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Supporting Information

Synthesis and evaluation of MGB polyamide-oligonucleotide conjugates as gene expression control compounds

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Figure S1: Mass, ¹H-NMR and ¹³C-NMR spectra of the synthesized compounds.

Py₄-OMe (**4**) **Elemental Composition Report** Single Mass Analysis Tolerance = 10.0 mDa / DBE: min = -1.5, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 3 Monoisotopic Mass, Even Electron Ions 5 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass) Elements Used: C: 0-300 H: 0-3000 N: 7-7 O: 5-5 mt1-P55 M-18745 297 (2.999) AM2 (Ar,22000.0,0.00,0.00); ABS; Cm (275:305) 1: TOF MS ES+ 3.53e+007 506.2151 100-507.2198 % 508.2238 509.2270 510.2303^{511.4729} 504.3937 0 501.3752 502.3802 501.0 502.0 5 512.2222 m/z 505.2041 512.0 503.0 504.0 505.0 506.0 507.0 508.0 509.0 510.0 511.0 Minimum: Maximum: -1.5 300.0 10.0 5.0 Mass Calc. Mass mDa PPM DBE i-FIT Norm Conf(%) Formula 506.2151 506.2152 -0.1 -0.2 15.5 671.3 n/a n/a C25 H28 N7 05 Elemental Composition Report Single Mass Analysis Tolerance = 10.0 mDa / DBE: min = -1.5, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 3 Monoisotopic Mass, Even Electron Ions 5 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass) Elements Used: C: 0-300 H: 0-3000 N: 7-7 O: 5-5 Na: 1-1 mt1-P55 M-18745 297 (2.999) AM2 (Ar,22000.0,0.00,0.00); ABS; Cm (275:305) 1: TOF MS ES+ 3.41e+007 528,1973 100 529 2020 %-525.1884 525.6909 526.1931 527.1954 530.2060 535.0548 ----- m/z 531.2098 532.2126 533.6880 534.2457 523.2187 524.3146 0 526.0 528.0 530.0 524.0 532.0 534.0 Minimum Maximum -1.5 300.0 10.0 5.0 -------· ____ ---- ---- -Py₄-OMe (4) mt1-P55 M-18745 297 (2.999) AM2 (Ar,22000.0,0.00,0.00); ABS; Cm (275:305) 275,1499 1: TOF MS ES+ 8.78e7 506.2151 28.1973 297.1318 230 0921 313.1136 384 778 20 544.1711 607.1341 .651.1604 770.8108 385.1683 186.1024 889.3732 911.3545 970.3742

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700 750 900 950 1000

150 200

25



, Py ₄ -OH Elemental	(carboxylic Composition	c acid) Report				· · ·			
Single Mas Tolerance = Element pre Number of i	ss Analysis 10.0 mDa / I ediction: Off sotope peaks u	DBE: min = -1.5 sed for i-FIT = 3	, max = 300	.0.					
Monoisotopic 5 formula(e) Elements Use C: 1-300 Py40H	: Mass, Even Elec evaluated with 1 ed: H: 1-1000 N: 7	tron lons results within limi 7-7 O: 5-5	ts (up to 50 cl	osest resi	ults for ea	ch mass)			
M-12583 221 (1.785) AM2 (Ar,2200	0.0,0.00,0.00); ABS	Cm (189:226) 492.1993					1:	TOF M3 ES+ 1.41e+007
123.04 0	919 234.0867 	326.1604 398.240 	48.2089 51 3 5 450 500	14.1815 15.1844 550 60	597.2578 00 650	737.7841760. 700 750	2813 0 800	895.4120 9 850 900	39.4017 קיראקיקי m/z 950
Minimum: Maximum:		10.0 20.0	-1.5 300.0						
Mass	Calc. Mass	mDa PPM	DBE i	i-FIT	Norm	Conf(%)	Formula	1	
492.1993	492.1995	-0.2 -0.4	15.5 9	553.4	n/a	n/a	C24 H26	5 N7 O5	







Py₄-OH (carboxylic acid) ¹³C-NMR (DMSO-d₆)



Py₃-OMe (6) Elemental Composition Report

Single Mass Analysis (displaying only valid results) Tolerance = 15.0 PPM / DBE: min = -1.5, max = 40.0 Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron lons 34 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)







Py₃-OH (carboxylic acid) Elemental Composition Report Single Mass Analysis Tolerance = 10.0 mDa / DBE: min = -1.5, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 3 Monoisotopic Mass, Even Electron Ions 4 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass) Elements Used: C: 0-300 H: 0-1000 N: 5-5 O: 4-4 py3OH M-11932 MeOH 162 (1.308) AM2 (Ar,22000.0,0.00,0.00); ABS; Cm (133:169) 1: TOF MS ES+ 1.22e+008 370.1509 100-%-371.1544 326.1611 327.1643 338.3413 352.1401 0 Minimum: Maximum: -1.5 300.0 10.0 20.0 Mass Calc. Mass mDa PPM DBE i-FIT Norm Conf(%) Formula 370.1509 370.1515 -0.6 -1.6 11.5 835.8 n/a n/a C18 H20 N5 O4

