Biology-oriented synthesis of a natural-product inspired oxepane collection yields a small molecule activator of the Wnt-pathway

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Supporting information

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Additional Figures, Schemes, and Tables

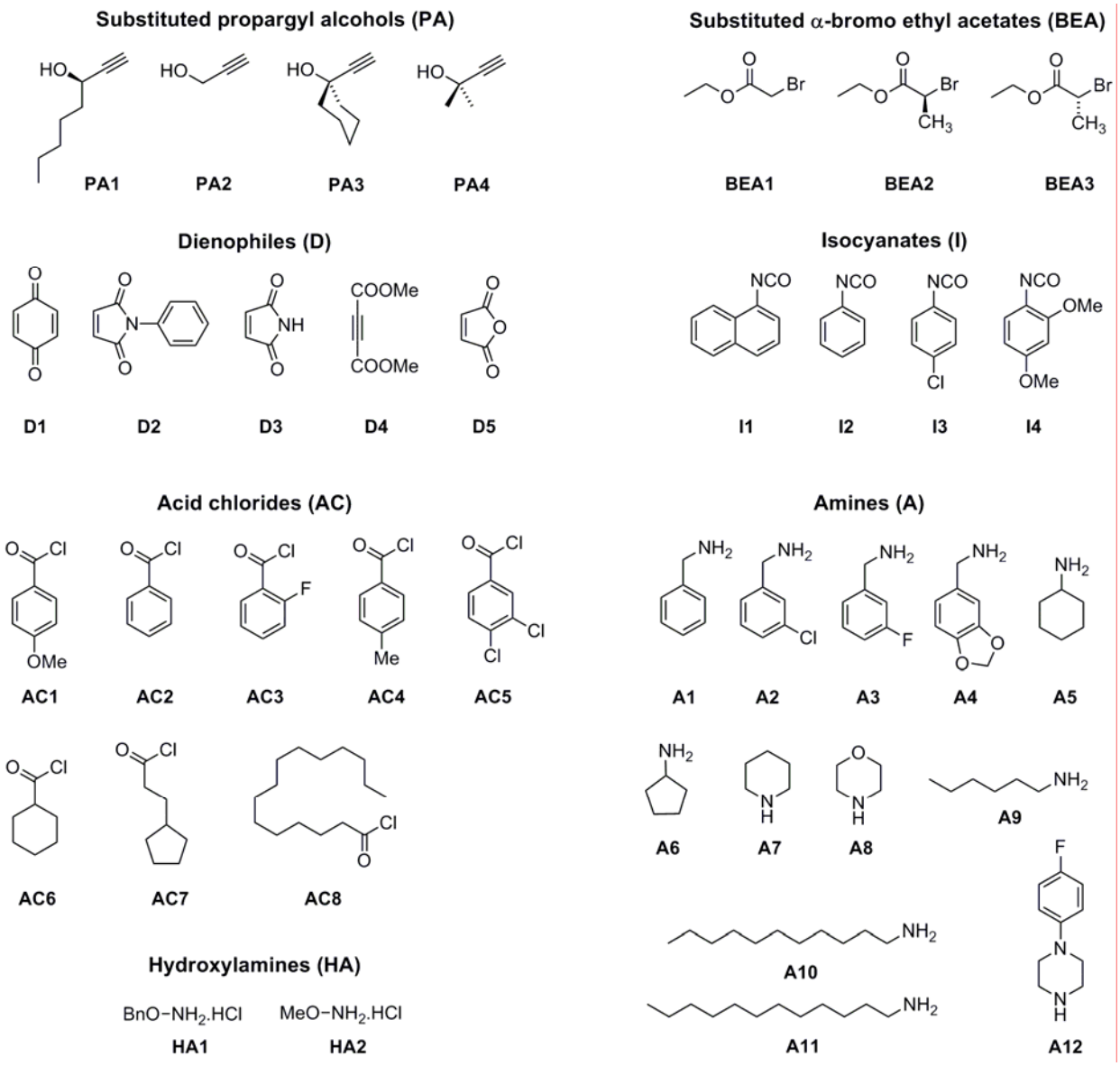
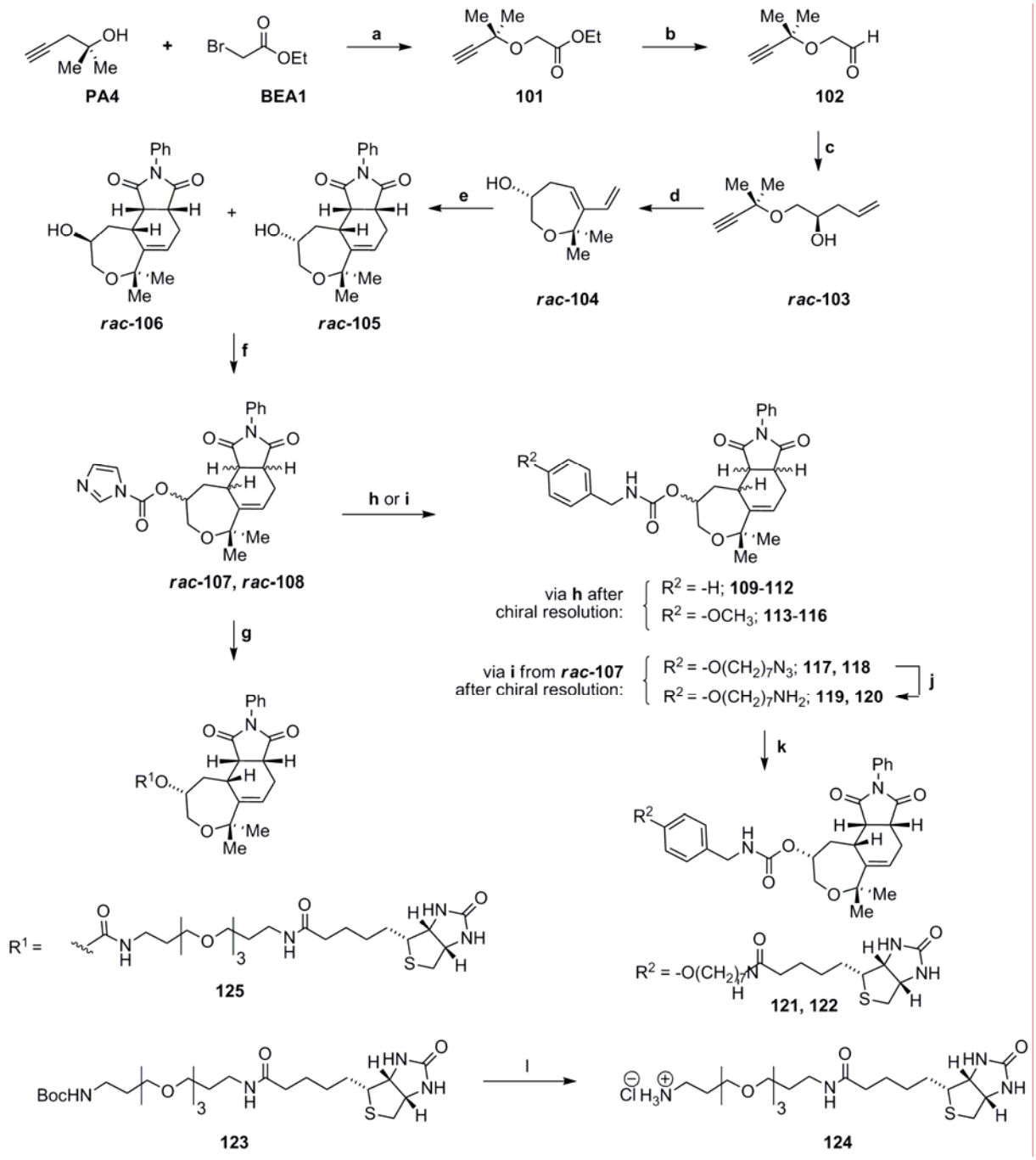
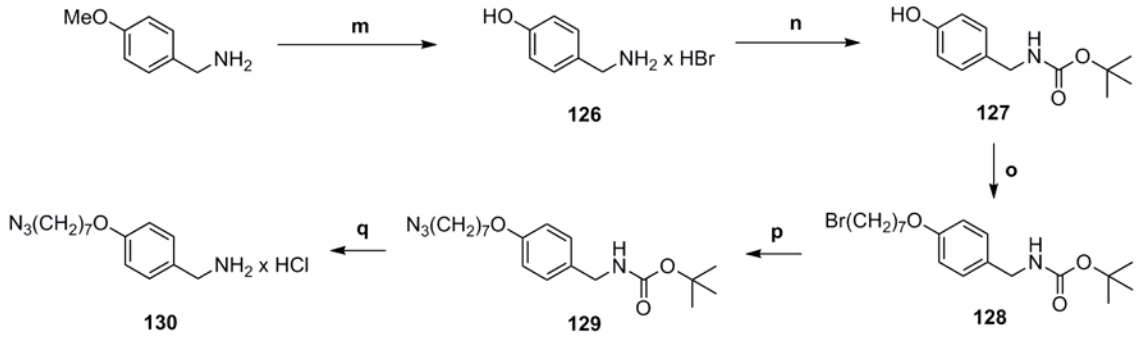


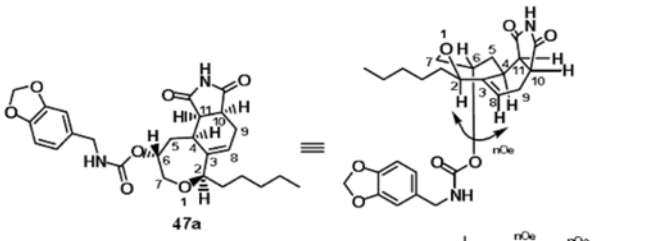
Figure S1. Building blocks used for the synthesis of the oxepane sub-libraries 1–3.

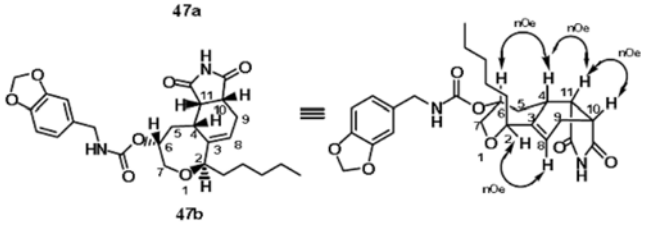


Scheme S1. Synthesis of the hit compounds **109**-**112** and derived biotinylated probes **121**, **122** and **125**. Reagents and conditions: a) NaH, THF, 0°C to r.t., 10h; b) DIBAL-H, Et_2O, -78°C, 20 min. then HCl 1M aq. sol., -78°C to r.t.; c) allylmagnesium chloride 2M sol. in THF, 0°C to r.t., 2h; d) 1st generation Grubbs catalyst (10% mol), dichloromethane, reflux, 18h; e) **D2**, toluene, 70 °C, 3h; f) carbonyldiimidazole, dichloromethane, r.t. overnight; g) ***rac*-7**, **124**, K_2CO_3, THF/DMF : 4/1, r.t., o.n.; h) benzyl amine or *p*-methoxybenzyl amine, K_2CO_3, DMF, r.t. overnight; i) **130** (see Scheme S2 for the synthesis), K_2CO_3, DMF, r.t. overnight; j) H_2 (1 atm), Pd/C 10% , MeOH, 45 min, r.t.; k) biotin, HBTU, EtN(*i*Pr)$_2$, DMF, r.t., overnight; l) (see reference 2).



Scheme S2. Synthesis of linker **130**. Reagents and conditions: m) aq. HBr (48%), reflux, 6 h; n) Boc_2O, $NaHCO_3$, MeOH, r.t., 16 h; o) $Br(CH_2)_7Br$, K_2CO_3, acetone, reflux, 20 h; p) NaN_3, DMF, 90°C, 8 h; q) 2M HCl in dioxane, 40 min, r.t.





Irradiation	Intensity
H^6	H^4 (5.0%), H^5 (3.0 %)
H^4	H^6 (6.0%), H^{11} (3.0%), H^5 (3.0%)
H^2	H^8 (4.0%), H^7 (2.0%)

Figure S2. Nuclear Overhauser Effect (nOe) study of compound **47**. When H2 was irradiated at resonance frequency, the intensity of H8 increased. This finding matches with both the possible isomers of **47**: **47a** and **47b** namely the *endo*-*trans*- and *cis*-isomers. However, when H6 was irradiated only the intensity of H4 increased, suggesting that H6 and H4 are close in space. This would be the case only for isomer **47b**. Moreover, when H4 was irradiated the intensity of both H6 and H11 increased, suggesting that H4 is closer in space to both H6 and H11. Hence, the most likely isomer for compound **47** is **47b**, indicating *endo*-selectivity for the Diels-Alder reaction.

