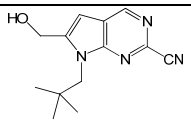
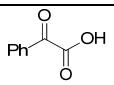
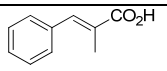
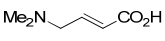
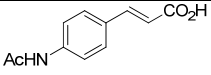
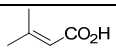
equiv) in 200µL of NMP. The reaction was shaken for 8 hours at r.t. Finally, the resin was washed with DMF and CH_2Cl_2.

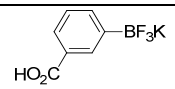
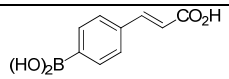
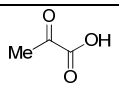
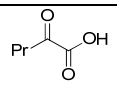
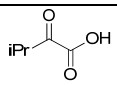
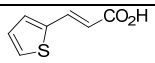
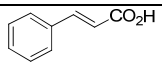
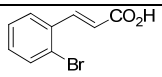
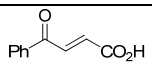
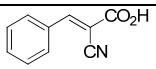
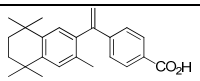
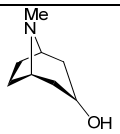
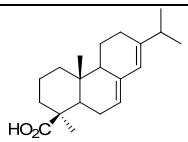
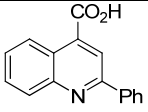
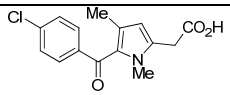
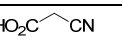
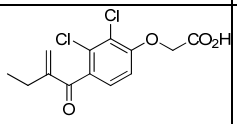
Nr.	PNA Sequence	Structure	Final [M+H$^+$]	
			calc	found
1	GCCG T*GG GTG* AGGC		4672.80 (+TFA)	4675.32
2	GCCG C*CG GTG* AGGC		4481.89	4479.17
3	GCCG G*CA GTG* AGGC		4550.04	4547.11
4	GCCG C*GA GTG* AGGC		4796.34	4792.74
5	GCCG G*GC GTG* AGGC		4635.16	4632.71
6	GCCG T*GG GCA* AGGC		4640.13	4645.32
7	GCCG C*CG GCA* AGGC		4788.31	4789.00
8	GCCG G*CA GCA* AGGC		4787.40	4787.55
9	GCCG C*GA GCA* AGGC		4766.32	4762.33
10	GCCG G*GC GCA* AGGC		4555.06	4547.43
11	GCCG T*GG ACG* AGGC		4588.13	4587.45
12	GCCG C*CG ACG* AGGC		4545.13	4545.94
13	GCCG G*CA ACG* AGGC		4729.40	4725.22
14	GCCG C*GA ACG* AGGC		4584.16	4581.27

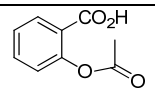
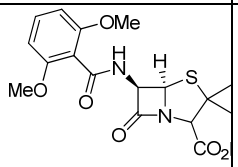
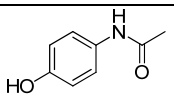
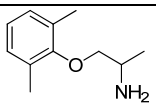
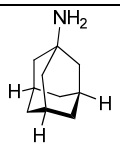
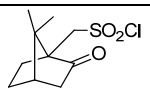
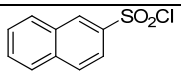
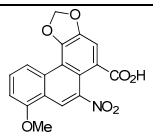
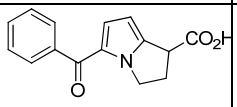
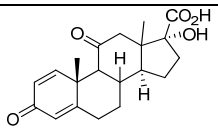
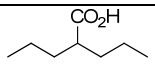
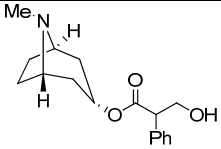
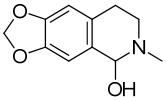
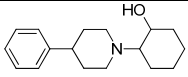
15	GCCG	G*GC	ACG*	AGGC		4624.17	4626.36
16	GCCG	T*GG	CGA*	AGGC		4719.28	4713.31
17	GCCG	C*CG	CGA*	AGGC		4547.17	4542.02
18	GCCG	G*CA	CGA*	AGGC		4559.16	4555.95
19	GCCG	C*GA	CGA*	AGGC		4577.22	4571.90
20	GCCG	G*GC	CGA*	AGGC		4597.19	4591.68
21	GCCG	T*GG	AGC*	AGGC		4719.67	4715.05
22	GCCG	C*CG	AGC*	AGGC		4660.66	4660.34
23	GCCG	G*CA	AGC*	AGGC		4683.28	4669.40
24	GCCG	C*GA	AGC*	AGGC		4661.28	4662.96
25	GCCG	G*GC	AGC*	AGGC		4561.03	4557.15
26	GGAA	T*GG	GTG*	AGGC		4747.60	4742.71
27	GGAA	C*CG	GTG*	AGGC		4559.30	4557.49
28	GGAA	G*CA	GTG*	AGGC		4686.43	4683.63