Py₃-OH (carboxylic acid)





$\mathsf{FmocNH}(\mathsf{CH}_2)_2\mathsf{NH}_2 \cdot \mathsf{HCI}\ (\mathbf{8})$

Elemental Composition Report Single Mass Analysis Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0 Element prediction: Off Number of isotope peaks used for i-FIT = 3 Monoisotopic Mass, Even Electron Ions 4 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass) Elements Used: C: 1-300 H: 1-1000 N: 2-2 O: 2-2 Fmoc C 205 M-10247 57 (1.198) AM2 (Ar,30000.0,0.00,0.00); ABS; Cm (56:64) 1: TOF MS ES+ 8.71e+006 283.1455 100-279.0947 % 144.9845 179.0890 303,1391 345.0666 391.2847 182.9865 219.1757 252.9746 413.2672 429.2429 482.6499 m/z n 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 Minimum: Maximum: -1.5 50.0 3.0 20.0 Mass PPM Calc. Mass mDa DBE i-FIT Norm Conf(%) Formula 283.1455 283.1447 0.8 2.8 9.5 897.5 n/a n/a C17 H19 N2 O2

FmocNH(CH₂)₂NH₂ · HCI (8)





S11

FmocNH(CH ₂) ₄ NH ₂ ·HCI (10)								
Elemental Composition Report								
Single Mass Analysis Tolerance = 20.0 PPM / DBE: min = -1.5, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 3								
Monoisotopic Mass, Even Electron Ions 4 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass) Elements Used: C: 1-300 H: 1-1000 N: 2-2 O: 2-2 EmerC4								
HINGCON M-11599 200 (4.166) AM2 (Ar,22000.0,0.00,0.00); ABS; Cm (199:213)								
100- %- 312.1765								
152.0621 179.0849 196.1115 273.1668 333.1570 391.1625405.1785 519.2001 563.1091.607.1346	621.3455 m/z							
150 200 250 300 350 400 450 500 550 60	0							
Minimum: -1.5 Maximum: 100.0 20.0 300.0								
Mass Calc. Mass mDa PPM DBE i-FIT Norm Conf(%) Formula								
311.1752 311.1760 -0.8 -2.6 9.5 836.2 n/a n/a C19 H23 N2 O2								

 $\mathsf{FmocNH}(\mathsf{CH}_2)_4\mathsf{NH}_2\cdot\mathsf{HCI}\ (\textbf{10})$





 $\label{eq:main_state} FmocNH(CH_2)_4NH_2 \cdot HCI~(\textbf{10}) ~~^1\text{H-NMR}~(CD_3OD)$

 $\mathsf{FmocNH}(\mathsf{CH}_2)_5\mathsf{NH}_2 \cdot \mathsf{HCl}\ (\textbf{11})$

Elemental Composition Report

Single Mass Analysis (displaying only valid results) Tolerance = 10.0 PPM / DBE: min = -1.5, max = 40.0 Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions 113 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

FMOC 5 SANO 004 1	9 (0.442) AM (C	Cen,4, 80.00, Ar	,5000.0,520	.33,1.00,LS	10); Sm (SG,	5x1.00); Cm (1:2	0)	26-Oct-2009 1: TOF MS ES+
100 					325.1	925		0.0164
149.02 0	64 180.1026	205.0912 2:	39.1431	276.1246 ³	01.1458	327.0823 374 328.0874	.1950 375.2004 _{396.1808}	472.6779
140	160 180	200 220	240 26	0 280	300 320	340 360	380 400 420 4	40 460
Minimum: Maximum:		10. 0	10.0	-1.5 40.0				
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula		
325. 1925	325. 1916	0.9	2.8	9.5	1	C20 H25 N2	02	

FmocNH(CH₂)₅NH₂ HCl (11)





Py₄-NH(CH₂)₄NHFmoc (13) Elemental Composition Report

Single Mass Analysis (displaying only valid results) Tolerance = 10.0 PPM / DBE: min = -1.5, max = 40.0 Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