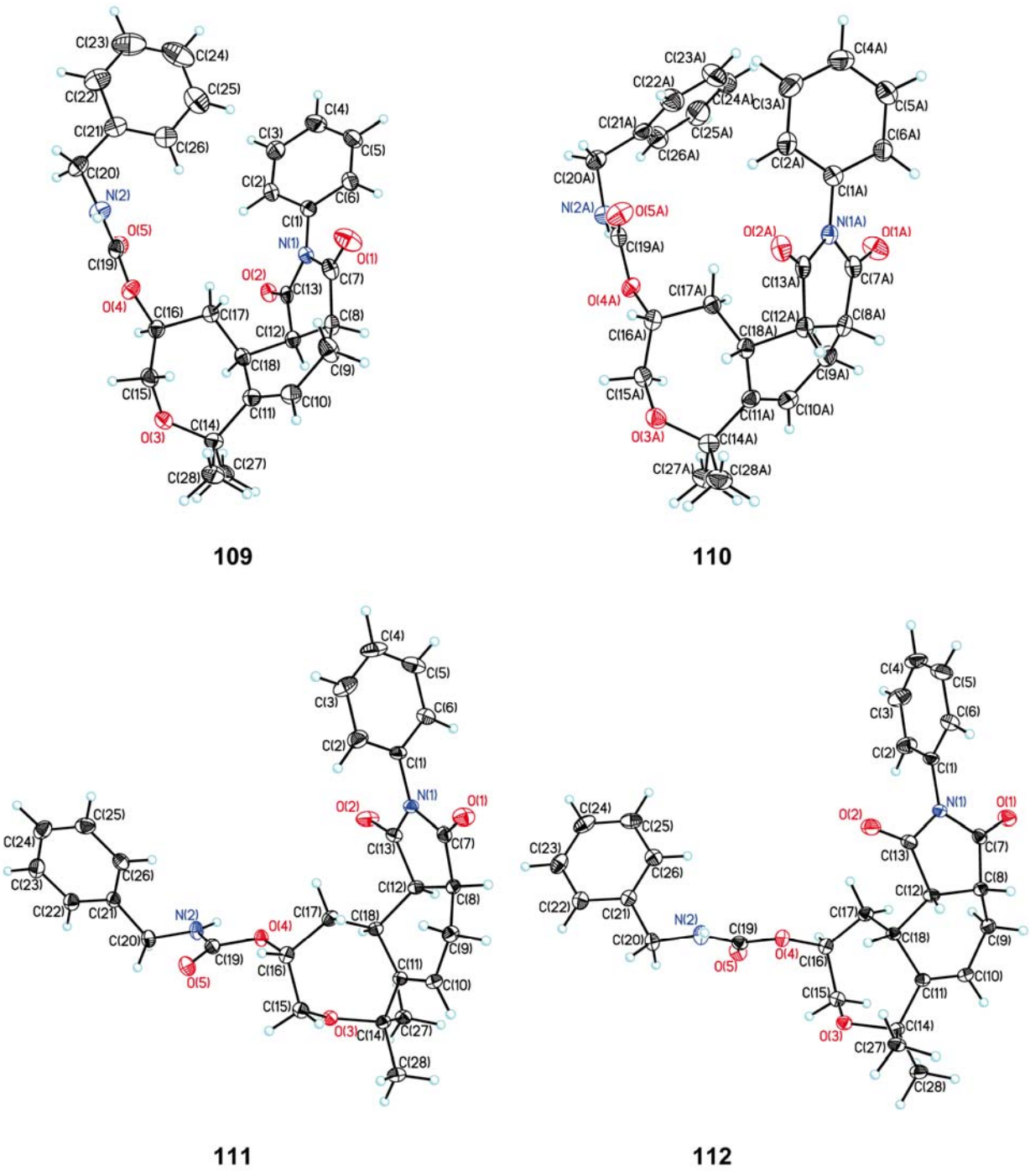


Figure S3. Molecular structures from X-ray crystal structure analysis of compounds **109-112**. Crystallographic data were deposited in the Cambridge Crystallographic Database Center (CCDC) and can be retrieved using the access codes CCDC 803785 (for **109**/**110**), CCDC 803786 (for **111**/**112**) or – alternatively – on request directly from the authors: hans.preut@tu-dortmund.de.

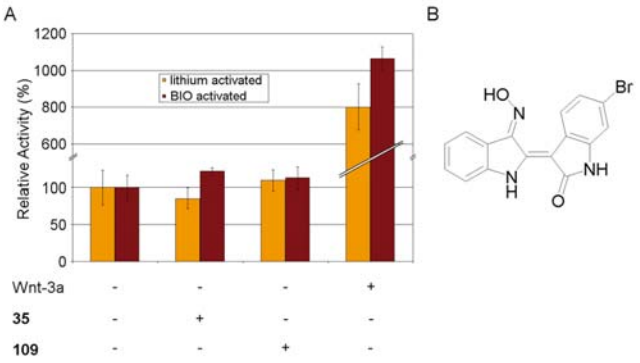


Figure S4. Synergistic Wnt pathway activation by **35** and **109** in combination with the alternative activators BIO or Li^+. *(A)* BIO and Li^+ where used at 5 nM and 20 mM respectively (corresponding to IC_{50} values). The data were normalized to cells treated with BIO or Li^+ alone. The compounds **35** and **109** were used at 15 µM concentration. The compounds fail to activate the Wnt pathway if used in combination with Li^+ or BIO. The cells were still highly responsible to further activation shown by addition of Wnt-3a. *(B)* Structure of (2'*Z*,3'*E*)-6-bromoindirubin-3'-oxime (BIO).

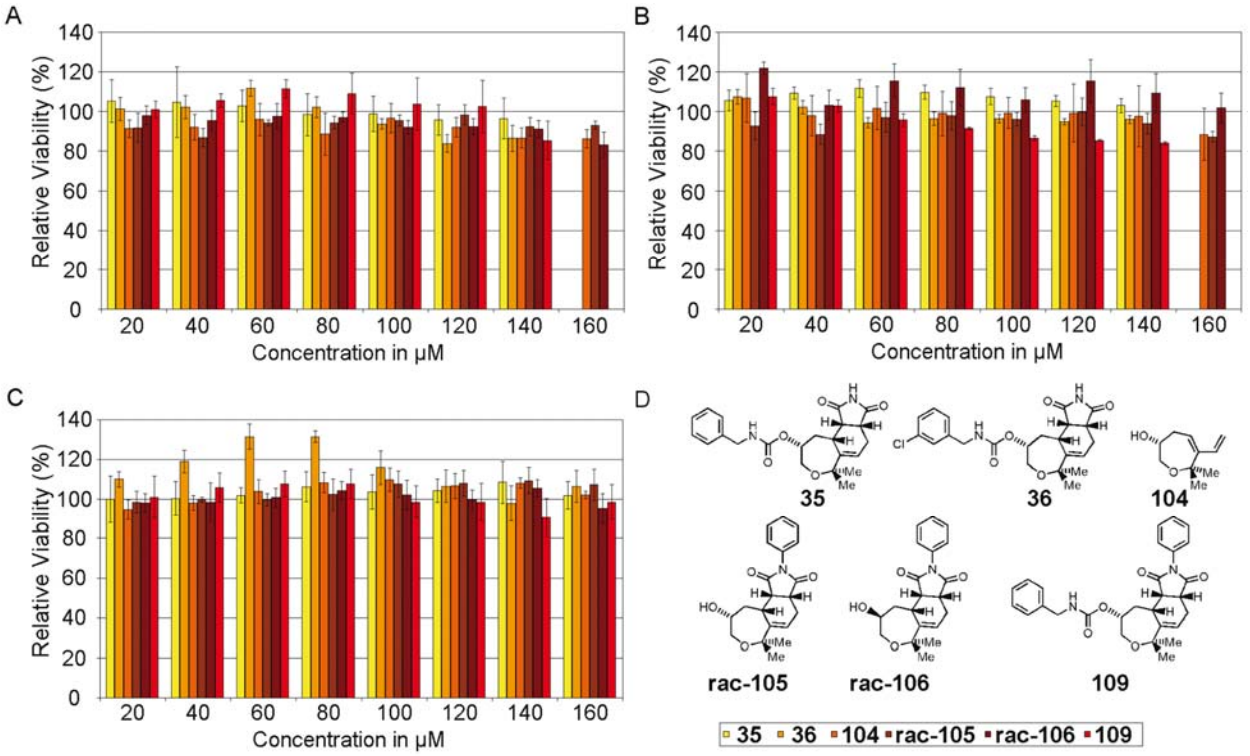


Figure S5. Cytotoxicity of **35**, **36**, **104**, ***rac*-105**, ***rac*-106**, **109** at high concentrations. *(A)* HeLa cell line. *(B)* Hek293 cell line. *(C)* HepG2 cell line. *(D)* Compound structures.

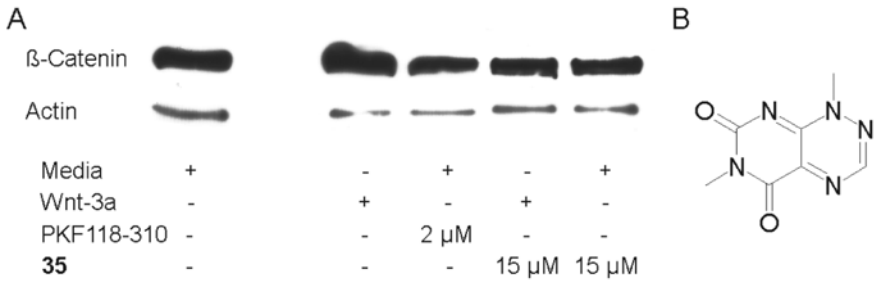
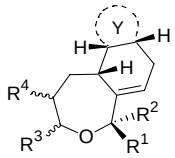
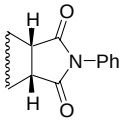
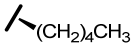
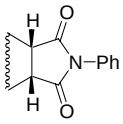
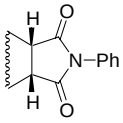
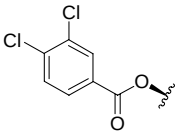
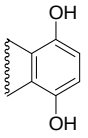
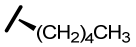
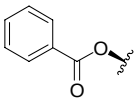
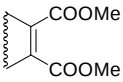
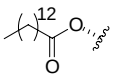
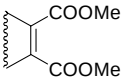
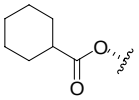
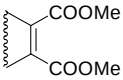
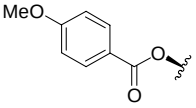
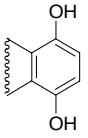
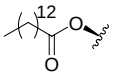
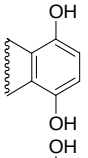
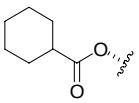
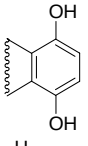
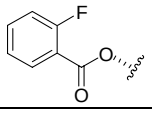
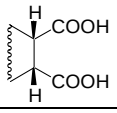
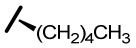
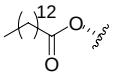
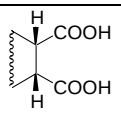
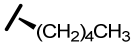
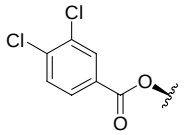
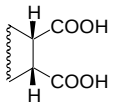
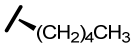
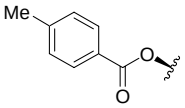
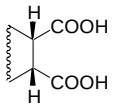
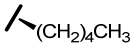
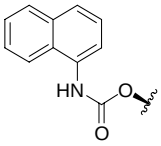
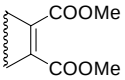
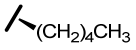
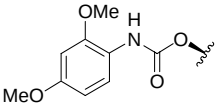
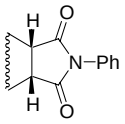
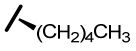
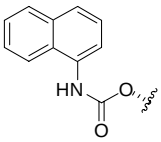
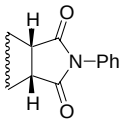
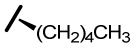
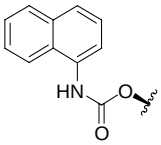
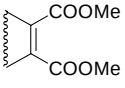
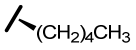
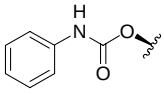
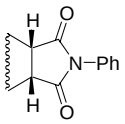
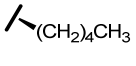
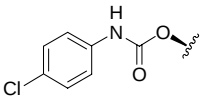
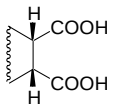
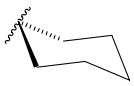
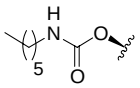
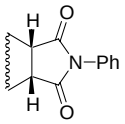
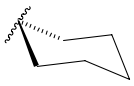
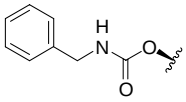
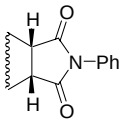
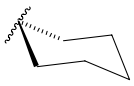
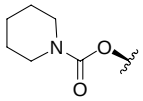
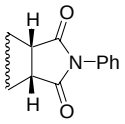
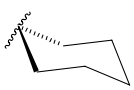


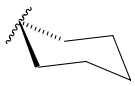
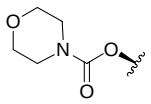
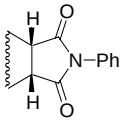
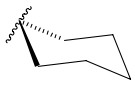
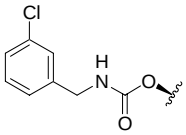
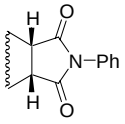
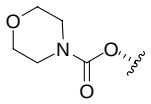
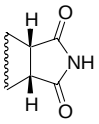
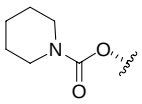
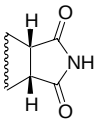
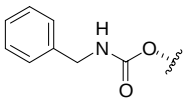
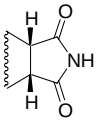
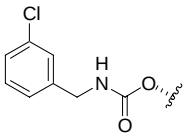
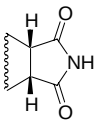
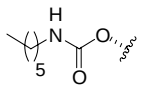
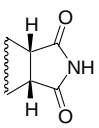
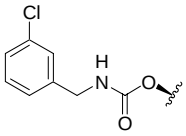
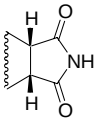
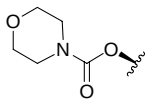
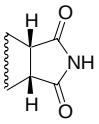
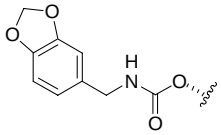
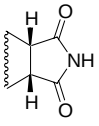
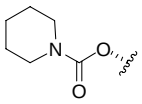
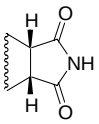
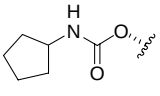
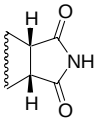
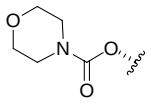
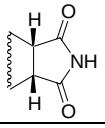
Figure S6. Western blot of total amount of ß-catenin protein. *(A)* The western blot was done using SW480 cell lysate. Wnt-3a does not activate the wnt pathway due to the mutated APC gene in the cancer cell line. Compound **35** has no activating effect if used in combination with Wnt-3a or alone pointing (see Fig. S3) to modulation of the Wnt signalling pathway at the level of the receptor complex. PKF118-310 is a known inhibitor and was used as a control (3). The control is of limited efficiency in this cancer cell line. *(B)* Structure of PKF118-310.

