

UNIVERSITÀ DEGLI STUDI DI MILANO

CORSO DI DOTTORATO IN SCIENZE FARMACEUTICHE (XXXVI ciclo)

DIPARTIMENTO DI SCIENZE FARMACEUTICHE (DISFARM)

Synthesis of pharmacologically active molecules through biocatalytic approaches: study of their stereochemistry and polymorphism by means of spectroscopic and crystallographic methods.

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A.A. 2022/2023

Supplementary Materials

INDEX

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Figure S1	¹ H NMR of compound 17
Figure S2	¹³ C NMR of compound 17
Figure S3	COSY of compound 17
Figure S4	¹ H- ¹³ C HSQC of compound 17
Figure S5	¹ H- ¹³ C HMBC of compound 17
Figure S6	¹ H- ¹⁵ N HMBC of compound 17
Figure S7	¹ H- ¹⁵ N HSQC of compound 17
Figure S8	¹ H NMR of compound 18
Figure S9	¹³ C NMR of compound 18
Figure S10	COSY of compound 18
Figure S11	¹ H- ¹³ C HSQC of compound 18
Figure S12	¹ H- ¹³ C HMBC of compound 18
Figure S13	¹ H- ¹⁵ N HMBC of compound 18
Figure S14	¹ H- ¹⁵ N HSQC of compound 18
Figure S15	¹ H NMR of compound 25
Figure S16	¹³ C NMR of compound 25
Figure S17	COSY of compound 25
Figure S18	¹ H- ¹³ C HSQC of compound 25
Figure S19	¹ H- ¹³ C HMBC of compound 25
Figure S20	¹ H- ¹⁵ N HMBC of compound 25
Figure S21	¹ H- ¹⁵ N HSQC of compound 25
Figure S22	¹ H NMR of compound 20
Figure S23	¹³ C NMR of compound 20
Figure S24	COSY of compound 20
Figure S25	¹ H- ¹³ C HSQC of compound 20
Figure S26	¹ H- ¹³ C HMBC of compound 20
Figure S27	¹ H- ¹⁵ N HMBC of compound 20
Figure S28	¹ H NMR of compound 21
Figure S29	¹³ C NMR of compound 21
Figure S30	COSY of compound 21
Figure S31	¹ H- ¹³ C HSQC of compound 21
Figure S32	¹ H- ¹³ C HMBC of compound 21
Figure S33	¹ H- ¹⁵ N HMBC of compound 21
Figure S34	¹ H NMR of compound 1
Figure S35	¹³ C NMR of compound 1
Figure S36	COSY of compound 1
Figure S37	¹ H- ¹³ C HSQC of compound 1
Figure S38	¹ H- ¹³ C HMBC of compound 1
Figure S39	¹ H- ¹⁵ N HMBC of compound 1
Figure S40	NOESY of compound 1
Figure S41	^1H NMR of compound $\boldsymbol{1}$ in DMSO-d_6
Figure S42	¹ H NMR of compound 1 in D_2O
Figure S43	¹ H NMR of compound 24
Figure S44	COSY of compound 24