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Monoisotopic Mass, Odd and Even Electron Ions 196 formula(e) evaluated with 4 results within limits (all results (up to 1000) for each mass) 26-Oct-2009 1: TOF MS ES+ 761 FMOC 4PY SANO 001 17 (0.313) AM (Cen,3, 80.00, HL5000.0,0.00,0.80); Sm (SG, 2x3.00); Cm (13:17) 784,3578 100-% 786.3648 806.3401 808.3523 787.3762 801.4028 823.3435 m/z _809.3569 ٥Ľ 772.4623 760.0 770.0 780.0 790.0 800.0 810.0 820.0 Minimum: Maximum: -1.5 40.0 10.0 10.0 Mass Calc. Mass mDa PPM DBE Score Formula 784. 3571 784. 3601 784. 3547 784. 3625 0.7 -2.2 3.1 -4.6 25. 5 26. 0 22. 5 29. 0 0.9 -2.8 4.0 -5.9 784. 3578 C43 H46 N9 O6 C48 H49 N4 O5 Na C41 H47 N9 O6 Na C50 H48 N4 O5 3 2 4 1

Py₄-NH(CH₂)₄NHFmoc (13)





Py₄-NH(CH₂)₅NHFmoc (14) Elemental Composition Report

Single Mass Analysis (displaying only valid results) Tolerance = 10.0 PPM / DBE: min = -1.5, max = 40.0 Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions 198 formula(e) evaluated with 5 results within limits (all results (up to 1000) for each mass)



Py₄-NH(CH₂)₅NHFmoc (14)





S19

Py₃-NH(CH₂)₄NHFmoc (15) **Elemental Composition Report**

Single Mass Analysis (displaying only valid results) Tolerance = 10.0 PPM / DBE: min = -1.5, max = 40.0 Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron lons 26 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)









4-O2NIm-OEt (21) **Elemental Composition Report**

Single Mass Analysis (displaying only valid results) Tolerance = 15.0 PPM / DBE: min = -1.5, max = 40.0 Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions 27 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)





4-O2NIm-OEt (21)



S23

5-O₂NIm-OEt (22) Elemental Composition Report

Single Mass Analysis (displaying only valid results) Tolerance = 7.0 PPM / DBE: min = -0.5, max = 100.0 Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Even Electron lons 213 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

saka-120 by SAKATA_00 100- 100- 100-	04 59 (1.359) AM 200.06	(Cen,4, 80.00 69	, Ar,5000.0	,556.28,0.00),LS 10); Sm	(SG, 2x3.00); Sb (1	,40.00); Cm	(32:63)	1: TOF MS ES+ 2.70e4
%-	179.0718	01.0705 1223.0557	345.	0218 399.	1231421.106	ⁱ⁶ 455.0674 51:	3.0741	614.2817	705.9622
0	150 200	250	300	350	400	450 500	550	600	650 700
Minimum: Maximum:		10. 0	7.0	-0.5 100.0					
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula			
200. 0669	200. 0671	-0. 2	-1.2	4.5	1	C7 H10 N3 (D4		







Im-OH (23) Elemental Composition Report

Single Mass Analysis Tolerance = 20.0 PPM / DBE: min = -1.5, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions 2 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass) Elements Used: C: 1-300 H: 1-1000 N: 2-2 O: 2-2 OTUKI-34 M-9682 55 (0.665) Cn (Cen,4, 80.00, Ar); Sm (SG, 2x3.00); Sb (1,40.00); Cm (22:56)



1: TOF MS ES+ 7.97e+003





Elemental Composition Report Single Mass Analysis Tolerance = 20.0 PPM / DBE: min = -1.5, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 3 Monoisotopic Mass, Even Electron Ions 2 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass) Elements Used: C: 1_300 H: 1-1000 N: 3-3 O: 2-2 M-1040 11, 1 (200 12, 200 12, 200 24) M-10184 15 (0.196) Cn (Cen.4, 80.00, Ar); Sm (SG, 2x3.00); Sb (1,40.00); Cm (15:41) 1: TOF MS ES+ 1.25e+004 170.0938 100-j 232.0153 142.0626 %-124.0495 234.0144 247.9750 168.0432 184.0735 186.0893 103.9510 263.0532 273.0444 215.0515 L.44 280 290 m/z 210 220 dul. Ĺ Load 190 200 110 120 130 140 150 160 170 180 230 240 250 260 270 Minimum: Maximum: -1.5 300.0 100.0 20. 0 Mass Calc. Mass mDa PPM DBE i-FIT Formula C7 H12 N3 O2 170.0938 170.0930 0.8 4. 7 3.5 n/a



4-H₂NIm-OEt (24)





4-BocHNIm-OEt (25) Elemental Composition Report

Single Mass Analysis (displaying only valid results) Tolerance = 15.0 PPM / DBE: min = -1.5, max = 40.0 Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%



4-BocHNIm-OEt (25)





4-BocHNIm-OH (26) Elemental Composition Report

Single Mass Analysis Tolerance = 20.0 PPM / DBE: min = -1.5, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 3

aki-81 M-10182 78 (0.942) AM (Cen,4, 80.00, Ar,8500.0,556.28,0.00,LS 10); Sm (SG, 2x3.00); Sb (1,40.00); Cm (78:104) 1: TOF MS ES+ 3.91e+004 264.0950 100 %-