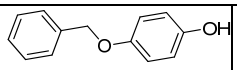
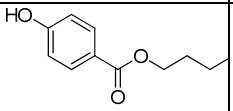
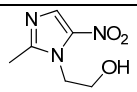
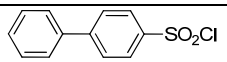
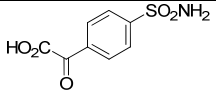
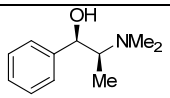
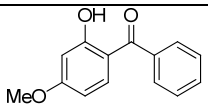
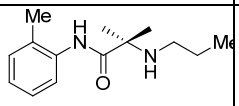
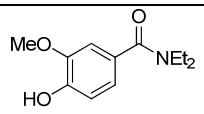
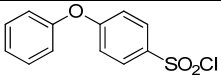
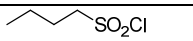
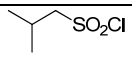
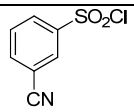
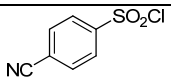
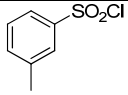
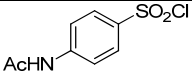
29	GGAA	C*GA	GTG*	AGGC		4713.10	4708.72
30	GGAA	G*GC	GTG*	AGGC		4725.50	4719.16
31	GGAA	T*GG	GCA*	AGGC		4720.32	4713.54
32	GGAA	T*CG	GCA*	AGGC		4767.57	4764.76
33	GGAA	G*CA	GCA*	AGGC		4611.34	4606.96
34	GGAA	C*GA	GCA*	AGGC		4704.51	4691.44
35	GGAA	G*GC	GCA*	AGGC		4593.06	4594.33
36	GGAA	T*GG	ACG*	AGGC		4664.40	4658.51
37	GGAA	C*CG	ACG*	AGGC		4627.47	4623.11
38	GGAA	G*CA	ACG*	AGGC		4503.25	4497.52
39	GGAA	C*GA	ACG*	AGGC		4612.41	4607.06
40	GGAA	G*GC	ACG*	AGGC		4588.30	4584.00
41	GGAA	T*GG	CGA*	AGGC		4677.11	4671.97

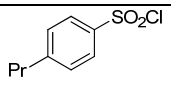
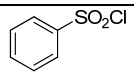
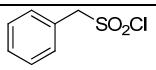
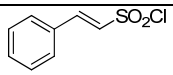
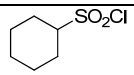
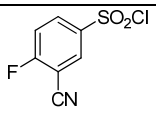
42	GGAA	C*CG	CGA*	AGGC		4571.34	4563.98
43	GGAA	G*CA	CGA*	AGGC		4825.64	4819.54
44	GGAA	C*GA	CGA*	AGGC		4845.32	4843.97
45	GGAA	G*GC	CGA*	AGGC		4719.46	4717.53
46	GGAA	T*GG	AGC*	AGGC		4629.34	4623.67
47	GGAA	C*CG	AGC*	AGGC		4672.52	4667.43
48	GGAA	G*CA	AGC*	AGGC		4718.54	4713.30
49	GGAA	C*GA	AGC*	AGGC		4682.51	4676.53
50	GGAA	G*GC	AGC*	AGGC		4754.07 ($-N_3$)	4755.89 ($-N_3$)
51	CGGC	T*GG	GTG*	AGGC		4643.20	4638.52
52	CGGC	C*CG	GTG*	AGGC		4540.19	4534.44
53	CGGC	G*CA	GTG*	AGGC		4523.10	4518.56
54	CGGC	C*GA	GTG*	AGGC		4567.07	4559.46

55	CGGC	G*GC	GTG*	AGGC		4588.55	4583.12
56	CGGC	T*GG	GCA*	AGGC		4885.56	4878.23
57	CGGC	C*CG	GCA*	AGGC		4699.33	4697.64
58	CGGC	G*CA	GCA*	AGGC		4656.22	4653.64
59	CGGC	C*GA	GCA*	AGGC		4734.27	4731.07
60	CGGC	G*GC	GCA*	AGGC		4593.02	4586.60
61	CGGC	T*GG	ACG*	AGGC		4556.13	4551.66
62	CGGC	C*CG	ACG*	AGGC		4452.05	4446.79
63	CGGC	G*CA	ACG*	AGGC		4715.77	4717.15
64	CGGC	C*GA	ACG*	AGGC		4518.15	4513.68
65	CGGC	G*GC	ACG*	AGGC		4631.22	4625.62
66	CGGC	T*GG	CGA*	AGGC		4507.90	4508.31
67	CGGC	C*CG	CGA*	AGGC		4465.04	4461.58
68	CGGC	G*CA	CGA*	AGGC		4427.99	4429.91
69	CGGC	C*GA	CGA*	AGGC		4532.09	4545.66
70	CGGC	G*GC	CGA*	AGGC		4442.99	4439.18