Modifications at the 4-position

Figure S45	Potential energy scan.
Figure S46	Comparison between the potential energy plots obtained optimizing the energy
	minima and the TS structures derived from the potential energy scan (Figure S45)
	in gas-phase (cyan line) and in PCM implicit water solvent model (orange line).
	Calculations were performed at DFT/B3LYP/6-31g(d,p) level.
Figure S47	¹ H NMR of compound 30
Figure S48	¹³ C NMR of compound 30
Figure S49	COSY of compound 30
Figure S50	¹ H- ¹³ C HSQC of compound 30
Figure S51	¹ H- ¹³ C HMBC of compound 30
Figure S52	HRMS analysis of compound 30
Figure S53	Chiral HPLC analysis of compound 30
Figure S54	¹ H NMR of compound 31
Figure S55	¹³ C NMR of compound 31
Figure S56	COSY of compound 31
Figure S57	¹ H- ¹³ C HSQC of compound 31
Figure S58	¹ H- ¹³ C HMBC of compound 31
Figure S59	HRMS analysis of compound 31
Figure S60	Chiral HPLC analysis of compound 31
Figure S61	¹ H NMR of compound 32
Figure S62	¹³ C NMR of compound 32
Figure S63	COSY of compound 32
Figure S64	¹ H- ¹³ C HSQC of compound 32
Figure S65	¹ H- ¹³ C HMBC of compound 32
Figure S66	HRMS analysis of compound 32
Figure S67	¹ H NMR of compound 33
Figure S68	¹³ C NMR of compound 33
Figure S69	COSY of compound 33
Figure S70	¹ H- ¹³ C HSQC of compound 33
Figure S71	¹ H- ¹³ C HMBC of compound 33
Figure S72	Superimposed spectra of I and II atropisomer of 33 , and equilibrium mixture
Figure S73	HRMS analysis of compound 33
Figure S74	¹ H NMR of compound 34
Figure S75	¹³ C NMR of compound 34
Figure S76	COSY of compound 34
Figure S77	¹ H- ¹³ C HSQC of compound 34
Figure S78	¹ H- ¹³ C HMBC of compound 34
Figure S79	¹ H NMR of compound 35
Figure S80	¹³ C NMR of compound 35
Figure S81	COSY of compound 35
Figure S82	¹ H- ¹³ C HSQC of compound 35
Figure S83	¹ H- ¹³ C HMBC of compound 35
Figure S84	Superimposed spectra of I and II atropisomer of 35 , and equilibrium mixture
Figure S85	HRMS analysis of compound 35
Figure S86	Chiral HPLC analysis of the obtained crude mixture of compounds 33 and 35
Figure S87	¹ H NMR of compound 3

Figure S88	¹³ C NMR of compound 3
Figure S89	COSY of compound 3
Figure S90	¹ H- ¹³ C HSQC of compound 3
Figure S91	¹ H- ¹³ C HMBC of compound 3
Figure S92	NOESY of compound 3
Figure S93	HRMS analysis of compound 3
Figure S94	¹ H NMR of compound 4
Figure S95	¹³ C NMR of compound 4
Figure S96	COSY of compound 4
Figure S97	¹ H- ¹³ C HSQC of compound 4
Figure S98	¹ H- ¹³ C HMBC of compound 4
Figure S99	NOESY of 4
Figure S100	HRMS analysis of compound 4
Figure S101	¹ H NMR of compound 29
Figure S102	¹³ C NMR of compound 29
Figure S103	COSY of compound 29
Figure S104	¹ H- ¹³ C HSQC of compound 29

Modifications at the 6-position

Figure S105	¹ H NMR of compound 36
Figure S106	¹ H NMR of compound 39
Figure S107	¹ H NMR of compound 47
Figure S108	¹ H NMR of compound 53
Figure S109	¹ H NMR of compound 56
Figure S110	¹ H NMR of compound 62
Figure S111	¹ H NMR of compound 5
Figure S112	¹ H NMR of compound 37
Figure S113	¹ H NMR of compound 40
Figure S114	¹ H NMR of compound 48
Figure S115	¹ H NMR of compound 54
Figure S116	¹ H NMR of compound 57
Figure S117	¹ H NMR of compound 60
Figure S118	¹ H NMR of compound 63
Figure S119	¹ H NMR of compound 6
Figure S120	¹ H NMR of compound 65
Figure S121	¹ H NMR of compound 38
Figure S122	¹ H NMR of compound 41
Figure S123	¹ H NMR of compound 49
Figure S124	¹ H NMR of compound 55
Figure S125	¹ H NMR of compound 58
Figure S126	¹ H NMR of compound 64
Figure S127	¹ H NMR of compound 7

Modifications at the 21-position

Figure S128	¹ H NMR of compound 14
Figure S129	¹³ C NMR of compound 14

Figure S130 COSY of compound 14 ¹H-¹³C HSQC of compound **14** Figure S131 ¹H-¹³C HMBC of compound **14** Figure S132 Figure S133 Chiral HPLC analysis of 14 Figure S134 ¹H NMR of compound **66** Figure S135 Chiral HPLC analysis of 66 Figure S136 ¹H NMR of compound **67** Figure S137 Chiral HPLC analysis of 67

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Figure S1. ¹H NMR of compound 17