0-+	224.001 	6	242.1 239.9736 240.0	247. 140 + - _†	9742 249.9735 263 250.0	264	1.3676 2 270.0	280.0708	286.0	0792 288.9993 290.0	304.037	78 <u>307.0363</u> 310.0
Minimum Maximum	:		100. 0	20. 0	-1.5 300.0							
Mass	Calc.	Mass	mDa	PPM	DBE	i-FIT	Fo	rmula				
264, 095	0 264.09	60	-1.0	-3.8	4.5	70.3	C1	0 H15	N3 04	l Na		







BocHNIm₂-OEt (27)

Elemental Composition Report

Single Mass Analysis (displaying only valid results) Tolerance = 20.0 mDa / DBE: min = -0.5, max = 100.0 Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron lons 4 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)











H₂NIm₂-OEt Elemental Composition Report

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Single Mass Analysis (displaying only valid results) Tolerance = 20.0 PPM / DBE: min = -0.5, max = 100.0 Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron lons 31 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

SAKA-199 SAKATA_007 614 (14.090) AM (Cen,4, 80.00, Ar,5000.0,556.28,0.00,LS 10); Sm (SG, 2x3.00); Sb (1,40.00); Cm (579:636) 1: TOF MS ES+ 293.1365 4.40e4 % 149.0071 147.0203 158.0047 315.1187 125 198.9851 241.0036 316.1214 198.9851 241.0036 316.1214 391.2860 437.1937.453.1647 532.7792 581.2328 m/z 200 252 250 275 300 325 350 375 400 425 450 475 500 525 550 575 122.0612 0-100 125 150 175 Minimum: Maximum: -0.5 100.0 10.0 20.0 PPM DBE mDa Mass Calc. Mass Score Formula 293. 1365 293. 1362 1.0 7.5 1 C12 H17 N6 O3 0.3






Im₃-OEt (28) Elemental Composition Report

Single Mass Analysis (displaying only valid results) Tolerance = 5.0 PPM / DBE: min = -0.5, max = 100.0 Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Even Electron Ions 42 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

SAKA-194 SAKATA_008 599 (13.741) AM (Cen,4, 80.00, Ar,5000.0,556.28,0.00,LS 10); Sm (SG, 2x3.00); Sb (1,40.00); Cm (581:611) 1: TOF MS ES+ 400, 401,1682 2.85e4

%-						
]					402.1725	⁵ 423.1496
323	.1129 349.065	362.9284	386.8771	398.9253	403.1726	6 424.1530 437.1936 453.1691 472.8527 492.8696
320 3	330 340 350	360 3	70 380	390 40	0 410	420 430 440 450 460 470 480 490
Minimum: Maximum:		10.0	5.0	-0,5 100.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
401. 1682	401.1686	-0.4	-0. 9	11.5	1	C17 H21 N8 O4









Im₃-OH (carboxylic acid)





Im₃-NH(CH₂)₃NHFmoc (29) Elemental Composition Report

Single Mass Analysis (displaying only valid results) Tolerance = 20.0 mDa / DBE: min = -0.5, max = 100.0 Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron lons 7 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

SAKA-147 SAKATA_01	0 92 (2.111) Cn (C	Cen,4, 80.00, Ar); Sm	n (SG, 2x3.00); Sb (1,40.00);	Cm (87:92) 651.2820 652.2867	1: TOF MS ES+ 6.44e3
		429.211	3		
301.1	424 337,1270	413.2625	37.1942	637.3115 653.2903	
0-4	···	·····	485.1773 515.2114	581.2156 712.	1794 753.2825
275	300 325 350	375 400 425	450 475 500 525 550	575 600 625 650 675 700	725 750 775
Minimum: Maximum:		20. 0 50. 0	-0.5 100.0		
Mass	Calc. Mass	mDa PPM	DBE Score	Formula	
651. 2820	651.2792	2.9 4.4	21.5 1	C33 H35 N10 05	

Im₃-NH(CH₂)₃NHFmoc (29)





Im₃-NH(CH₂)₄NHFmoc (**30**) **Elemental Composition Report**

Single Mass Analysis (displaying only valid results) Tolerance = 20.0 mDa / DBE: min = -0.5, max = 100.0 Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions 7 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