71	CGGC	T*GG	AGC*	AGGC		4641.81 (+TFA)	4638.31
72	CGGC	C*CG	AGC*	AGGC		4494.84	4500.19
73	CGGC	G*CA	AGC*	AGGC		4414.94	4412.73
74	CGGC	C*GA	AGC*	AGGC		4442.99	4441.29
75	CGGC	G*GC	AGC*	AGGC		4458.98	4459.86
76	AAGG	T*GG	GTG*	AGGC		4591.08	4586.79
77	AAGG	C*CG	GTG*	AGGC		4530.04	4524.98
78	AAGG	G*CA	GTG*	AGGC		4632.94	4629.51
79	AAGG	C*GA	GTG*	AGGC		4582.06	4584.59
80	AAGG	G*GC	GTG*	AGGC		4594.94	4584.84
81	AAGG	T*GG	GCA*	AGGC		4754.37	4751.86
82	AAGG	C*CG	GCA*	AGGC		4608.17	4602.29
83	AAGG	G*CA	GCA*	AGGC		4677.35	4677.25
84	AAGG	C*GA	GCA*	AGGC		4624.11	4618.83
85	AAGG	G*GC	GCA*	AGGC		4682.58	4677.23
86	AAGG	T*GG	ACG*	AGGC		4490.25	4488.14
87	AAGG	C*CG	ACG*	AGGC		4461.99	4460.05
88	AAGG	G*CA	ACG*	AGGC		4678.04	4672.26

89	AAGG	C*GA	ACG*	AGGC		4513.02 (-Ac)	4509.96
90	AAGG	G*GC	ACG*	AGGC		4771.31	4776.79
91	AAGG	T*GG	CGA*	AGGC		4601.32	4596.31
92	AAGG	C*CG	CGA*	AGGC		4574.38	4571.75
93	AAGG	G*CA	CGA*	AGGC		4570.38	4564.29
94	AAGG	C*GA	CGA*	AGGC		4625.64	4620.35
95	AAGG	G*GC	CGA*	AGGC		4617.57	4610.36
96	AAGG	T*GG	AGC*	AGGC		4747.16	4745.93
97	AAGG	C*CG	AGC*	AGGC		4606.16	4604.94
98	AAGG	G*CA	AGC*	AGGC		4733.32	4735.18
99	AAGG	C*GA	AGC*	AGGC		4519.11	4512.64
100	AAGG	G*GC	AGC*	AGGC		4724.50	4719.85
101	GAAC	T*GG	GTG*	AGGC		4648.34	4642.61
102	GAAC	C*CG	GTG*	AGGC		4645.50	4639.77

103	GAAC	G*CA	GTG*	AGGC		4610.39	4604.26
104	GAAC	C*GA	GTG*	AGGC		4604.39	4597.79
105	GAAC	G*GC	GTG*	AGGC		4597.30	4589.80
106	GAAC	T*GG	GCA*	AGGC		4600.17	4598.16
107	GAAC	C*CG	GCA*	AGGC		4512.08	4516.21
108	GAAC	G*CA	GCA*	AGGC		4658.23	4657.36
109	GAAC	C*GA	GCA*	AGGC		4679.20	4680.10
110	GAAC	G*GC	GCA*	AGGC		4687.23	4688.75
111	GAAC	T*GG	ACG*	AGGC		4633.43	4627.48
112	GAAC	C*CG	ACG*	AGGC		4561.16	4554.47
113	GAAC	G*CA	ACG*	AGGC	$Me_2N\text{-}SO_2Cl$	4460.04	4559.68
114	GAAC	C*GA	ACG*	AGGC		4473.08	4468.33
115	GAAC	G*GC	ACG*	AGGC		4489.08	4482.19
116	GAAC	T*GG	CGA*	AGGC		4549.07	4552.30
117	GAAC	C*CG	CGA*	AGGC		4494.07	4494.72
118	GAAC	G*CA	CGA*	AGGC		4507.10	4505.81
119	GAAC	C*GA	CGA*	AGGC		4550.12	4553.38

120	GAAC	G*GC	CGA*	AGGC		4551.15	4550.41
121	GAAC	T*GG	AGC*	AGGC		4524.07	4519.79
122	GAAC	C*CG	AGC*	AGGC		4483.09	4478.41
123	GAAC	G*CA	AGC*	AGGC		4519.11	4516.13
124	GAAC	C*GA	AGC*	AGGC		4499.12	4498.36
125	GAAC	G*GC	AGC*	AGGC		4552.07	4547.79