Figure S2. ¹³C NMR of compound 17



Figure S3. COSY of compound 17



Figure S4. ¹H-¹³C HSQC of compound 17



Figure S5. ¹H-¹³C HMBC of compound **17**



Figure S6. ¹H-¹⁵N HMBC of compound 17



Figure S7. ¹H-¹⁵N HSQC of compound **17**



Figure S8. ¹H NMR of compound 18



Figure S9. ¹³C NMR of compound 18



Figure S10. COSY of compound 18



Figure S11. ¹H-¹³C HSQC of compound 18



Figure S12. ¹H-¹³C HMBC of compound 18



Figure S13. ¹H-¹⁵N HMBC of compound 18



Figure S14. 1H-15N HSQC of compound 18



Figure S15. ¹H NMR of compound 25



Figure S16. ¹³C NMR of compound 25



Figure S17. COSY of compound 25



Figure S18. ¹H-¹³C HSQC of compound 25



Figure S19. ¹H-¹³C HMBC of compound 25



Figure S20. ¹H-¹⁵N HMBC of compound 25



Figure S21. ¹H-¹⁵N HSQC of compound 25



Figure S22. ¹H NMR of compound 20



Figure S23. ¹³C NMR of compound 20



Figure S24. COSY of compound 20



Figure S25. ¹H-¹³C HSQC of compound 20



Figure S26. ¹H-¹³C HMBC of compound 20



Figure S27. ¹H-¹⁵N HMBC of compound 20



Figure S28. ¹H NMR of compound 21


Figure S29. ¹³C NMR of compound 21



Figure S30. COSY of compound 21



Figure S31. ¹H-¹³C HSQC of compound 21



Figure S32. ¹H-¹³C HMBC of compound 9



Figure S33. ¹H-¹⁵N HMBC of compound 21



Figure S34. ¹H NMR of compound 1



Figure S35. ¹³C NMR of compound 1



Figure S36. COSY of compound 1



Figure S37. ¹H-¹³C HSQC of compound 1



Figure S38. ¹H-¹³C HMBC of compound 1



Figure S39. ¹H-¹⁵N HMBC of compound 1



Figure S40. NOESY of compound **1**



Figure S41. ¹H NMR of compound 1 in DMSO-d₆



Figure S42. ¹H NMR of compound 1 in D₂O



Figure S43. ¹H NMR of compound 24



Figure S44. COSY of compound 24

Modifications at the 4-position



Figure S45. Potential energy scan obtained rotating the torsion angle indicated by the red arrow. The red dots highlight the atoms selected for the scan.



Figure S46. Comparison between the potential energy plots obtained optimizing the energy minima and the TS structures derived from the potential energy scan (Figure S45) in gas-phase (cyan line) and in PCM implicit water solvent model (orange line). Calculations were performed at DFT/B3LYP/6-31g(d,p) level.



Figure S47. ¹H NMR of compound 30



Figure S48. ¹³C NMR of compound 30



Figure S49. COSY of compound 30



Figure S50. ¹H-¹³C HSQC of compound 30



Figure S51. ¹H-¹³C HMBC of compound 30

Elemental Composition Report

Single Mass Analysis Tolerance = 5.0 PPM / DBE: min = -5.0, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 5

Monoisotopic Mass, Even Electron Ions 13 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass) Elements Used: C: 20-20 H: 14-16 N: 2-2 O: 2-2 Na: 0-4 S: 1-1 K: 0-2 F: 5-5

INT10Z 30 (0.603) AM2 (Ar,40000.0,0.00,0.00); Cm (30:50)												1: TOF MS ES+ 1 44e+007
100-		465.0674										
% 457	2776 459	.0799	461.0	348	463.3031	466	.0704	467.0669	469.3289 47	1.0714	473.3449	474.3482 475.7417
0 11 11	458.0	46	0.0	462.0	464.0	46	6.0	468.0	470.0	472.0	474.0	476.0
Minimum:			5.0	5.0	-5.0							
Maximum,			5.0	5.0	300.0							
Mass	Calc. M	Mass	nDa	PPM	DBE	1-FIT	Nors	n Conf	(%) Formul	a		
465,0674	465.06	72	0.2	0.4	11.5	1840.8	n/a	n/a	C20 H1	5 N2 O2 N	a S F5	