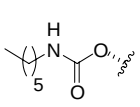
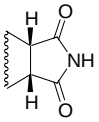
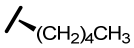
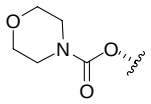
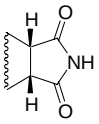
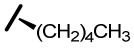
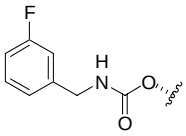
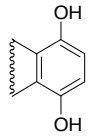
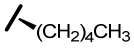
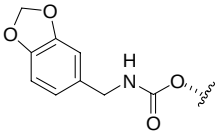
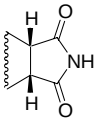
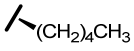
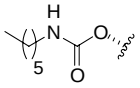
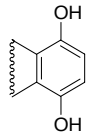
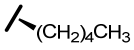
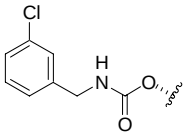
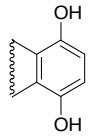
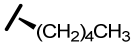
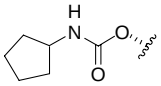
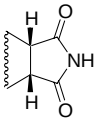
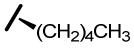
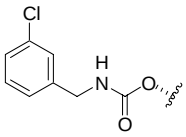
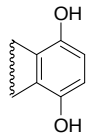
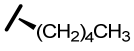
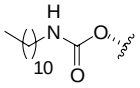
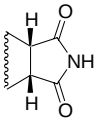
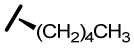
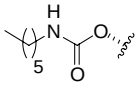
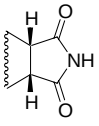
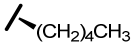
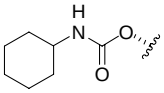
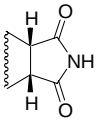
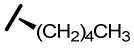
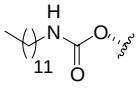
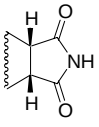
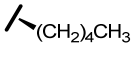
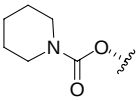
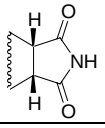
Table S1. Oxepane sub-library 1.

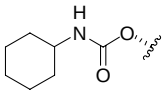
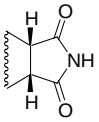
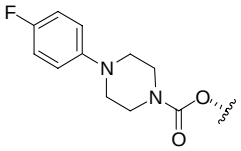
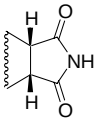
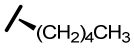
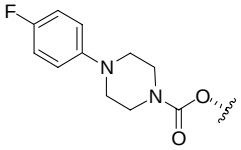
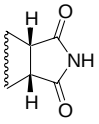
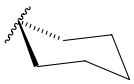
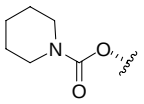
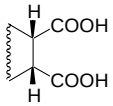
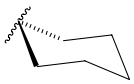
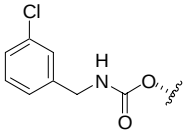
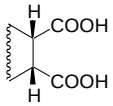
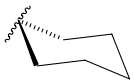
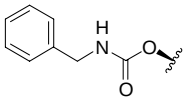
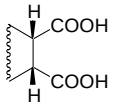
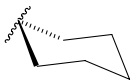
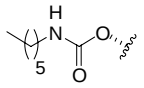
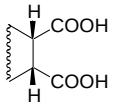
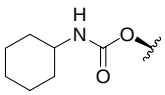
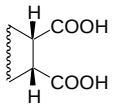
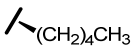
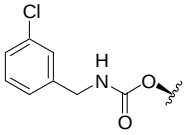
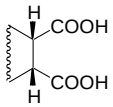
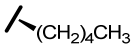
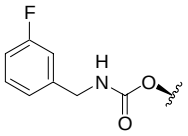
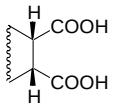
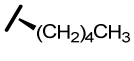
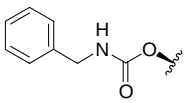
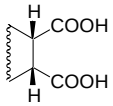
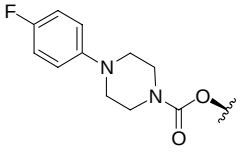
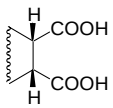
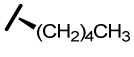
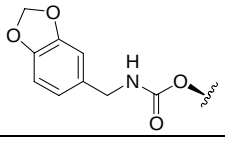
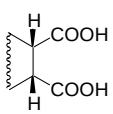


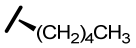
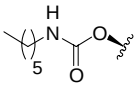
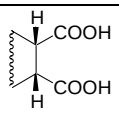
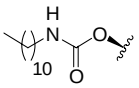
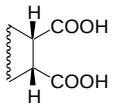
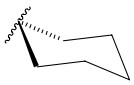
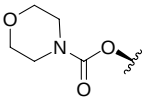
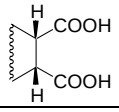
compound	R^1	R^2	R^3	R^4	Y	yield[a]
7		-H				70% (after 4 steps)
8		-H	-H			65% (after 4 steps) d.r. = 20:1
9		-H				50% (after 4 steps)
10		-H	-H			30% (after 5 steps) d.r. = 4:1
11		-H				26% (after 5 steps)
12		-H	-H			28% (after 5 steps)
13		-H	-H			32% (after 5 steps)
14		-H	-H			17% (after 5 steps)
15		-H	-H			23% (after 5 steps)
16		-H	-H			20% (after 5 steps)
17		-H	-H			21% (after 6 steps) d.r. = 8:1

18		-H	-H			25% (after 6 steps) d.r. = 9:1
19		-H	-H			24% (after 6 steps) d.r. = 6.5:1
20		-H	-H			20% (after 6 steps) d.r. = 6:1
21		-H				32% (after 5 steps) d.r. = 8:1
22		-H				50% (after 5 steps) d.r. = 8:1
23		-H				60% (after 5 steps)
24		-H	-H			16% (after 5 steps)
25		-H	-H			32% (after 5 steps)
26		-H	-H			26% (after 6 steps) d.r. = 6:1
27		-H			32% (after 6 steps) d.r. = 8:1
28		-H			40% (after 6 steps) d.r. = 7.5:1
29		-H			32% (after 6 steps) d.r. = 8:1
30		-H			32% (after 6 steps) d.r. = 8:1

31			-H			34% (after 6 steps) d.r. = 8:1
32			-H			32% (after 6 steps) d.r. = 6:1
33	$-CH_3$	$-CH_3$	-H			30% (after 6 steps)
34	$-CH_3$	$-CH_3$	-H			22% (after 6 steps)
35	$-CH_3$	$-CH_3$	-H			20% (after 6 steps)
36	$-CH_3$	$-CH_3$	-H			16% (after 6 steps)
37	$-CH_3$	$-CH_3$	-H			31% (after 6 steps) d.r. = 3:1
38	$-CH_3$	$-CH_3$	-H			22% (after 6 steps) d.r. = 4:1
39	$-CH_3$	$-CH_3$	-H			15% (after 6 steps) d.r. = 3:2
40	$-CH_3$	$-CH_3$	-H			30% (after 6 steps) d.r. = 3:1
41	-H	-H	-H			20% (after 6 steps)
42	-H	-H	-H			26% (after 6 steps)
43	-H	-H	-H			30% (after 6 steps)

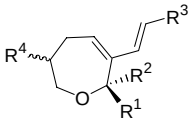
44	-H	-H	-H			20% (after 6 steps)
45		-H	-H			16% (after 6 steps)
46		-H	-H			15% (after 6 steps)
47		-H	-H			16% (after 6 steps)
48		-H	-H			15% (after 6 steps)
49		-H	-H			15% (after 6 steps)
50		-H	-H			16% (after 6 steps) d.r. = 25:1
51		-H	-H			20% (after 6 steps)
52		-H	-H			23% (after 6 steps)
53		-H	-H			29% (after 6 steps) d.r. = 2.6:1
54		-H	-H			31% (after 6 steps)
55		-H	-H			24% (after 6 steps)
56		-H	-H			20% (after 6 steps)

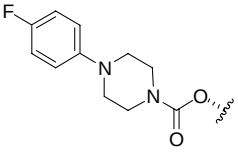
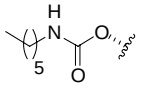
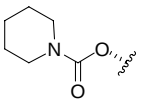
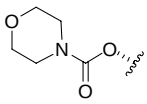
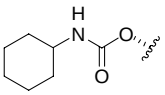
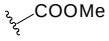
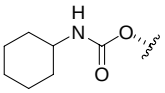
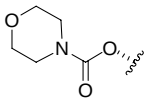
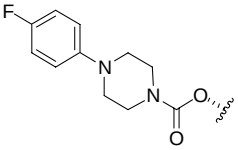
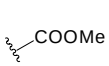
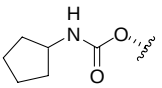
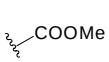
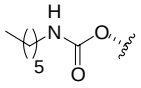
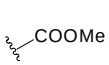
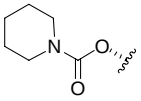
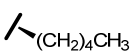
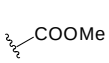
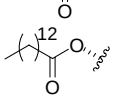
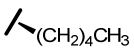
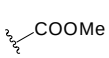
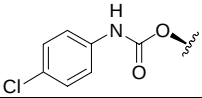
57	-H	-H	-H			20% (after 6 steps)
58	-H	-H	-H			28% (after 6 steps)
59		-H	-H			22% (after 6 steps)
60			-H			34% (after 7 steps) d.r. = 4:1
61			-H			15% (after 7 steps) d.r. = 5:1
62			-H			20% (after 7 steps) d.r. = 4:1
63			-H			15% (after 7 steps) d.r. = 7:1
64	$-CH_3$	$-CH_3$	-H			16% (after 7 steps)
65		-H	-H			15% (after 7 steps) d.r. = 3:2
66		-H	-H			20% (after 7 steps)
67		-H	-H			41% (after 7 steps) d.r. = 3:2
68	$-CH_3$	$-CH_3$	-H			25% (after 7 steps) d.r. = 6:1
69		-H	-H			15% (after 7 steps) d.r. = 4:1

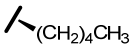
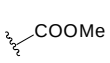
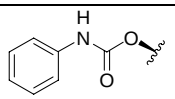
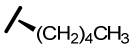
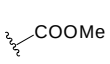
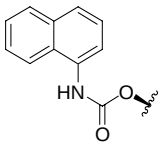
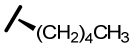
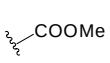
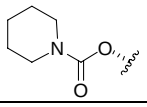
70		-H	-H			27% (after 7 steps) d.r. = 4:1
71	$-CH_3$	$-CH_3$	-H			20% (after 7 steps) d.r. = 6:1
72		-H			10% (after 7 steps) d.r. = 4:1

[a]Isolated yield after column chromatography, d.r. = diastereomeric ratio.

Table S2. Oxepane sub-library 2.