SAKA SAKA	-164 TA_01	1 251 (5.	762) Cn ((Cen,4, 80	.00, Ar); Sm (SG, 2x3.00)); Sb (1,40.00); Cm (249:2	71) 665	5.2973	1: TOF MS ES+ 2.10e4
%-			443.2	2272				651	.2813	666.3003	
0		429. 402.207	2116 6	444.2300 (469.2) 511 039 ^{485.1731}	542.25 3.2515 54	23 \$3.2560 	641.29 12 637.3088		667.3026 688.2905	755.4022
Minim	38 :	0 400	420 440	0 460	480 500	520 540 -0 5	560 580	600 620	640 66	60 680 700 720	740 760
Maxim	ເທີ			20. 0	50.0	100.0					
Mass		Calc. M	lass	mDa	PPM	DBE	Score	Formula			
665. 2	973	665. 294	18	2.5	3. 7	21.5	1	C34 H37	N10 0	5	









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Im₃-NH(CH₂)₅NHFmoc (**31**) Elemental Composition Report

Single Mass Analysis (displaying only valid results) Tolerance = 20.0 mDa / DBE: min = -0.5, max = 100.0 Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions 7 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)









Py3-NH(CH2)3CO2Et (32) **Elemental Composition Report**

Single Mass Analysis Tolerance = 20.0 PPM / DBE: min = -1.5, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions 5 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass) Elements Used: C: 1-300 H: 1-1000 N: 6-6 O: 5-5 yu-Py3GABA M-8434 98 (1.167) AM (Cen,4, 80.00, Ar,8500.0,556.28,0.00,LS 10); Sm (SG, 2x3.00); Sb (1,40.00); Cm (95:129)



Py3-NH(CH2)3CO2Et (32)





Elemental Composition Report Single Mass Analysis Tolerance = 20.0 PPM / DBE: min = 0.5, max = 500.0 Element prediction: Off Number of isotope peaks used for i-FIT = 4 Monoisotopic Mass, Even Electron Ions 5 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass) Elements Used: C: 1-300 H: 1-1000 N: 6-6 O: 5-5 koga-156 KOGAWARA_002 44 (0.537) Cn (Cen,4, 80.00, Ar); Sm (SG, 2x3.00); Cm (2:44) 1: TOF MS ES+ 1.18e+005 455,2039 100 %-456.2155 <u>______353.1508</u> 393.0825 415.2184 437.2000 ___________ 340 360 300 477.1916 509.1299 508.1256 511.1322 541.1267_{573.2}147<u>581.2505 625.3006</u> 50 480 500 520 540 560 580 600 620 0-460 Minimum: Maximum: 0.5 500.0 100.0 20.0 PPM DBE i-FIT -0.9 12.5 1368.3 mDa PPM Mass Calc. Mass Formula 455. 2039 455. 2043 -0.4 C22 H27 N6 O5



Py3-NH(CH2)3CO2H





BocHNIm₃-OEt (**33**) **Elemental Composition Report**

Single Mass Analysis Tolerance = 20.0 PPM / DBE: min = 0.5, max = 500.0 Element prediction: Off Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions 8 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass) Elements Used: C: 1-300 H: 1-1000 N: 9-9 O: 6-6 1271: 0-1

koga-147 OGASAHARA_023 82 (0.982) Cn (Cen,4, 80.00, Ar); Sm (SG, 2x3.00); Cm (71:112) 1: TOF MS ES+ 1.97e+004 516.2318

100-7				10.2010					
-									
%-				517	2355		538.	2145	
-					2000			520 2177	
4	97.1862 499.2000	507.1181.50 .501.6994	9.1191		523.1273 5	25.1239	532.7549	555.2111	544.7370 554.1888
0-L	م بالبلاية باب به	للبريدية بالغانغ	ىرە بەنۇ بال ىيار. 510.0	بد پنالالار ب ب 5	հեփ խի սին 20.0		(+-+- - +-+-+-+	יי קו קי קי קייון ול, 540.0	m/z - _{זייליטע} נייעייייייייייייייייייייי 550.0
Min incont								0.000	
Max imum:		100. 0	20. 0	500. 0					
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula			
516. 2318	516. 2319	-0.1	-0. 2	12.5	523.7	C22 H30	N9 06		

BocHNIm₃-OEt (33)





H₂NIm₃-OEt Elemental Composition Report Single Mass Analysis Tolerance = 10.0 mDa / DBE: min = -1.5, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 3 Monoisotopic Mass, Even Electron Ions 4 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass) Elements Used: C: 0-300 H: 0-1000 N: 9-9 O: 4-4 NH2Im3OEt M-12011 192 (1.549) AM2 (Ar,22000.0,0.00,0.00); ABS; Cm (192:213) 1: TOF MS ES+ 8.81e+007 416.1788 100-% 417.1812 293.1355319.1144 170.0923 208.5932 564.0922 636.1185 708.3082 746.9890.791.4328 550 600 650 700 750 800 467.0276 10 500 150 200 250 450 350 400 Minimum: Maximum: -1.5 10.0 20.0 300.0 Mass Calc. Mass mDa PPM DBE i-FIT Norm Conf(%) Formula 416.1788 416.1795 -0.7 -1.7 11.5 724.1 n/a n/a C17 H22 N9 04