Figure S52. HRMS analysis of compound 30

Page 1



Figure S53. Chiral HPLC analysis of compound 30



Figure S54. ¹H NMR of compound 31



Figure S55. ¹³C NMR of compound 31



Figure S56. COSY of compound 31



Figure S57. ¹H-¹³C HSQC of compound 31



Figure S58. ¹H-¹³C HMBC of compound 31

Elemental Composition Report

Single Mass Analysis Tolerance = 5.0 PPM / DBE: min = -5.0, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 5 Monoisotopic Mass, Even Electron lons 12 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass) Elements Used: C: 33-33 H: 31-33 N: 3-3 O: 4-4 Na: 0-4 S: 1-1 K: 0-2 F: 5-5 INT5Z 41 (0.829) AM2 (Ar,40000.0,0.00,0.00); Cm (30.50) 1: TOF MS ES+ 9.09e+006 684.1933
 100
 649.4504
 659.2876.662.2109
 668.2153
 683.4340
 685.1963
 700.1675
 706.1750
 716.1805
 722.1525
 735.2977,738.2827

 645
 650
 655
 660
 665
 670
 675
 680
 685
 700
 705
 710
 715
 720
 725
 730
 735
 740
 745
735.2977_738.2827 m/z Minimum: -5.0 Maximum: 5.0 5.0 300.0 Calc. Mass mDa PPM DBE i-FIT Norm Conf(%) Formula Mass 684.1933 684.1931 0.2 0.3 16.5 1281.5 n/a n/a C33 H32 N3 O4 Na S F5

Figure S59. HRMS analysis of compound 31

Page 1



Figure S60. Chiral HPLC analysis of compound 31



Figure S61. ¹H NMR of compound 32



Figure S62. ¹³C NMR of compound 32



Figure S63. COSY of compound 32



Figure S64. ¹H-¹³C HSQC of compound 32


Figure S65. ¹H-¹³C HMBC of compound 32

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -5.0, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 5

Monoisotopic Mass, Even Electron lons 12 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass) Elements Used: C: 28-28 H: 23-25 N: 3-3 O: 2-2 Na: 0-4 S: 1-1 K: 0-2 F: 5-5

INT5Zdep 4	19 (0.984) AM2 (Ar,40	000.0,0.00	,0.00); C	m (30:50)							1: TOF MS ES+ 1.440+007
100	537.1451 545.13	22 551.355	6	562.158956	5.1609571.1	277 581.1	584.1411	1437	600.114	6606. <u>1223</u> 609.1241	622.0974
530.0	540.0	550.0	5	60.0	570.0	580.0	0 5	90.0	600.0	610.0	620.0
Minimum: Maximum:		5.0	5.0	-5.0 300.0							
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula			
584.1411	584.1407	0.4	0.7	15.5	1472.7	n/a	n/a	C28 H24	N3 02	Na S F5	

Elemental Composition Report

Single Mass Analysis Tolerance = 5.0 PPM / DBE: min = -5.0, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 5

Monoisotopic Mass, Even Electron Ions 14 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass) Elements Used: C: 28-28 H: 23-25 N: 3-3 O: 2-2 Na: 0-4 S: 1-1 K: 0-2 F: 5-5

INT5Zdep 49 (0.984) AM2 (Ar,40000.0,0.00,0.00); Cm (30:50)

Page 1

1: TOF MS ES+ 1.44e+007

100-	584.1411											
%	537.1451 545.1322 551.3556			562.1589565.1609571.1277			581.1320 585.1437			6606.1223/609.1241	622.0974 m/z	
530.0	540.0	550.0		560.0	570.0	580.0	5	90.0	600.0	610.0	620.0	
Minimum: Maximum:		5.0	5.0	-5.0 300.0								
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula				
562.1589	562.1588	0.1	0.2	15.5	1553.9	n/a	n/a	C28 H25	N3 02	s F5		

Figure S66. HRMS analysis of compound 32



Figure S67. ¹H NMR of compound 33



Figure S68. ¹³C NMR of compound 33



Figure S69. COSY of compound 33



Figure S70. ¹H-¹³C HSQC of compound 33



Figure S71. ¹H-¹³C HMBC of compound 33



Figure S72. Superimposed spectra of I and II atropisomer of 33, and equilibrium mixture

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -5.0, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 7

Monoisotopic Mass, Even Electron Ions 4 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass) Elements Used: C: 34-34 H: 34-35 N: 3-3 O: 4-4 Na: 0-4 S: 1-1 F: 5-5