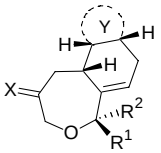


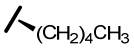
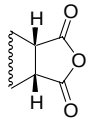
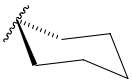
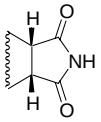
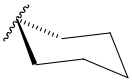
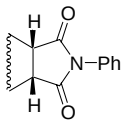
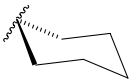
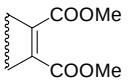
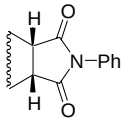
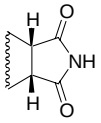
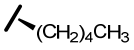
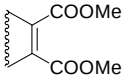
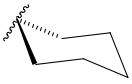
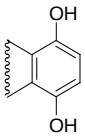
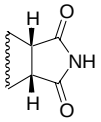
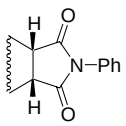
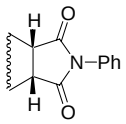
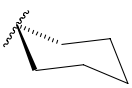
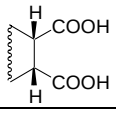
compound	R^1	R^2	R^3	R^4	yield[a]
73	$-CH_3$	$-CH_3$	-H		40% (after 5 steps)
74	$-CH_3$	$-CH_3$	-H		25% (after 5 steps)
75	$-CH_3$	$-CH_3$	-H		29% (after 5 steps)
76	$-CH_3$	$-CH_3$	-H		40% (after 5 steps)
77	$-CH_3$	$-CH_3$	-H		45% (after 5 steps)
78	$-CH_3$	$-CH_3$			35% (after 6 steps)
79	$-CH_3$	$-CH_3$			36% (after 6 steps)
80	$-CH_3$	$-CH_3$			38% (after 6 steps)
81	$-CH_3$	$-CH_3$			35% (after 6 steps)
82	$-CH_3$	$-CH_3$			35% (after 6 steps)
83	$-CH_3$	$-CH_3$			30% (after 6 steps)
84		-H			75% (after 5 steps)
85		-H			47% (after 5 steps)

86		-H			47% (after 5 steps)
87		-H			40% (after 5 steps)
88		-H			34% (after 6 steps)

[a]Isolated yield after column chromatography, d.r. = diastereomeric ratio.

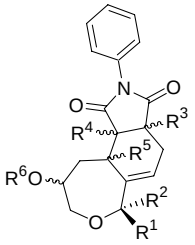
Table S3. Oxepane sub-library 3.

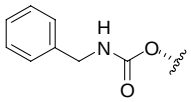
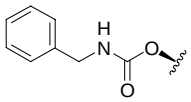
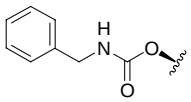
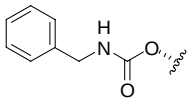
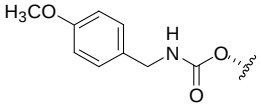
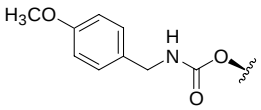
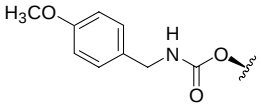
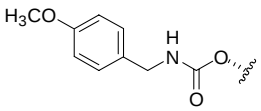
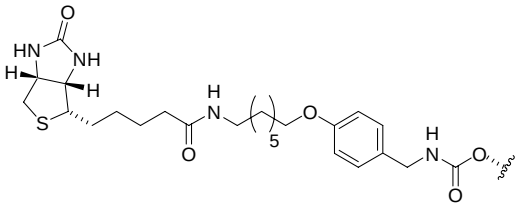
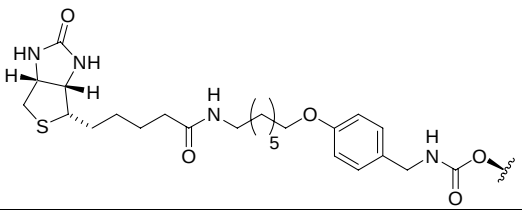


compound	R^1	R^2	X	Y	yield
89		-H	O		25%[a] (after 5 steps)
90			O		15%[a] (after 5 steps)
91			O		10%[a] (after 5 steps)
92			O		15%[a] (after 5 steps)
93	$-CH_3$	$-CH_3$	O		12%[a] (after 5 steps)
94	$-CH_3$	$-CH_3$	O		15%[a] (after 5 steps)
95		-H	O		13%[a] (after 5 steps)
96			N〰OMe		10%[b] (after 6 steps) d.r. = 8:1
97	$-CH_3$	$-CH_3$	N〰OBn		15%[b] (after 6 steps)
98	$-CH_3$	$-CH_3$	N〰OBn		13%[b] (after 6 steps) d.r. = 4.3:1
99		-H	N〰OBn		10%[b] (after 6 steps) d.r. = 20:1
100			N〰OMe		10%[b] (after 6 steps) d.r. = 6:1

[a]Isolated yield after column chromatography, [b]Isolated yield after preparative thin layer chromatography (PTLC), d.r. = diastereomeric ratio.

Table S4. Hit compounds and related probes.



compound	R^1	R^2	R^3	R^4	R^5	R^6
***rac*-105**	- CH_3	$-CH_3$	H	H	H	H
***rac*-106**	- CH_3	$-CH_3$	H	H	H	H
109	- CH_3	$-CH_3$	H	H	H	
110	- CH_3	$-CH_3$	H	H	H	
111	- CH_3	$-CH_3$	H	H	H	
112	- CH_3	$-CH_3$	H	H	H	
113	- CH_3	$-CH_3$	H	H	H	
114	- CH_3	$-CH_3$	H	H	H	
115	- CH_3	$-CH_3$	H	H	H	
116	- CH_3	$-CH_3$	H	H	H	
121	- CH_3	$-CH_3$	H	H	H	
122	- CH_3	$-CH_3$	H	H	H	

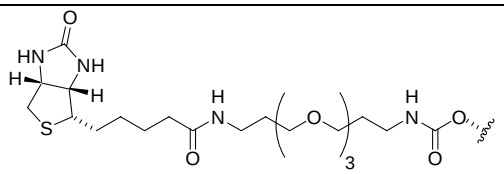
125	- CH_3	$-CH_3$				

Table S5. Screening results. Wnt-3a synergistic activity measurements were done using Hek293 reporter cells (4). All data given in %, Wnt-3a treated Hek293 reporter cells were set to 100%. Cytotoxicity was tested with Hek293 cells (ATCC: CRL-1573). The data obtained at 20 μM compound concentration were used for SAR studies.

	Apparent Wnt reporter gene activation at			Cell viability at		
Comp.	30 μM	20 μM	10 μM	30 μM	20 μM	10 μM
7	113.5±12.1	136.0±29.4	150.4±12.8	59.7±7.5	69.2±3.9	80.9±6.3
8	126.8±14.1	161.2±24.9	154.9±8.0	76.8±6.6	84.8±8.5	84.8±5.6
9	145.3±5.8	136.3±10.7	118.4±14.9	107.2±6.5	101.6±9.5	102.5±7.0
10	82.5±40.4	86.8±17.2	120.2±6.8	111.4±7.7	120.5±7.3	129.9±9.8
11	136.3±15.9	106.8±16.9	124.0±13.9	118.1±9.6	122.6±9.2	124.1±10.2
12	130.4±5.2	138.7±9.3	131.0±7.8	112.1±23.4	102.8±8.5	110.1±17.1
13	119.6±15.9	176.3±47.0	180.8±24.4	91.4±25.4	116.1±17.2	122.4±6.6
14	130.6±11.3	100.3±9.7	117.1±7.1	96.0±4.2	98.3±9.9	85.3±9.9
15	145.1±7.3	75.6±13.8	113.8±22.9	73.0±3.4	77.5±6.7	90.3±10.9
16	122.6±11.0	132.4±27.3	135.6±24.2	61.7±5.3	79.9±6.7	86.6±8.7
17	115.3±6.2	106.6±8.7	139.3±7.5	91.8±6.0	102.0±11.4	106.2±2.4
18	137.8±27.8	136.8±8.7	104.7±14.1	78.3±12.3	65.1±3.0	85.6±8.9
19	151.3±14.7	107.7±11.2	118.8±10.8	85.4±13.1	97.3±19.5	103.4±17.6
20	97.0±16.7	145.1±32.9	130.9±5.5	89.7±5.2	91.5±6.0	89.4±8.7
21	131.6±13.0	131.0±35.7	150.8±7.9	91.6±6.7	96.2±12.1	102.1±17.3
22	113.0±12.0	79.3±7.4	130.6±10.3	113.1±4.0	104.4±4.5	91.0±15.2
23	124.4±15.4	137.7±33.8	181.2±12.3	120.4±9.9	121.7±10.6	108.7±8.9
24	188.4±20.4	191.4±36.2	203.6±7.2	97.8±9.1	77.9±9.8	103.1±8.9
25	127.7±12.7	135.8±26.3	200.2±15.6	87.1±9.6	63.4±7.4	107.4±2.0
26	208.4±9.0	127.6±29.8	113.1±14.5	92.7±6.1	77.0±8.8	104.8±16.2
27	99.0±2.2	158.0±21.9	148.4±12.2	99.9±23.2	98.7±27.9	130.7±28.2
28	164.1±22.0	154.1±18.2	170.2±8.4	107.0±6.1	136.0±21.3	188.7±35.4
29	134.5±11.7	169.5±36.7	126.0±19.0	109.6±46.0	122.8±9.9	145.6±16.4
30	120.3±34.7	159.4±11.1	163.6±20.1	90.4±7.3	107.3±7.0	103.3±16.1
31	120.2±15.4	162.2±9.8	109.0±9.0	117.9±23.3	123.7±26.6	125.5±20.9
32	151.5±67.4	159.5±27.6	147.8±3.0	79.2±15.2	124.6±24.1	110.0±42.5
33	118.1±15.6	138.9±9.3	76.6±25.6	94.2±6.0	91.6±5.5	100.9±7.7
34	133.3±6.0	180.5±9.6	135.1±9.7	107.9±27.3	114.0±19.5	110.0±21.0
35	96.5±14.1	171.8±22.2	96.0±4.8	147.4±25.2	151.7±54.2	121.4±16.8
36	143.0±14.2	208.7±8.9	145.3±8.8	105.4±15.9	100.6±7.8	120.5±7.7
37	143.3±16.1	168.4±20.4	155.0±26.8	116.0±24.9	115.7±23.8	125.8±26.6
38	113.3±29.4	151.8±7.4	150.7±12.2	113.5±9.7	111.9±12.2	128.8±8.1

39	103.3±11.1	136.6±15.3	113.1±19.7	106.6±9.7	95.2±8.0	107.3±10.3
40	126.5±43.6	186.8±14.3	124.9±11.6	84.6±6.2	94.7±13.4	101.0±13.7
41	121.0±5.3	126.2±6.6	119.5±21.0	111.7±8.1	102.9±7.9	102.3±18.6
42	155.1±11.2	192.0±23.4	123.4±10.6	87.5±17.0	94.2±6.7	92.9±8.9
43	105.2±11.7	184.0±17.8	111.2±25.9	102.9±7.7	111.9±8.4	109.4±8.7
44	115.9±12.8	167.1±33.8	118.8±17.0	83.7±16.3	105.3±37.9	158.0±87.4
45	89.0±31.0	143.6±13.6	128.9±16.6	111.7±10.5	113.1±2.4	116.0±6.7
46	128.0±8.6	168.8±29.2	102.7±11.1	48.9±21.3	62.2±13.1	67.3±11.2
47	66.3±14.3	151.6±20.0	141.9±26.0	99.1±9.3	98.4±1.8	92.9±5.4
48	48.8±7.7	135.4±16.4	112.1±5.3	152.2±46.0	113.3±4.4	114.4±12.5
49	235.2±67.1	213.5±23.0	219.6±31.0	16.5±5.7	64.0±5.4	100.6±8.4
50	112.0±4.8	178.6±13.1	137.1±7.6	80.4±11.7	89.7±16.0	94.5±14.6
51	79.8±14.2	151.7±14.4	158.6±30.3	81.7±8.8	77.5±9.4	115.5±20.0
52	112.3±25.5	186.1±9.4	131.3±11.0	70.7±9.7	71.9±17.0	75.2±17.1
53	154.9±37.5	166.6±20.0	163.6±24.0	66.5±7.3	109.4±8.9	105.8±13.8
54	180.7±10.8	217.3±20.8	166.2±21.6	94.0±5.7	110.2±5.9	119.0±11.5
55	98.1±10.5	150.2±8.4	129.6±11.7	81.1±12.8	81.8±7.9	69.5±5.7
56	3.8±3	159.6±22.6	132.8±9.6	101.7±7.7	111.1±4.6	124.0±10.0
57	152.0±15.1	136.4±10.5	122.4±17.2	74.1±5.0	86.0±2.5	98.6±7.5
58	104.2±35.9	108.5±10.0	136.2±9.9	50.3±13.1	59.8±8.0	65.8±13.1
59	57.0±4.9	158.7±14.0	142.5±5.9	101.0±5.5	124.3±21.1	131.2±6.1
60	83.9±22.2	140.3±12.5	129.5±0.8	107.3±8.5	87.7±10.2	111.0±8.3
61	131.9±16.5	161.5±12.8	136.4±6.8	91.5±7.1	95.1±5.2	114.3±18.8
62	81.7±21.0	141.9±10.1	99.7±3.3	105.5±8.9	104.9±5.2	122.5±7.4
63	98.2±17.6	154.8±11.9	116.9±19.2	127.2±8.4	132.9±8.2	130.6±54.4
64	161.8±13.3	161.5±16.4	105.9±22.3	111.0±27.1	113.7±19.0	107.7±25.9
65	61.3±3.8	159.2±19.9	114.8±14.4	121.8±7.8	121.1±15.9	126.4±16.0
66	159.8±82.9	147.6±19.7	104.0±5.8	88.4±23.1	89.9±46.2	74.1±16.4
67	111.4±37.2	128.5±11.0	121.2±20.7	91.2±4.6	97.8±7.5	122.4±9.7
68	79.1±14.4	149.8±19.3	77.6±19.8	114.7±11.8	112.8±7.5	112.1±6.7
69	91.9±28.7	146.0±9.6	114.2±7.1	118.5±5.7	94.7±5.7	103.7±17.5
70	143.9±41.7	144.4±14.9	115.4±7.8	106.4±18.6	95.4±2.8	89.2±8.3
71	129.4±11.2	145.7±25.0	121.6±3.9	110.3±26.7	105.8±28.3	108.0±31.5
72	124.8±16.4	155.8±30.9	98.3±10.0	125.8±26.1	138.8±30.4	183.4±60.9
73	129.9±42.4	172.8±9.2	140.9±22.0	100.3±24.1	115.0±18.7	90.4±13.6
74	130.3±16.0	187.2±10.0	126.0±21.1	98.2±8.7	134.4±28.5	171.5±47.0
75	142.0±7.2	150.1±11.2	128.5±6.2	63.3±11.6	92.6±14.1	115.8±23.0
76	116.8±12.3	153.1±8.5	123.0±5.3	79.2±6.3	76.6±20.7	80.4±9.9
77	86.4±21.9	190.1±20.4	150.8±21.6	93.9±28.1	105.4±7.4	121.4±9.5