Py₃-γ-Im₃-OEt (**34**) Elemental Composition Report

Single Mass Analysis Tolerance = 20.0 PPM / DBE: min = -1.5, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 3

 Monoisotopic Mass, Even Electron Ions

 8 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)

 Elements Used:

 C: 1-300
 H: 1-1000

 N: 15-15
 O: 8-8

 yu-199cru

 M:7395-179 (0.551) AM (Cen,4, 80.00, Ar,8500.0,556.28,0.00,LS 10); Sm (SG, 2x3.00); Sb (1,40.00); Cm (79:119)
 1: TOF N



Py₃-γ–Im₃-OEt (**34**)













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Py₃-γ-Im₃-NH(CH₂)₂NHFmoc (**35**) **Elemental Composition Report**

Single Mass Analysis Tolerance = 20.0 PPM / DBE: min = -1.5, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions 10 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass) Elements Used: C: 1-300 H: 1-1000 N: 17-17 O: 9-9 P160C2 M-9249 187 (2.223) AM (Cen.4, 80.00, Ar,8500.0,556.28,0.00,LS 10); Sm (SG, 2x3.00); Sb (1,40.00); Cm (183:216) 1: TOF MS ES+ 1.20e+004

100-	1089,4625									
					1090.47	05				
1				1088.460	4					
%-					1091.4	802				
					1092	.4832				
0 1056	5.5332 1062.4	150	1081.465	1087.4541	10	93.4824	105.4641	1111.4550	1121.4625	m/z
	1060.0	1070.0	່ 108 [່]	0.0	1090.0	1100.0	. 111 111	D.O '	1120.0	
Minimum: Maximum:		100. 0	20. 0	-1.5 300.0						
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula				
1088. 4604	1088. 4603	0.1	0.1	34. 5	4443. 2	C54 H58	N17 09			

Py₃-γ-Im₃-NH(CH₂)₂NHFmoc (35)





Py₃-γ-Im₃-NH(CH₂)₃NHFmoc (**36**) **Elemental Composition Report**

Single Mass Analysis Tolerance = 20.0 PPM / DBE: min = -1.5, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions 10 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass) Elements Used: C: 1-300 H: 1-1000 N: 17-17 O: 9-9

M-8017 616 (7.298) AM (Cen.4, 80.00, Ar,8500.0,556.28,0.00,LS 10); Sm (SG, 2x3.00); Sb (1,40.00); Cm (596.630) 1: TOF MS ES+ 3.22e+004 437.1893 100₇

%- - - - - - - - - - - - - - - - - - -	415.2107 344 321.1278) 	655 637.3041 453.1706 551.7335 551.7335 600	9.2857 660.2913 851.3 851.3	856.3890 970 974 1 1 1 1	1102.47 11 11 11 11 11 11 11 11 11 1	775 103.4811 1170.6761 1171.6788 1192.6592 1200	82,7693 1400	1516.6946 173 1600	8.7720 1800	m/z
Minimum: Maximum:		100. 0	20. 0	-1.5 300.0						
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula				
1102. 4775	1102. 4760	1.5	1.4	34. 5	31.9	C55 H60 N	117 09			

 $Py_3-\gamma-Im_3-NH(CH_2)_3NHFmoc$ (36)





 $Py_{3}-\gamma-Im_{3}-NH(CH_{2})_{4}NHFmoc$ (37) Elemental Composition Report

Single Mass Analysis Tolerance = 20.0 PPM / DBE: min = -1.5, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions 11 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass) Elements Used: C: 1-300 H: 1-1000 N: 17-17 O: 9-9 yu-181 M-8004 145 (1.727) AM (Cen,4, 80.00, Ar,8500.0,556.28,0.00,LS 10); Sm (SG, 2x3.00); Sb (1,40.00); Cm (144:175)

1: TOF MS ES+ 7.42e+004 100-1116,4918

1007					1117.4988	3				
%- 	482.2510 437.1953	558.7440 559.75	560 852.3	704	1118.5111 1119.5203	4240	1674.74	126_1753.81	75 1968.8781	m/z
Minimum: Maximum:	400	100. 0	20. 0	-1.5 300.0	1200	1400	1000	1000	2000	
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula				
1116. 4918	1116, 4916	0. 2	0. 2	34. 5	207.8	C56 H62	N17 09			

Py₃-y-Im₃-NH(CH₂)₄NHFmoc (**37**)









Figure S3: HPLC charts of MGB polyamide-oligonucleotide conjugates.

(A) HPLC chart of crude products of the preparation of modified DNA.