INT6ZIII 49 (0	.984) AM2 (Ar,4000	00.0,0 .00,0	00); Cn	n (10:50)						1:1	OF MS ES+
100-3			676.2	267							2.0201001
652.16	65 658.2294 664.2	186 674.	2111	677.2297	683.5431	698	8.2083 701.2115	5 711.5740 714.	2003	726.2020 730.1971	739.6055
650.0	660.0	670.0)	680.0	690.0	erel au	700.0	710.0	720.0	730.0	740.0
Minimum: Maximum:		5.0	5.0	-5.0 300.0							
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula			
676.2267	676.2268	-0.1	-0.1	16.5	2248.4	n/a	n/a	C34 H35 N3 0	04 S F	5	

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -5.0, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 7

Monoisotopic Mass, Even Electron Ions 3 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass) Elements Used:

C: 34-34 H: 34-35 N: 3-3 O: 4-4 Na: 0-4 S: 1-1 F: 5-5

600 0000

INT6ZIII 49 (0.984) AM2 (Ar,40000.0,0.00,0.00); Cm (10:50)

1: TOF MS ES+ 1.02e+007

100-			000	2003							
%	696.1924 697.1967			698.2768 699.2114			00.2108	701.2115	702.2131	703.2214	m/z
0 11 1	696.00 6	97.00	698.0	0	699.00	700	.00	701.00	702.00	703.00	704.00
Minimum: Maximum:		5.0	5.0	-5.0 300.0							
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula			
698.2083	698.2088	-0.5	-0.7	16.5	1635.3	n/a	n/a	C34 H34 N3	04 Na S F5	5	

Figure S73. HRMS analysis of compound 33



Figure S74. ¹H NMR of compound 34



Figure S75. ¹³C NMR of compound 34



Figure S76. COSY of compound 34



Figure S77. ¹H-¹³C HSQC of compound 34



Figure S78. ¹H-¹³C HMBC of compound 34



Figure S79. ¹H NMR of compound 35



Figure S80. ¹³C NMR of compound 35



Figure S81. COSY of compound 35



Figure S82. ¹H-¹³C HSQC of compound 35



Figure S83. ¹H-¹³C HMBC of compound 35



Figure S84. Superimposed spectra of I and II atropisomer of 35, and equilibrium mixture

Mass

Single Mass Analysis Tolerance = 5.0 PPM / DBE: min = -5.0, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 5

Monoisotopic Mass, Even Electron lons 13 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass) Elements Used: C: 34-34 H: 34-35 N: 3-3 O: 4-4 Na: 0-4 S: 1-1 K: 0-2 F: 5-5

%- 649.443	3	670.17	74	676.22	266	2202	692.	698. 2213	2086	9.2115	71	4.2018	716 20	26	700 170	e 7	36.184	0 7	46.170	3 754.1933
0 ⁻¹	5 660	665	670	675	680	685	690	695	700	705	710	715	720	725	730	735	740	745	750	755 r
inimum: aximum:			5.0)	5.0	-5 30	.0).0													
ass	Calc.	Mass	mDa		PPM	DB	s	1-FI	Т	Norm	Cor	f(%)	For	nula						
76.2266	676.22	268	-0.	2	-0.3	16	. 5	1290	.6	n/a	n/a	e -	C34	H35	N3 04	S	F5			
lemental	Comp	ositio	n Re	port	6															Page
umber of it	ouropo l																			
Number of i Aonoisotopic 1 formula(e)	Mass, E evaluat	ven El ed with	ectron 1 resi	lons ults w	ithin lir	nits (a	resu	ults (up	to 10	000) fo	each	mass								
Number of i Monoisotopic 1 formula(e) Elements Use 2 34-34 H:	Mass, E evaluate ed: 34-35	ed with N: 3-3	ectron 1 resi O: 4	lons ults w 4-4	ithin lir Na: 0-4	n <mark>its (a</mark> 1 S:	l resi 1-1	ults (up K: 0-2	to 10 F:	000) fo 5-5	each	mass								
Number of i Monoisotopic 1 formula(e) Elements Use 34-34 H: NT6Z 49 (0.98	Mass, E evaluate ed: 34-35 34) AM2 (/	ven El ed with N: 3-3 Ar,4000	O: 4	lons ults w 4-4 0,0.00	ithin lir Na: 0-4); Cm (:	nits (a 1 S: 30:50)	l resi 1-1	ults (up K: 0-2	to 10 F:	000) foi 5-5	each	mass)	1						1	TOF MS E 9.750+(
Iumber of i Ionoisotopic 1 formula(e) lements Use : 34-34 HEZ 49 (0.98 00 649.443 04	Mass, E evaluate ed: 34-35 34) AM2 (/	Even El ed with N: 3-3 Ar,4000 670.1	ectron 1 resi 0: 4 0.0,0.0	10ns ults w 4-4 0,0.00 676.2	ithin lir Na: 0-4); Cm (: 266	nits (a 4 S: 30:50)	l resi 1-1 692.	ults (up K: 0-2 698 2213	to 10 F: .2086	000) fo 5-5 99.2115	71	mass) 4.2018	716.20	26	730.178	5 7	36.184	0 7	1: 46.1708	TOF MS E 9.756+4 3 754.1933
Number of i Monoisotopic 1 formula(e) 2 ements Use 3 4-34 H: NT6Z 49 (0.98 0 650 65	Mass, E evaluate ed: 34-35 34) AM2 (/ 33	Even El ed with N: 3-3 Ar,4000 670.1	ectron 1 resi 0:4 0.0,0 0 774 670	lons ults w 4-4 0,0.00 676.2 675	ithin lir Na: 0-4); Cm (: 266 679 680	nits (a 4 S: 30:50) 9.2292 685	l resu 1-1 692.	698 698 2213	to 10 F: 2086	000) for 5-5 99.2115 705	71	mass) 4.2018 117 715	716.20	26	730.178	5 7 735	36.184 740	0 7 745	1: 46.1708 750	TOF MS E 9.750+ 754.1933