78	109.2±12	171.1±8.7	114.9±27.4	127.9±8.7	105.2±10.3	108.9±22.4
79	130.7±8.7	156.6±13.6	101.5±14.2	138.2±24.7	120.6±27.3	125.9±28.7
80	111.7±27.3	125.9±15.0	109.5±26.8	53.8±18.3	50.7±11.5	57.0±14.7
81	112.8±5.4	136.6±29.9	108.0±3.2	110.0±24.0	112.2±17.4	118.4±22.5
82	150.0±39.3	153.7±15.1	110.1±9.0	90.2±20.5	167.8±41.3	185.1±48.0
83	117.7±3.1	118.3±37.3	96.6±5.9	95.0±16.4	106.9±12.3	110.1±11.9
84	147.3±10.6	116.1±14.4	115.6±11.2	59.0±4.9	118.5±9.3	74.3±2.4
85	128.3±24.8	95.3±31.6	226.1±14.3	103.1±1.3	102.9±6.7	87.2±7.6
86	138.6±10.6	140.3±6.6	180.2±30.9	85.1±4.3	90.6±2.5	93.8±3.4
87	162.9±9.7	136.4±22.5	131.7±10.4	103.4±4.1	121.5±21.5	90.5±14.7
88	97.2±5.6	131.1±22.0	150.9±12.4	77.7±4.9	88.8±4.5	106.8±5.1
89	147.7±13.4	133.1±14.0	122.0±12.3	80.6±8.7	90.1±9.7	107.6±4.2
90	122.8±9.7	120.1±39.3	97.2±20.2	95.0±20.2	80.3±10.7	92.1±12.3
91	121.7±6.1	132.2±13.4	172.1±18.8	81.6±11.2	75.4±9.8	87.0±16.2
92	123.5±12.2	173.0±18.5	94.3±14.6	83.2±19.6	89.8±18.6	101.4±25.3
93	122.1±11.8	146.5±10.3	113.4±5.6	117.2±6.2	126.6±13.2	133.6±14.4
94	111.1±14.8	138.3±14.3	149.5±11.6	83.3±9.0	91.6±9.2	93.5±11.0
95	86.7±36.8	137.7±16.2	121.7±19	114.7±1.7	113.3±6.2	121.4±20.6
96	98.8±27.2	150.5±7.7	153.1±18.8	145.3±4.3	137.0±3.5	145.0±6.6
97	71.5±2.4	173.7±1.2	85.4±7.6	112.0±44.0	134.3±47.1	140.0±58.8
98	154.8±7.8	229.4±42.4	126.3±15.0	84.9±10.0	83.6±9.1	96.2±16.0
99	139.2±8.3	140.1±7.9	97.5±7.9	109.4±18.0	112.2±19.0	108.4±15.8
100	116.8±43.1	124.7±6.4	144.9±19.4	86.8±18.0	103.5±11.1	96.4±8.5
***rac*-105**	114.7±8.0	119.4±8.4	97.5±7.1	91.3±11.4	97±6.9	n.d.
***rac*-106**	114.7±5.8	108.8±10.3	109.6±	100.4±1.8	112.5±3.9	n.d.
109	n.d.	206.1±15.1	249.3±45.4	93.8±2.6	102.5±4.5	n.d.
110	n.d.	157.0±32.7	143.4±37.2	n.d.	n.d.	n.d.
111	n.d.	172.6±29.8	174.2±64.3	n.d.	n.d.	n.d.
112	n.d.	266.3±48.6	201.3±15.6	n.d.	n.d.	n.d.
113	n.d.	196.3±11.1	161.9±11.9	n.d.	n.d.	n.d.
114	n.d.	109.8±4.4	110.5±13.3	n.d.	n.d.	n.d.
115	n.d.	122.5±17.6	107.3±4.7	n.d.	n.d.	n.d.
116	n.d.	112.3±12.4	89.5±12.8	n.d.	n.d.	n.d.

Additional Materials and Methods

General methods for synthesis. All solvents, when not purchased in suitable purity or dryness, were distilled. Deionized water was used for all experiments. All reagents were purchased from commercial suppliers and used without purification. Thin layer chromatography (TLC) was carried out on silica gel plate (60F-254) using ultra violet light irradiation 254 nm and $KMnO_4$ solution as staining reagent. Preparative HPLC was performed on a Waters machine using a Macherey-Nagel C18 gravity 5 µm reversed phase column. The separations were started at 40% MeCN in H_2O, and the MeCN proportion was linearly increased to 100% over a period of 50 min with a flow of 20 mL/min (Method A). Preparative chiral HPLC was performed on a Ultimate 3000 system using a Chiralpack IC 10mm chiral phase column. The separations were started at 70% dichloro-methane/EtOH (100:2, eluent A) in *iso*-hexane (eluent B), and eluent A was increased to 100% over a period of 30 min with a flow of 3 mL/min (Method B). Melting points were determined with a Büchi Melting Point B-540 apparatus (uncorrected). Optical rotations were measured at 23°C in a Schmidt+Haensch Polartronic HH8 polarimeter at 589 nm, with values given in 10^{-1} deg cm^2 g^{-1} and concentrations *c* given in g/100 mL. ^1H and ^{13}C-NMR spectra were recorded on a Varian Mercury VX 400 (400.1 MHz (^1H) and 100.6 MHz (^{13}C) spectrometer at room temperature. Chemical shifts are expressed in part per million (ppm) and the spectra are calibrated to residual solvent signals of $CDCl_3$ (7.26 ppm for ^1H-spectra and 77.16 ppm for ^{13}C-spectra). Coupling constants are given in Hertz (Hz) and the following notations indicate the multiplicity of the signals: s (singlet), d (doublet), t (triplet), dd (double of doublet), m (multiplet), br (broad signal). Fourier transform infrared spectroscopy (FT-IR) spectra were obtained with a Bruker Tensor 27 spectrometer (ATR, neat or as a thin film). High Resolution Mass Spectra were measured by using electron impact (EI),

fast atom bombardment (FAB) or electrospray ionisation techniques (ESI). Chromatography was performed using silica gel under approximately 0.5 bar pressure.

General procedure for the solution phase synthesis of oxepanes using polymer-supported scavenging reagents. To a cooled suspension (0°C) of sodium hydride (1.5 equiv., 95% dispersion in mineral oil) in THF (50 mL), a solution of a selected building block **PA1-4** (1 equiv.) in THF (20 mL) was added dropwise over 20 min. The mixture was warmed to 25°C and stirred for 15 min. After cooling to 0°C, a solution of selected building block **BEA1-3** (1.5 equiv.) in THF (10 mL) was added dropwise over 30 min. and the resulting mixture was warmed to 25°C and stirred for 6 h. Water (20 mL) was added, the mixture was further diluted with water (100 mL) and diethyl ether (100 mL) and the resulting layers were separated. The aqueous layer was extracted with diethyl ether (2 × 200 mL). The combined ether layers were washed with brine (2 × 20 mL), dried with Na_2SO_4, filtered, concentrated under reduced pressure, and purified by silica gel chromatography (cyclohexane/ethyl acetate 9:1), to furnish ethers **5** in 70-80% yield.

5 was dissolved in anhydrous diethyl ether, the solution was cooled to -78°C, and diisobutylaluminium hydride (1M solution in hexane, 1.5 equiv.) was added slowly by syringe pump over 30 min. The mixture was stirred at -78°C for 20 min. 1M aq. HCl was added, the cooling bath was removed, and the mixture was stirred for 1 h before being diluted with diethyl ether (50 mL). The aqueous layer was extracted with diethyl ether (2 × 20 mL) and the combined extracts were washed with water (2 × 10 mL) and brine (2 × 10 mL), dried with Na_2SO_4, filtered and concentrated under reduced pressure to afford the aldehyde **4**.

In a two-neck round-bottom flask (+)- or (–)-Diisopinocampheyl boron chloride (DIPCl) was dissolved in THF. The solution was cooled to -78°C, allylmagnesium chloride (2M in THF, 1.5 equiv.) was added dropwise and the mixture was stirred at -78°C for 1 h. The reaction mixture

was warmed to 20°C and stirred for 1 h. After cooling to -78°C, crude aldehyde **4** (dissolved in anhydrous THF) was added dropwise and the mixture was stirred at -78°C for 1 h. The reaction mixture was allowed to warm to room temperature, stirred for 1 h and then diluted with methanol (10 mL). Sulfonic acid resin (**S1**) was added and the mixture was shaken at room temperature for 6 h. The resin was drained, washed with methanol and dichloromethane and the combined eluates were evaporated to afford crude homoallylic alcohol **2**. The alcohol was dissolved in dichloromethane (0.002 M) in a two neck round bottom flask with attached reflux condenser. Ar gas was bubbled through the solution using a stainless steel cannula for 30 min. 1st generation Grubbs catalyst (20 mol%) was added and the reaction mixture was heated to reflux. After the reaction was complete (TLC control), scavenger resin **S2** (4 equiv.) was added and the mixture was shaken at room temperature for 10 h. The resin was filtered over a short silica gel pad, washed with dichloromethane, and the combined eluates were evaporated to obtain the crude product **1**.

General procedure for the synthesis of sub-library 1. Crude alcohol **1** (1 equiv.) was dissolved in THF in a two-neck round-bottom flask. Pyridine (1.5 equiv.) and either acyl chloride **AC1-8** (1.5 equiv.) or isocyanate **I1-4** (1.5 equiv.) was added. The reaction mixture was stirred for 6 h at room temperature. When the reaction was complete (TLC control), aminomethylated polystyrene **S3** (6 equiv. relative to acyl chloride or 3 equiv. relative to the isocyanate) was added and the reaction mixture was stirred at room temperature for 4 h. The resin was filtered, washed with dichloromethane, and the combined eluates were evaporated to obtain crude esters **10**-**20** or carbamates **21**-**26**.

Alternatively, carbamates **27**-**72** were prepared by dissolving **1** in dichloromethane, then adding 1,1'-carbonyldiimidazole (1.5 equiv.) and stirring the mixture at room temperature for 10 h. When the starting material was consumed (TLC control), the solvent was evaporated and the crude residue

was dissolved in THF/DMF (4:1). Potassium carbonate (1.5 equiv.) and amine **A1-12** (1.2 equiv.) was added. The reaction mixture was stirred at room temperature for 5 h. When the transformation was complete (monitored by TLC), sulfonic acid resin **S1** (6 equiv. relative to K_2CO_3 and excess amine) was added and the resulting suspension was stirred at room temperature for 4 h. The resin was filtered, washed with dichloromethane, and the combined eluates were concentrated to obtain crude carbamates. Esters and carbamates were then dissolved in a minimum volume of toluene and heated at 70°C in the presence of the dienophiles **D1-4** (1.2 equiv) for 3–10 h. When the starting material was consumed (monitored by TLC), the solvent was evaporated and the crude products were purified by silica gel chromatography to obtain the Diels-Alder adducts (**10-16, 21-25, 27-59**) in 15–70% overall yield over 4–6 steps.

A series of diacids was prepared by heating esters and carbamates at 70°C with maleic anhydride **D5** (1.2 equiv.) in a minimum volume of toluene for 3 h, followed by addition of a 20% solution of water in THF (5 mL). Stirring was continued for 10 h at room temperature. Excess EtOH was added, and all volatiles were evaporated. The crude product was purified by silica gel chromatography to afford acids **17-20, 26, 60-72** in 10–34% overall yield over 6–7 steps.

General procedure for the synthesis of sub-library 2. Diene **1** (1 equiv.) and methyl acrylate (1.5 equiv.) was dissolved in dichloromethane (0.002 M) in a two-neck round-bottom flask under Ar. The solution was deoxygenated by introducing Ar for 30 min via a cannula. 2nd generation Grubbs catalyst (15 mol%) was added and the reaction mixture was refluxed until the diene **1** was consumed (monitored by TLC). Scavenger resin **S2** (20 equiv. with respect to catalyst) was added and the mixture was shaken at room temperature for 10 h. The resin was filtered through a short silica gel pad and washed with dichloromethane. The solvent was evaporated to afford the crude

cross metathesis products, which were then derivatized as esters and carbamates as described above to afford derivatives **78-88** in 30–75% overall yield after 5–6 steps.