5'-d(CGGAATTTGGC)-3'

0

25

Time (min)

0

(B) HPLC chart of the modified DNA isolated from crude products (A).

HPLC conditions: Detection: UV 260 nm, flow rate: 1.0 mL/min, mobile phase: 5-50% CH₃CN in water (0.01 M TEAA, pH 7), column: µBONDASPHARE C18 5 µm 100A (3.9 mm ID x 150 mm L)





S67

25

Time (min)



Figure S4: Mass spectra of MGB polyamide-oligonucleotide conjugates.





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 $Py_4-NH(CH_2)_4-ON$ [**ON 1** (n = 4)]





 $Py_4-NH(CH_2)_5-ON$ [**ON 1** (n = 5)]



Py₃-NH(CH₂)₄-ON [ON 2 (n = 4)]


$Im_3-NH(CH_2)_3-ON $ [ON 3 (n = 3)]									
Elemental Composition Report									
Single Mass Analysis Tolerance = 100.0 mDa / DBE: min = -1.5, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 3									
Monoisotopic Mass, Even Electron Ions 1 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass) Elements Used: C: 123-123 H: 1-3000 N: 51-51 O: 67-67 P: 10-10 saka-146A SAKATA 001 807 (9.566) M3 (Ev-75272,150,En11 (25000,0.4,Peo.Cmo): Mk (Ev-2295,150,En11 (25000,0.4,Peo.Cmo): Sm (SG, 3/3,00): Sb (10.10.00)									
100-900.4573	3728.7866								
1000 1200 1400	1600 1800	2000 2200	2400 2600 28	00 3000 3200	3400 3600				
Minimum: Maximum:	100.0 10.0	-1.5 300.0							
Mass Calc. Mass	mDa PPM	DBE i-FIT	Norm Conf() Formula					
3728.7866 3728.7744	12.2 3.3	76.5 28.6	n/a n/a	C123 H156 N51	067 P10				

 $Im_3-NH(CH_2)_3-ON$ [**ON 3** (n = 3)]



Im₃-NH(CH₂)₄-ON [**ON 3** (n = 4)] **Elemental Composition Report Single Mass Analysis** Tolerance = 100.0 mDa / DBE: min = -1.5, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 3 Marcinetia Mars. Ever Electron Icon

 Monoisotopic Mass, Even Electron lons:
 1 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass):

 Elements Used:
 C: 124-124
 H: 1-3000
 N: 51-51
 O: 67-67
 P: 10-10

 saka-f67
 SAKATA 003
 403 (4.782) M3 (Ev-246599, H50, En1) (25000, 0.4, Pep, Cmp); Sm (SG, 3x3.00); Sb (10, 10.00); Cm (365.412)
 1: TOF MS ES+699e+002

 1001
 1200
 1400
 1800
 2000
 2400
 2600
 2800
 3000
 3226.5327
 3523.6554
 m/z

 1001
 1200
 1400
 1800
 2000
 2400
 2600
 2800
 3000
 3200
 3400
 3600

 Minimum:
 100.0
 10.0
 300.0
 -1.5
 -1.5
 -1.5
 -1.5
 -1.5

 Mass
 Calc. Mass
 mDa
 PPM
 DBE
 i-FIT
 Norm
 Conf (%)
 Formula

 3742.7827
 3742.7900
 -7.3
 -2.0
 76.5
 26.4
 n/a
 n/a
 C124
 H158
 N51 067
 P10

Im-NH(CH)-ON	ON 3	(n = 4)	
		U – TJ	



 $Im_3-NH(CH_2)_5-ON$ [ON 3 (n = 5)] Elemental Composition Report Single Mass Analysis Tolerance = 100.0 mDa / DBE: min = -1.5, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 3 Monoisotopic Mass, Even Electron Ions 1 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass) Elements Used: C: 125-125 H: 1-3000 N: 51-51 O: 67-67 P: 10-10 Saka-169 Saka-169 SakaTA 006 81 (0.972) M3 [Ev125406,150,En1] (25000.0.4,Pep.Cmp); Sm (SG, 3x3.00); Sb (10,10.00); Cm (57:90) 1: TOF MS ES+ 3.38e+002 3756.8152 100-%-3755.9470 925.4698 3754.6025 m/z 2400 2600 2800 3000 1000 1200 1400 1600 1800 2000 2200 3200 3400 3600 Minimum: Maximum: -1.5 300.0 100.0 10.0 Mass Calc. Mass mDa PPM DBE i-FIT Norm Conf(%) Formula 3756.8152 3756.8057 9.5 2.5 76.5 29.1 n/a n/a C125 H160 N51 067 P10