Figure S85. HRMS analysis of compound 35

Calc. Mass mDa PPM DBE i-FIT Norm Conf(%) Formula 698.2086 698.2088 -0.2 -0.3 16.5 1333.5 n/a n/a C34 H34 N3 O4 Na 5 F5 Page 1



Figure S86. Chiral HPLC analysis of the obtained crude mixture of compounds 33 and 35



Figure S87. ¹H NMR of compound 3



Figure S88. ¹³C NMR of compound 3



Figure S89. COSY of compound 3



Figure S90. ¹H-¹³C HSQC of compound 3



Figure S91. ¹H-¹³C HMBC of compound 3



Figure S92. NOESY of compound 3

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -5.0, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 7

Monoisotopic Mass, Even Electron Ions 4 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass) Elements Used: C: 32-32 H: 30-31 N: 3-3 O: 4-4 Na: 0-4 S: 1-1 F: 5-5

SC1iso2 11 (0.242) AM2 (Ar,40000.0,0.00,0.00); Cm (10:50)

100		64	8.1959								2.200100
%	640.1666	645.5558	649	.1989_652	1672,655.1	716,658.1770	663.466	4 669.168	670.1774	672.1794	676.2267 679.2296
0-111	640.0	645.0	650.0)	655.0	660.0	6	65.0	670.0	675.0	680.0
Minimum: Maximum:		5.0	5.0	-5.0 300.0							
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula			
648.1959	648.1955	0.4	0.6	16.5	2386.6	n/a	n/a	C32 H31	N3 04 S	F5	

Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -5.0, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 7

Monoisotopic Mass, Even Electron Ions 3 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass) Elements Used: C: 32-32 H: 30-31 N: 3-3 O: 4-4 Na: 0-4 S: 1-1 F: 5-5

SC1iso2 11 (0.242) AM2 (Ar,40000.0,0.00,0.00); Cm (10:50)

1.27e+007 670.1774 100-669.1681 669.4642 671.1804 % 667.7882 674.5967 668,1608 670.4656 672.1794 673,1797 674.1814 m/z 668.00 671.00 669.00 670.00 672.00 673.00 674.00 675.00 Minimum: -5.0Maximum: 5.0 5.0 300.0 Mass Calc. Mass mDa PPM DBE i-FIT Norm Conf(%) Formula 670.1774 670.1775 -0.1 -0.1 16.5 1650.2 n/a C32 H30 N3 O4 Na S F5 n/a