General procedure for the synthesis of sub-library 3. Pyridinium chlorochromate (3 equiv) was suspended in CH_2Cl_2 at room temperature. A solution of alcohol **1** in dichloromethane was added dropwise and the reaction mixture was stirred for 10 h at room temperature. When the reaction was complete (monitored by TLC), the mixture was filtered through a pad of Celite which was thoroughly washed with CH_2Cl_2. The combined eluates were evaporated to afford the crude ketones which were then treated with dienophiles **D1-5**, under the conditions described for the synthesis of sub-library 1, to afford adducts **89-95** in 10–25% overall yield after 5 steps**.**

A selection of keto-oxepanes was dissolved in EtOH/H_2O (2:1). *O*-methyl hydroxylamine or *O*-benzyl hydroxylamine hydrochloride (1.5 equiv.) was added and the reaction mixture was stirred at room temperature for 10 h. When the ketone was consumed (monitored by TLC), excess EtOH was added, and all volatiles were evaporated. The crude materials were purified by preparative thin layer chromatography affording oximes **96-100** in 10–15% overall yield after 6 steps.

General procedure for the synthesis of enantiopure oxepanes 109-112. Racemic **1** ($R^1 = R^2 = CH_3$, $R^3 = H$) was subjected to Diels-Alder reaction with diene **D3** and the resulting diastereomers ***rac*-105** and ***rac*-106** were separated and converted into carbamates **109-110** and **111-112** respectively using amine **A1**. Finally, the enantiomers of these urethanes were separated by preparative HPLC on a chiral stationary phase. The relative configuration of the stereoisomers was successfully determined by nOe studies (Fig. S2) and by crystal structure analyses of racemic mixtures **109/110** and **111**/**112** (see Fig. S3), which confirmed that the Diels-Alder reaction

proceeded via an *endo*-transition state. The absolute configuration of the oxepanes was assigned by analogy to the well-established stereochemical course of the Brown allylation reaction (1).

Procedures and characterization for hit compounds and derived probes

Ethyl 2-(2-methyl-3-butyn-2-yloxy)acetate (101). A solution of 2-methyl-3-butyn-2-ol (11.6 mL, 119 mmol) in 20 mL of THF was added dropwise at 0°C over 15 min to a suspension of sodium hydride (60% in mineral oil) (9.51 g, 238 mmol) in dry THF (200 mL). The resulting mixture was warmed to room temperature, stirred for 30 minutes and cooled again to 0°C. A solution of ethyl bromoacetate (19.7 mL, 178 mmol) in THF (10 mL) was added dropwise over 30 min. The mixture was warmed to room temperature and stirred for 8 h. Water was added (20 mL + 100 mL), and teh mixture was extracted with diethyl ether (3 × 200 mL). The combined organic extracts were washed with brine, dried with $MgSO_4$ and concentrated. The residue was purified by flash chromatography (95:5, cyclohexane/ethyl acetate) to give ether **101** as colorless oil (11.1 g, 55%). R_f = 0.34 (cyclohexane/ethyl acetate 9:1); IR (film) 3260, 2986, 2938, 2110, 1757, 1732, 1445, 1380, 1365, 1281, 1204, 1187, 1153, 1112, 1031, 945, 888, 850 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 4.25 – 4.19 (m, 4H), 2.46 (s, 1H), 1.51 (s, 6H), 1.28 (t, *J* = 7.1, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ= 170.8, 85.1, 73.3, 71.5, 63.1, 61.0, 28.8 (2xC), 14.4 ppm; GC-MS (EI) t_R = 4.70 min, *m/z* = 170 $[M]^+$. HR-MS (FAB, 70 eV)**:** m/z calculated for $C_9H_{14}O_3$ = 171.1015, found = 171.1014 $[M]^+$

2-(2-methylbut-3-yn-2-yloxy)acetaldehyde (102). Ester **101** (10 g, 58.8 mmol) was dissolved in anhydrous diethyl ether (200 mL) at -78°C, and a solution of diisobutylaluminium hydride (1M in hexanes, 88.1 mL, 88.1 mmol) was added dropwise over 20 min. The solution was stirred at -78°C for 40 min. An excess of aq. HCl (1M) was added, and the mixture was stirred for 1 hour. The

layers were separated, and the aqueous layer was extracted with diethyl ether (2 × 100 ml). The combined extracts were washed with water (2 × 100 ml) and brine (1 × 50 ml), dried with $MgSO_4$ and concentrated. Purification of the residue by silica gel chromatography (cyclohexane/ethyl acetate 98:2) gave aldehyde **102** as colourless oil (5.77 g, 78%). R_f = 0.41 (cyclohexane/ethyl acetate 9:1); IR (film) 3446, 3291, 2985, 2937, 2874, 2110, 1736, 1466, 1380, 1364, 1275, 1226, 1188, 1155, 1082, 945, 911, 872 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 9.75 (d, *J* = 1.1 Hz, 1H), 4.17 (d, *J* = 1.2 Hz, 2H), 2.49 (s, 1H), 1.52 (s, 6H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$); δ = 201.8, 85.1, 73.8, 71.5, 70.6, 28.7 ppm (2xC); GC-MS (EI) t_R = 3.39 min, *m/z* = 126 $[M]^+$; HRMS (EI) *m/z* calculated for $C_7H_{10}O_2$ 126.0675, found 126.0674 $[M]^+$.

1-(2-methylbut-3-yn-2-yloxy)pent-4-en-2-ol (*rac*-103). A stirred solution of **102** (5.50 g, 43.7 mmol) in dry THF (200 mL) was cooled to 0 °C, and allyl magnesium chloride (2M in THF, 32.7 mL, 65.5 mmol) was added dropwise. The solution was allowed to warm to room temperature and stirred for 1 h. Water (100 mL) was added, and the mixture was extracted with ethyl acetate (3 × 100 ml). The combined organic extracts were washed with water (2 × 100 ml) and brine (1 × 50 ml), dried with $MgSO_4$, and concentrated. The residue was purified by flash chromatography (cyclohexane/ethyl acetate 94:6) to give racemic homoallylalcohol ***rac*-103** as a colorless oil (4.47 g, 61%). R_f = 0.35 (cyclohexane/ethyl acetate 4:1); IR (film) 3452, 3301, 3077, 2986, 2934, 2873, 2322, 2112, 1641, 1466, 1436, 1380, 1362, 1337, 1265, 1227, 1187, 1159, 1072, 997, 945, 915, 872 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.85 (ddt, *J* = 17.2, 10.2, 7.1 Hz, 1H), 5.20 – 4.99 (m, 2H), 3.82 (ddd, *J* = 13.6, 6.5, 3.5 Hz, 1H), 3.59 (dd, *J* = 9.2, 3.5 Hz, 1H), 3.45 (dd, *J* = 9.2, 7.3 Hz, 1H), 2.42 (s, 1H), 2.27 (t, *J* = 6.7 Hz, 2H), 1.47 (s, 6H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 134.6, 117.6, 85.9, 72.5, 70.5, 70.1, 67.9, 38.1, 28.8, 28.8 ppm; GC-MS (EI) t_R = 4.77 min, *m/z* = 168 $[M]^+$; HRMS (EI) *m/z* calculated for $C_{10}H_{16}O_2$ 168.1145, found 168.1137 $[M]^+$.

7,7-dimethyl-6-vinyl-2,3,4,7-tetrahydrooxepin-3-ol (*rac*-104). A solution of ***rac*-103** (3.00 g, 17.9 mmol) in anhydrous dichloromethane (1 L) was deoxygenated by purging with argon for 30 min. via cannula. 1st Generation Grubbs catalyst (1.47 g, 1.79 mmol) was added and the reaction mixture was heated to reflux for 8 h. More catalyst (1.47 g, 1.79 mmol) was added and the mixture was heated to reflux for additional 8 h. The solvent was evaporated and the residue was purified by flash chromatography (cyclohexane/ethyl acetate 80:20) to give racemic oxepene ***rac*-104** as colorless oil (2.86 g, 96%). R_f = 0.27 (cyclohexane:ethyl acetate, 8:2); IR (film) 3384, 3083, 2974, 2932, 2875, 1613, 1451, 1413, 1379, 1360, 1274, 1182, 1109, 1088, 1068, 1036, 1008, 982, 937, 911, 867, 826 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 6.13 (ddd, *J* = 16.9, 10.7, 1.0 Hz, 1H), 5.75 – 5.61 (m, 1H), 5.28 (dd, *J* = 16.9, 1.9 Hz, 1H), 4.95 (dd, *J* = 10.7, 1.9 Hz, 1H), 4.06 (dtd, *J* = 7.1, 5.3, 3.7 Hz, 1H), 3.97 (dd, *J* = 13.1, 5.1 Hz, 1H), 3.60 (dd, *J* = 13.1, 3.7 Hz, 1H), 2.63 – 2.41 (m, 2H), 2.00 (s, 2H), 1.38 (s, 3H), 1.32 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 147.6, 137.6, 120.1, 114.8, 81.0, 71.1, 68.3, 32.7, 27.4, 26.1 ppm; GC-MS (EI) t_R = 3.32 min, *m/z* = 168 $[M]^+$; HRMS (EI) *m/z* calculated for $C_{10}H_{16}O_2$ 168.1145, found 168.1141 $[M]^+$.

9-Hydroxy-6,6-dimethyl-2-phenyl-3a,4,6,8,9,10,10a,10b-octahydro-7-oxa-2-azacyclohepta[e] indene-1,3-dione (*rac*-105 and *rac*-106). A solution of ***rac*-104** (2.80 g, 16.7 mmol) and *N*-phenylmaleimide (4.33 g, 25.0 mmol) in anhydrous toluene (100 mL) was stirred at 70°C for 3 h. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (cyclohexane/EtOAc 3:2) to provide a mixture of racemic endo-cyclohexenes ***rac*-105** (3.41 g, 60%) and ***rac*-106** (1.99 g, 35%) as colorless solids.

***Endo-rac*-105:** mp: 123–124 °C; R_f = 0.42 (cyclohexane/ethyl acetate 3:2); IR (film) 3402, 3055, 2973, 2922, 2851, 1770, 1706, 1595, 1496, 1454, 1381, 1292, 1261, 1184, 1137, 1100, 1081, 1049,

1017, 993, 973, 945, 930, 909, 859, 841, 826 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.53 – 7.44 (m, 2H), 7.43 – 7.36 (m, 1H), 7.33 – 7.28 (m, 2H), 5.85 (dd, *J*=2.4, 7.1, 1H), 3.85 – 3.76 (m, 1H), 3.51 (ddd, *J* = 1.6, 3.7, 12.4, 1H), 3.27 – 3.16 (m, 3H), 3.13 (dd, *J*=6.6, 10.1, 1H), 2.85 – 2.73 (m, 1H), 2.52 – 2.42 (m, 1H), 2.04 – 1.96 (m, 1H), 1.66 (br. s, 1H), 1.37 (s, 3H), 1.26 (s, 3H), 1.19 – 1.07 (m, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 179.3, 177.8, 148.0, 131.9, 129.4 (2xC), 128.9, 126.6 (2xC), 121.4, 78.2, 70.5, 68.0, 44.8, 38.5, 36.0, 31.4, 27.6, 24.0, 22.2 ppm; LC-MS (C4, ESI) t_R = 5.72 min, *m/z* = 342 $[M+H]^+$; HRMS (ESI) *m/z* calculated for $C_{20}H_{24}NO_4$ 342.1700, found 342.1701 $[M+H]^+$.

***Endo*-rac-106:** mp: 147–148 °C; R_f = 0.36 (cyclohexane:ethyl acetate 3:2); IR (film) 3588, 3074, 2972, 2923, 2851, 1769, 1703, 1597, 1497, 1455, 1380, 1313, 1293, 1260, 1238, 1183, 1151, 1103, 1080, 1067, 1048, 1017, 995, 968, 956, 944, 931, 911, 854, 828 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.51 – 7.44 (m, 2H), 7.42 – 7.37 (m, 1H), 7.33 – 7.29 (m, 2H), 5.83 (dd, *J* = 2.3, 7.1, 1H), 3.71 (dd, *J* = 3.2, 6.2, 1H), 3.61 (ddd, *J* = 1.9, 3.5, 12.9, 1H), 3.46 – 3.37 (m, 2H), 3.29 – 3.14 (m, 2H), 2.83 – 2.71 (m, 1H), 2.51 – 2.41 (m, 1H), 2.30 (br. s, 1H), 1.80 (dd, *J* = 2.1, 12.8, 1H), 1.38 (s, 3H), 1.31 (s, 3H), 1.29 – 1.22 (m, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 179.4, 177.4, 147.7, 132.0, 129.4 (2xC), 128.8, 126.5 (2xC), 121.1, 77.7, 67.6, 65.8, 44.7, 38.4, 35.3, 30.8, 27.6, 23.9, 21.9 ppm; LC-MS (C4, ESI) t_R = 5.83 min, *m/z* = 342 $[M+H]^+$; HRMS (ESI) *m/z* calculated for $C_{20}H_{23}NO_4$ 342.1700, found 342.1701 $[M+H]^+$.