 $Im_3-NH(CH_2)_5-ON$ [**ON 3** (n = 5)]

saka	169	1 (N 972) M	13 (Ev1254)	06 H50 En11	(25000.0.4	Pen Cmn)	Sm (SG 3v	3 00) [,] Sh (1	- 0 10 00 \• 0	(57·90)				1. TOF	MS ES+
100-	UN 000 0	1 (0.312) 1	10 [2012040	50,000,0011	(20000.0,4	, ep,omp),	011 (00, 02	5.00), 05 (1	0,10.00), 0	m (07.50)				3756.8152	338
														57400122	
	925.4698													3755.9470 3754.6025 3754.2786	
머니	1000	1200	1400	1600	1800	2000	2200	2400	2600	2800	3000	3200	3400	3600	🗕 mass







 $Py_{3}\text{-}\gamma\text{-}Im_{3}\text{-}NH(CH_{2})_{3}\text{-}ON \ \, [\textbf{ON 4} \ (n=3)] \\ \textbf{Elemental Composition Report}$

Single Mass Analysis Tolerance = 5.0 PPM / DBE: min = -1.5, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 3

Monolscipic Mass, Even Electron Ions 29 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass) Elements Used: C: 1-300 H: 1-1000 N: 58-58 O: 71-71 P: 10-10 yu2:182b M-8218 154 (1.832) M3 [Ev-804306,II50,En1] (0.030,200.00.0.100,1000.00,2,Cmp); Sm (SG, 8x3.00); Sb (1,40.00); Cm (148:276)



 $Py_{3}-\gamma-Im_{3}-NH(CH_{2})_{3}-ON$ [**ON 4** (n = 3)]





$Py_{3}-\gamma-Im_{3}-NH(CH_{2})_{4}-ON$ [ON 4 (n = 4)]



Figure S5: UV melting curves of modified dsDNA (4.3 μ M) in 10 mM sodium phosphate buffer (pH 7.0) containing 10 mM NaCl and 0.1 mM Na₂EDTA. The melting temperature (T_m) was obtained using a TMSPC-8 with T_m analysis software.

Modified dsDNA: 5'-d(CGGAATTTGGC)-3'/complementary DNA

ON 2 (n=4)/complementary DNA ON 1 (n=3)/complementary DNA *T*m = 50.8 °C *T*m = 59.5 °C 1.4 1.4 Absorbance (260 nm) Absorbance (260 nm) 0.6 0.6 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 Temp. (°C) Temp. (°C) unmodified DNA/complementary DNA ON 1 (n=4)/complementary DNA *T*m = 34.1 °C *T*m = 60.2 °C 1.4 1.4 Absorbance (260 nm) Absorbance (260 nm) 0.6 0.6 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 Temp. (°C) Temp. (°C) ON 1 (n=5)/complementary DNA *T*m = 52.9 °C 1.4 Absorbance (260 nm) 0.6 $20 \hspace{0.1in} 25 \hspace{0.1in} 30 \hspace{0.1in} 35 \hspace{0.1in} 40 \hspace{0.1in} 45 \hspace{0.1in} 50 \hspace{0.1in} 55 \hspace{0.1in} 60 \hspace{0.1in} 65 \hspace{0.1in} 70 \hspace{0.1in} 75 \hspace{0.1in} 80 \hspace{0.1in} 85 \hspace{0.1in} 90 \hspace{0.1in} 95$

ON 1 [\underline{G} = Py₄-NH(CH₂)₄-G], **ON 2** [\underline{G} = Py₃-NH(CH₂)₄-G]





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Modified dsDNA: 5'-d(CGGACCCTGGC)-3'/complementary DNA

ON 3 [\underline{G} = Im₃-NH(CH₂)₄-G], **ON 4** [\underline{G} = Py₃- γ -Im₃-NH(C₂)₂-G]



5'-d(CGGACCCTGGC)-3'/mismatch DNA



Figure S6: CD spectra of modified dsDNAs (5.8 μM) in 10 mM sodium phosphate buffer (pH 7.0) containing 10 mM NaCl and 0.1 mM Na₂EDTA.



CD spectra of unmodified and modified dsDNAs.

 $\begin{array}{l} \mbox{modified DNA: 5'-d(CG\underline{G}AATTTGGC)-3' \\ \mbox{ON 1 } [\underline{G} = Py_4\mbox{-}NH(CH_2)_n\mbox{-}G], \mbox{ON 2 } [\underline{G} = Py_3\mbox{-}NH(CH_2)_n\mbox{-}G] \end{array}$





CD spectra of unmodified and modified dsDNAs. modified DNA : 5'-d(CG<u>G</u>ACCCTGGC)-3' **ON 3** [<u>G</u> = Im₃-NH(CH₂)_n-G], **ON 4** [<u>G</u> = Py₃- γ -Im₃-NH(CH₂)_n-G]