Figure S93. HRMS analysis of compound 3

1: TOF MS ES+

Page 1

1: TOF MS ES+



Figure S94. ¹H NMR of compound 4



Figure S95. ¹³C NMR of compound 4



Figure S96. COSY of compound **4**



Figure S97. ¹H-¹³C HSQC of compound 4



Figure S98. ¹H-¹³C HMBC of compound 4



Figure S99. NOESY of compound **4**

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -5.0, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 5

Monoisotopic Mass, Even Electron Ions 14 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass) Elements Used: C: 32-32 H: 28-31 N: 3-3 O: 4-4 F: 5-5 Na: 0-4 S: 1-1 K: 0-2 SC1_isomero 33 (0.674) AM2 (Ar,40000.0,0.00); Cm (30:49)

100	6171	100	648	8.1958	670.	1776 671 18076	86.1709	592 1593	7	0141410		740 1	055	
03 m	11/1	-11-11-11-11-1-1-1-1-1-1-1-1-1-1-1-1-1	646.1797		664.1905	finnin			708.1520	14.1410	730.1330	149.1	754.1	335 m/z
	620	630	640	650	660 67	680	690	700	710	720	730	740	750	760
Minim	um:				-5.0									
Maxim	um:		5.0	5.0	300.0									
Mass		Calc. Ma	ss mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formul	a				
648.1	958	648.1955	Ε.Ο	0.5	16.5	1498.5	n/a	n/a	C32 H3	1 N3 04	4 F5 S			

Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -5.0, max = 300.0 Element prediction: Off Number of isotope peaks used for i-FIT = 5

Monoisotopic Mass, Even Electron Ions 12 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass) Elements Used: C: 32-32 H: 28-31 N: 3-3 O: 4-4 F: 5-5 Na: 0-4 S: 1-1 K: 0-2 SC1 isomero 33 (0.674) AM2 (Ar,40000 0,0.00,0.00); Cm (30:49)

670.1776 100 % 648.1958 686.1709 .671.1807 692.1593 637.3061 714.1410 664.1905 685 690 695 700 705 710 715 720 725 730 708.1520 646.1797 651.1981 630 635 640 645 650 655 660 665 670 675 680 Minimum: -5.0 Maximum: 5.0 5.0 300.0 Calc. Mass mDa i-FIT Norm Conf(%) Formula Mass PPM DBE 670.1776 670.1775 0.1 0.1 16.5 1497.2 n/a n/a C32 H30 N3 O4 F5 Na S

Figure S100. HRMS analysis of compound 4

1: TOF MS ES+ 3.65e+007

Page 1

1: TOF MS ES+ 3.650+007


Figure S101. ¹H NMR of compound 29



Figure S102. ¹³C NMR of compound 29



Figure S103. COSY of compound 29



Figure S104. ¹H-¹³C HSQC of compound 29

Modifications at the 6-position



Figure S105. ¹H NMR of compound 36



Figure S106. ¹H NMR of compound 39



Figure S107. ¹H NMR of compound 47



Figure S108. ¹H NMR of compound 53



Figure S109. ¹H NMR of compound 56



Figure S110. ¹H NMR of compound 62



Figure S111. ¹H NMR of compound 5



Figure S112. ¹H NMR of compound 37



Figure S113. ¹H NMR of compound 40



Figure S114. ¹H NMR of compound 48



Figure S115. ¹H NMR of compound 54



Figure S116. ¹H NMR of compound 57



Figure S117. ¹H NMR of compound 60



Figure S118. ¹H NMR of compound 63



Figure S119. ¹H NMR of compound 6



Figure S120. ¹H NMR of compound 65



Figure S121. ¹H NMR of compound 38



Figure S122. ¹H NMR of compound 41



Figure S123. ¹H NMR of compound 49



Figure S124. ¹H NMR of compound 55



Figure S125. ¹H NMR of compound 58



Figure S126. ¹H NMR of compound 64



Figure S127. ¹H NMR of compound 7

Modifications at the 21-position



Figure S128. ¹H NMR of compound **14**



Figure S129. ¹³C NMR of compound 14



Figure S130. COSY of compound 14



Figure S131. ¹H-¹³C HSQC of compound 14



Figure S132. ¹H-¹³C HMBC of compound 14



Figure S133. Chiral HPLC analysis of compound 14

.40



Figure S134. ¹H NMR of compound 66


Figure S135. Chiral HPLC analysis of compound 66



Figure S136. ¹H NMR of compound 67



Figure S137. Chiral HPLC analysis of compound 67