Imidazole-1-carboxylic acid 6,6-dimethyl-1,3-dioxo-2-phenyl-2,3,3a,4,6,8,9,10,10a,10b-deca-hydro-1*H*-7-oxa-2-aza-cyclohepta[e]inden-9-yl-ester (*rac*-107). A solution of racemic alcohol ***rac*-105** (2.00 g, 5.86 mmol) and 1,1'-carbonyldiimidazole (1.42 g, 8.79 mmol) in anhydrous dichloromethane (100 ml) was stirred at room temperature overnight. The solvent was removed under reduced pressure and the residue was purified by silica gel chromatography

(cyclohexane/ethyl acetate 1:4) to give the racemic imidazoyl carbamate **107** as a colorless solid (2.54 g, quantitative yield). mp: 186–188°C; R_f = 0.32 (cyclohexane/ethyl acetate 1:4); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.20 – 8.14 (m, 1H), 7.51 – 7.42 (m, 3H), 7.39 (ddd, *J* = 7.4, 3.9, 1.3 Hz, 1H), 7.31 – 7.21 (m, 2H), 7.04 (dd, *J* = 1.7, 0.8 Hz, 1H), 5.89 (dd, *J* = 7.1, 2.4 Hz, 1H), 4.99 (d, *J* = 3.0 Hz, 1H), 4.01 – 3.88 (m, 1H), 3.61 (dd, *J* = 12.9, 5.1 Hz, 1H), 3.53 (d, *J* = 14.1 Hz, 1H), 3.34 – 3.17 (m, 2H), 2.84 (dt, *J* = 24.3, 8.1 Hz, 1H), 2.52 – 2.34 (m, 1H), 2.03 (dd, *J* = 13.9, 2.0 Hz, 1H), 1.60 – 1.45 (m, 1H), 1.40 (s, 3H), 1.33 (s, 3H), 0.92 – 0.82 (m, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 178.73, 176.97, 148.06, 147.67, 137.19, 131.51, 130.58, 129.23, 128.73, 126.22, 121.25, 117.17, 77.55, 77.32, 77.00, 76.68, 75.12, 63.06, 44.11, 38.19, 32.72, 31.08, 27.62, 23.22, 22.02 ppm; LC-MS (ESI) t_R = 5.86 min, *m*/*z* = 436 $[M+H]^+$. HRMS (ESI) *m/z* calculated for $C_{24}H_{25}N_3O_5$ 436.1794, found 436.1859 $[M+H]^+$.

Imidazole-1-carboxylic acid 6,6-dimethyl-1,3-dioxo-2-phenyl-2,3,3a,4,6,8,9,10,10a,10b-deca-hydro-1*H*-7-oxa-2-aza-cyclohepta[e]inden-9-yl-ester (*rac*-108). The racemic carbamate ***rac*-108** was prepared as described above from ***rac*-106** (1.90 g, 5.57 mmol). Colorless solid (2.42 g, quantitative yield). mp: 192–193 °C; R_f = 0.29 (cyclohexane/ethyl acetate 1:4); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.19 – 8.15 (m, 1H), 7.50 – 7.42 (m, 3H), 7.41 – 7.35 (m, 1H), 7.30 – 7.22 (m, 2H), 7.04 (dd, *J* = 1.6, 0.8 Hz, 1H), 5.88 (dd, *J* = 7.2, 2.4, 1H), 5.28 (d, *J* = 6.4, 1H), 4.98 (d, *J* = 2.9, 1H), 4.00 – 3.92 (m, 1H), 3.60 (dd, *J* = 12.7, 5.1, 1H), 3.53 (d, *J* = 14.1, 1H), 3.28 – 3.19 (m, 2H), 2.83 (dt, *J* = 16.8, 8.1, 1H), 2.50 – 2.37 (m, 1H), 2.08 – 1.97 (m, 1H), 1.57 – 1.46 (m, 1H), 1.39 (s, 3H), 1.31 (d, *J* = 8.8, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 178.7, 177.0, 148.1, 147.7, 137.2, 130.6, 129.3 (2xC), 126.2 (2xC), 121.2, 77.6, 75.1, 63.1, 53.4, 44.1, 38.2, 32.8, 27.6, 23.3, 22.9 ppm; LC-MS (ESI) t_R = 7.26 min, *m*/*z* = 436 $[M+H]^+$. HRMS (ESI) *m/z* calculated for $C_{24}H_{25}N_3O_5$ 436.1894, found 436.1867 $[M+H]^+$.

(3a*R*,9*R*,10a*R*,10b*S*)-6,6-dimethyl-1,3-dioxo-2-phenyl-2,3,3a,4,6,8,9,10,10a,10b-decahydro-1*H*-oxepino[4,3-e]isoindol-9-yl-benzylcarbamate (109) and **(3a*S*,9*S*,10a*S*,10b*R*)-6,6-dimethyl-1,3-dioxo-2-phenyl-2,3,3a,4,6,8,9,10,10a,10b-decahydro-1*H*-oxepino[4,3-e]isoindol-9-yl-benzyl-carbamate (110).** To a solution of compound ***rac*-107** (0.3 g, 0.69 mmol) in DMF (10 ml), benzylamine hydrochloride (0.3 g, 2.07 mmol) and K_2CO_3 (0.62 g, 4.48 mmol) was added and the reaction mixture was stirred at room temperature overnight. The mixture was diluted with water (50 ml) and extracted with EtOAc (3 × 25 ml). The combined organic extracts were dried with Mg_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (cyclohexane/ethyl acetate 3.2) to give a racemic mixture of compounds **109**,**110** (0.23 g, 72%) as colorless solids. An aliqut of the racemate (35.0 mg) was purified by preparative RP-HPLC (Method A) to give a colorless powder after lyophilization (27.0 mg) and then resolved into the enantiomers by preparative chiral HPLC (Method B) to afford enantiomerically pure compounds **109** (12.0 mg) and **110** (13.0 mg). Another aliquot of the racemate was crystallized from cyclohexane:ethylacetate (3:2) for single crystal X-ray analysis by slow evaporation. mp: 123–124°C; **109** $[\alpha]_D^{20}$ = +18.1 (C = 1, $CHCl_3$, 20°C); **110** $[\alpha]_D^{20}$ = -18.0 (C = 1, $CHCl_3$, 20°C); R_f = 0.40 (cyclohexane/ethyl acetate 7:3); IR (film) 3341, 3063, 2974, 2930, 2163, 1785, 1710, 1685, 1597, 1529, 1493, 1455, 1373, 1305, 1277, 1252, 1186, 1170, 1155, 1137, 1111, 1074, 1063, 1025, 1003, 968, 948, 934, 895, 883, 859, 838, 806 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.51 – 7.43 (m, 2H), 7.40 (d, *J* = 7.4, 1H), 7.35 – 7.20 (m, 7H), 5.86 (dd, *J* = 2.9, 6.7, 1H), 4.92 – 4.74 (m, 2H), 4.36 (s, 2H), 4.34 – 4.28 (m, 1H), 3.65 – 3.56 (m, 1H), 3.35 – 3.09 (m, 4H), 2.81 – 2.66 (m, 1H), 2.61 – 2.43 (m, 1H), 2.16 – 2.07 (m, 1H), 1.35 (s, 3H), 1.28 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.8, 176.8, 155.0, 147.4, 138.1, 131.5, 128.9 (2xC), 128.8, 128.4 (2xC), 128.3, 127.2 (2xC), 126.0 (2xC), 121.3, 78.0, 72.1,

64.7, 44.8, 44.5, 38.2, 32.1, 29.4, 27.0 (2xC), 21.8 ppm; LC-MS (ESI) t_R = 9.09 min, *m/z* = 475 $[M+H]^+$; HRMS (ESI) *m/z* calculated for $C_{28}H_{31}N_2O_5$ 475.2228, found 475.2223 $[M+H]^+$.

(3a*S*,9*R*,10a*R*,10b*S*)-6,6-dimethyl-1,3-dioxo-2-phenyl-2,3,3a,4,6,8,9,10,10a,10b-decahydro-1*H*-oxepino[4,3-e]isoindol-9-yl benzylcarbamate (111) and **(3a*R*,9*S*,10a*S*,10b*R*)-6,6-dimethyl-1,3-dioxo-2-phenyl-2,3,3a,4,6,8,9,10,10a,10b-decahydro-1*H*-oxepino[4,3-e]isoindol-9-yl benzylcarbamate (112).** Compounds **111** and **112** (0.27 g, 82%) were obtained as colorless solids following the procedure described above starting from ***rac*-108** (0.30 g, 0.69 mmol. An aliquot of the racemate (0.040 g) was purified by preparative RP-HPLC (Method A) to give a colorless powder after lyophilization (0.034 g) and then resolved into the enantiomers by preparative chiral HPLC (Method B) to afford enantiomerically pure compounds **111** (0.015 g) and **112** (0.016 g). A further aliquot of the racemate was crystallized from cyclohexane/ethylacetate (3:2) for single crystal X-ray analysis by slow evaporation. mp: 138–140 °C; **111** $[\alpha]_D^{20}$ = -23.4 (C = 1, $CHCl_3$, 20°C); **112** $[\alpha]_D^{20}$ = +23.5 (C = 1, $CHCl_3$, 20°C); R_f = 0.36 (cyclohexane/ethyl acetate 7:3); IR (film) 3318, 3063, 3031, 2973, 2927, 1948, 1785, 1711, 1682, 1621, 1493, 1568, 1531, 1492, 1469, 1453, 1426, 1365, 1304, 1248, 1186, 1074, 1061, 1024, 1002, 947, 934, 882, 858, 806 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.48 (t, *J* = 7.6 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 1H), 7.26 (dt, *J* = 8.3, 6.7 Hz, 8H), 5.83 (dd, *J* = 7.0, 2.3 Hz, 1H), 5.21 (t, *J* = 5.1 Hz, 1H), 4.80 (s, 1H), 4.35 (qd, *J* = 15.0, 6.0 Hz, 2H), 3.81 (d, *J* = 13.8 Hz, 1H), 3.58 (dd, *J* = 12.1, 5.9 Hz, 1H), 3.46 (d, *J* = 13.8 Hz, 1H), 3.30 – 3.12 (m, 2H), 2.87 – 2.70 (m, 1H), 2.51 – 2.37 (m, 1H), 1.91 (d, *J* = 13.4 Hz, 1H), 1.62 (s, 1H), 1.38 (s, 3H), 1.32 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.07, 177.35, 155.85, 148.20, 138.20, 131.62, 129.27 (2xC), 128.70, 128.60 (2xC), 127.46, 127.34 (2xC), 126.40 (2XC), 120.56, 70.74, 64.32, 45.05, 44.40, 38.30, 33.00, 31.35, 27.73, 23.23, 21.86 ppm; LC-MS (ESI) t_R = 9.26 min, *m/z* = 475 $[M+H]^+$; HRMS (ESI) *m/z* calculated for $C_{28}H_{31}N_2O_5$ 475.2228, found 475.2225 $[M+H]^+$.

(3a*R*,9*R*,10a*R*,10b*S*)-6,6-dimethyl-1,3-dioxo-2-phenyl-2,3,3a,4,6,8,9,10,10a,10b-decahydro-1*H*-oxepino[4,3-e]isoindol-9-yl-4-methoxybenzylcarbamate (113) and **(3a*S*,9*S*,10a*S*,10b*R*)-6,6-dimethyl-1,3-dioxo-2-phenyl-2,3,3a,4,6,8,9,10,10a,10b-decahydro-1*H*-oxepino[4,3-e]isoindol-9-yl-4-methoxybenzylcarbamate (114).** To a solution of compound ***rac*-107** (0.30 g, 0.69 mmol) in DMF (10 mL), 4-methoxy-benzylamine hydrochloride (0.36 g, 2.07 mmol) and K_2CO_3 (0.62 g, 4.48 mmol) was added and the reaction mixture was stirred at room temperature overnight. The mixture was diluted with water (50 mL) and extracted with EtOAc (3 × 25 mL). The combined organic extracts were dried with $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography (cyclohexane/ethyl acetate 1:1) to give a racemic mixture of compounds **113** and **114** as a colorless solid (0.26 g, 76%, mp: 132–133°C). An aliquot of the racemate (0.040 g) was purified by preparative RP-HPLC (Method A) to give a colorless powder after lyophilization (0.032 g) and then resolved into the enantiomers by preparative chiral HPLC (Method B) to afford enantiomerically pure compounds **113** (0.015 g) and **114** (0.014 g). **113** $[\alpha]_D^{20}$ = +16.2 (*c* = 1.5, $CHCl_3$, 20°C); **114** $[\alpha]_D^{20}$ = -16.5 (*c* = 1.5, $CHCl_3$, 20°C); R_f = 0.3 (cyclohexane/ethyl acetate 7:3); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.52 – 7.41 (m, 2H), 7.41 – 7.33 (m, 1H), 7.32 – 7.23 (m, 2H), 7.14 (s, 2H), 6.88 – 6.74 (m, 2H), 5.83 (d, *J* = 2.8 Hz, 1H), 4.82 (d, *J* = 33.6 Hz, 2H), 4.23 (s, 2H), 3.83 – 3.67 (m, 3H), 3.58 (d, *J* = 12.2 Hz, 1H), 3.22 (dd, *J* = 18.2, 8.4 Hz, 3H), 3.15 – 3.07 (m, 1H), 2.70 (s, 1H), 2.52 (s, 1H), 2.10 (s, 1H), 1.34 (d, *J* = 2.5 Hz, 3H), 1.27 (d, *J* = 2.8 Hz, 4H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.96, 177.00, 158.92, 155.13, 147.56, 131.66, 130.44, 129.10, 128.79, 128.50, 126.20, 121.45, 113.95, 78.13, 72.13, 64.92, 55.18, 44.64, 44.43, 38.39, 32.25, 31.54, 27.20, 21.97 ppm; LC-MS (ESI) t_R = 9.55 min, *m/z* = 505 $[M+H]^+$; HRMS (ESI) *m/z* calculated for $C_{29}H_{32}N_2O_6$ 505.2260, found 505.2327 $[M+H]^+$.

(3a*S*,9*R*,10a*R*,10b*S*)-6,6-dimethyl-1,3-dioxo-2-phenyl-2,3,3a,4,6,8,9,10,10a,10b-decahydro-1*H*-oxepino[4,3-e]isoindol-9-yl-4-methoxybenzylcarbamate (115) and **(3a*R*,9*S*,10a*S*,10b*R*)-6,6-dimethyl-1,3-dioxo-2-phenyl-2,3,3a,4,6,8,9,10,10a,10b-decahydro-1*H*-oxepino[4,3-e]isoindol-9-yl-4-methoxybenzylcarbamate (116).** Compounds **115** and **116** (0.26 g, 76%) were obtained as colorless solids following the procedure described above, starting from ***rac*-108** (0.30 g, 0.69 mmol, mp: 157–159°C). An aliquot of the racemate (0.040 g) was purified by preparative RP-HPLC (Method A) to give a colorless powder after lyophilization (0.035 g) and then resolved into the enantiomers by preparative chiral HPLC (Method B) to afford enantiomerically pure compounds **115** (0.013 g) and **116** (0.014 g). **115** $[\alpha]_D^{20}$ = -34.5 (c = 1.5, $CHCl_3$, 20°C); **116** $[\alpha]_D^{20}$ = +34.7 (c = 1.5, $CHCl_3$, 20°C); R_f = 0.26 (cyclohexane/ethyl acetate 7:3); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.42 (dd, *J* = 25.5, 7.4 Hz, 3H), 7.36 – 7.10 (m, 4H), 6.80 (d, *J* = 8.2 Hz, 2H), 5.80 (s, 1H), 5.57 – 5.23 (m, 1H), 4.76 (s, 1H), 4.40 – 4.07 (m, 2H), 3.83 – 3.68 (m, 4H), 3.59 (s, 1H), 3.42 (d, *J* = 13.2 Hz, 1H), 3.21 (d, *J* = 4.2 Hz, 2H), 2.76 (d, *J* = 7.6 Hz, 1H), 2.45 (s, 1H), 1.88 (d, *J* = 13.2 Hz, 1H), 1.31 (dd, *J* = 22.5, 9.0 Hz, 6H), 0.88 (s, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.33, 177.60, 159.11, 156.08, 148.41, 131.95, 130.80, 129.44, 129.12, 128.84, 126.62, 120.80, 114.19, 77.61, 77.30, 76.98, 74.65, 70.88, 64.38, 55.47, 48.30, 44.75, 44.60, 38.53, 33.23, 31.69, 29.92, 27.91, 23.53, 22.05 ppm; LC-MS (ESI) t_R = 9.42 min, *m/z* = 505 $[M+H]^+$; HRMS (ESI) *m/z* calculated for $C_{29}H_{32}N_2O_6$ 505.5742, found 505.5737 $[M+H]^+$.

(3a*R*,9*R*,10a*R*,10b*S*)-6,6-dimethyl-1,3-dioxo-2-phenyl-2,3,3a,4,6,8,9,10,10a,10b-decahydro-1*H*-oxepino[4,3-e]isoindol-9-yl-4-(7-azidoheptyloxy)benzylcarbamate (117) and **(3a*S*,9*S*,10a*S*,10b*R*)-6,6-dimethyl-1,3-dioxo-2-phenyl-2,3,3a,4,6,8,9,10,10a,10b-decahydro-1*H*-oxepino[4,3-e]isoindol-9-yl-4-(7-azidoheptyloxy)benzylcarbamate (118).** To a solution of ***rac*-107** (1.30 g, 2.99 mmol) in DMF (50 mL), compound **129** (2.68 g, 8.97 mmol) and K_2CO_3 (2.68 g,

19.4 mmol) was added and the reaction mixture was stirred at room temperature overnight. The mixture was diluted with water (200 mL) and extracted with EtOAc (3 × 75 mL). The organic layer was dried with $MgSO_4$ and concentrated. The residue was purified by flash chromatography (cyclohexane/ethyl acetate 4:1) to give a racemic mixture of compounds **117** + **118** (1.48 g, 79%) as colorless oil. An aliquot of the racemate (0.050 g) was resolved by preparative chiral HPLC (Method B) to afford enantiomerically pure compounds **117** (0.022 g) and **118** (0.023 g). **117** $[\alpha]_D^{20}$ = +29.4 (c = 1.0, $CHCl_3$, 20°C); **118** $[\alpha]_D^{20}$ = -29.5 (c = 1.0, $CHCl_3$, 20°C); R_f = 0.38 (cyclohexane/ethyl acetate 9:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.48 (dd, *J* = 10.4, 4.8 Hz, 2H), 7.40 (ddd, *J* = 7.4, 3.8, 1.2 Hz, 1H), 7.28 (dd, *J* = 10.7, 3.5 Hz, 2H), 7.17 (d, *J* = 8.4 Hz, 2H), 6.80 (d, *J* = 8.5 Hz, 2H), 5.81 (dd, *J* = 7.0, 2.2 Hz, 1H), 5.21 (t, *J* = 5.9 Hz, 1H), 4.78 (s, 1H), 4.26 (qd, *J* = 14.6, 5.8 Hz, 2H), 3.90 (t, *J* = 6.5 Hz, 2H), 3.79 (d, *J* = 13.9 Hz, 1H), 3.62 – 3.51 (m, 1H), 3.45 (d, *J* = 13.8 Hz, 1H), 3.26 (t, *J* = 6.9 Hz, 2H), 3.22 – 3.13 (m, 2H), 2.86 – 2.69 (m, 1H), 2.50 – 2.36 (m, 1H), 1.90 (d, *J* = 13.0 Hz, 1H), 1.80 – 1.70 (m, 2H), 1.66 – 1.55 (m, 2H), 1.52 – 1.37 (m, 7H), 1.37 – 1.33 (m, 4H), 1.33 – 1.25 (m, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 178.99, 177.03, 158.51, 155.13, 147.66, 131.71, 130.28, 129.16, 128.84, 128.55, 126.26, 121.50, 114.60, 77.32, 77.00, 76.68, 72.21, 67.84, 64.97, 51.41, 44.71, 44.54, 38.45, 32.32, 29.11, 28.86, 28.74, 27.26, 26.62, 25.89, 22.04 ppm; LC-MS (ESI) t_R = 11.05 min, *m/z* = 629 $[M+H]^+$; HRMS (ESI) *m/z* calculated for $C_{35}H_{43}N_5O_6$ 630.3213, found 630.3284 $[M+H]^+$.

(3a*R*,9*R*,10a*R*,10b*S*)-6,6-dimethyl-1,3-dioxo-2-phenyl-2,3,3a,4,6,8,9,10,10a,10b-decahydro-1*H*-oxepino[4,3-e]isoindol-9-yl-4-(7-aminoheptyloxy)benzylcarbamate (119). To a solution of **117** (0.20 g, 0.32 mmol) in MeOH (10 ml), Pd/C 10% was added (0.045 g, 0.032 mmol) and the flask was purged and filled with H_2. The mixture was stirred under H_2 (1 atm) for 40 min. The catalyst was removed by filtration through a pad of Celite. The filtrate was concentrated and the residue was

purified by silica gel chromatography (dichloromethane/MeOH/NH_4OH 95:5:0.5) to give amine **119** as a colorless oil (0.178 g, 93%). $[\alpha]_D^{20}$ = +37.8 (c = 1.0, $CHCl_3$, 20°C); R_f = 0.16 (dichloromethane/MeOH 9:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.53 – 7.44 (m, 2H), 7.40 (ddd, *J* = 8.4, 2.4, 1.2 Hz, 1H), 7.30 (d, *J* = 7.8 Hz, 2H), 7.16 (d, *J* = 8.1 Hz, 2H), 6.82 (d, *J* = 8.6 Hz, 2H), 5.87 (dd, *J* = 6.6, 2.8 Hz, 1H), 4.80 (s, 2H), 4.25 (d, *J* = 4.4 Hz, 2H), 3.93 (t, *J* = 6.4 Hz, 2H), 3.61 (dd, *J* = 12.3, 3.4 Hz, 1H), 3.38 – 3.19 (m, 5H), 3.15 (dd, *J* = 9.8, 6.4 Hz, 1H), 2.73 (d, *J* = 7.7 Hz, 1H), 2.53 (dd, *J* = 16.4, 5.6 Hz, 1H), 2.13 (dd, *J* = 10.9, 5.7 Hz, 1H), 1.85 – 1.71 (m, 2H), 1.69 – 1.55 (m, 2H), 1.48 (dd, *J* = 14.1, 8.2 Hz, 2H), 1.41 (dd, *J* = 11.0, 7.1 Hz, 4H), 1.36 (s, 3H), 1.29 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 178.99, 177.03, 158.51, 155.13, 147.66, 131.71, 130.28, 129.16, 128.84, 128.55, 126.26, 121.50, 114.60, 77.32, 77.00, 76.68, 72.21, 67.84, 64.97, 51.41, 44.71, 44.54, 38.45, 32.32, 29.11, 28.86, 28.74, 27.26, 26.62, 25.89, 22.04 ppm; LC-MS (ESI) t_R = 7.85 min, *m/z* = 604 $[M+H]^+$; HRMS (ESI) *m/z* calculated for $C_{35}H_{45}N_3O_6$ 604.3308, found 604.3374 $[M+H]^+$.

(3a*S*,9*S*,10a*S*,10b*R*)-6,6-dimethyl-1,3-dioxo-2-phenyl-2,3,3a,4,6,8,9,10,10a,10b-decahydro-1*H*-oxepino[4,3-e]isoindol-9-yl-4-(7-aminoheptyloxy)benzylcarbamate (120). Amine **120** was prepared as described above from azide **118** (0.20 g, 0.318 mmol). Colorless oil (0.182 g, 95%). $[\alpha]_D^{20}$ = -37.5 (c = 1.0, $CHCl_3$, 20°C); R_f = 0.16 (dichloromethane/MeOH 9:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.54 – 7.44 (m, 2H), 7.40 (ddd, *J* = 7.3, 3.7, 1.2 Hz, 1H), 7.30 (d, *J* = 7.6 Hz, 2H), 7.16 (d, *J* = 8.1 Hz, 2H), 6.83 (d, *J* = 8.6 Hz, 2H), 5.87 (dd, *J* = 6.6, 2.8 Hz, 1H), 4.79 (dd, *J* = 12.1, 7.1 Hz, 2H), 4.26 (d, *J* = 4.5 Hz, 2H), 3.93 (t, *J* = 6.4 Hz, 2H), 3.61 (dd, *J* = 12.1, 3.4 Hz, 1H), 3.36 – 3.19 (m, 5H), 3.16 (dd, *J* = 9.8, 6.4 Hz, 1H), 2.74 (d, *J* = 7.7 Hz, 1H), 2.54 (dd, *J* = 17.5, 6.9 Hz, 1H), 2.13 (dd, *J* = 11.1, 5.7 Hz, 1H), 1.83 – 1.72 (m, 2H), 1.69 – 1.53 (m, 3H), 1.53 – 1.44 (m, 2H), 1.41 (dd, *J* = 9.9, 6.1 Hz, 4H), 1.36 (s, 3H), 1.30 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ =

178.99, 177.03, 158.51, 155.13, 147.66, 131.71, 130.28, 129.16, 128.84, 128.55, 126.26, 121.50, 114.60, 77.32, 77.00, 76.68, 72.21, 67.84, 64.97, 51.41, 44.71, 44.54, 38.45, 32.32, 29.11, 28.86, 28.74, 27.26, 26.62, 25.89, 22.04 ppm; LC-MS (ESI) t_R = 7.85 min, *m/z* = 604 $[M+H]^+$; HRMS (ESI) *m/z* calculated for $C_{35}H_{45}N_3O_6$ 604.3308, found 604.3374 $[M+H]^+$.

(3a*R*,9*R*,10a*R*,10b*S*)-6,6-dimethyl-1,3-dioxo-2-phenyl-2,3,3a,4,6,8,9,10,10a,10b-decahydro-1*H*-oxepino[4,3-e]isoindol-9-yl-4-(7-(5-((4*S*)-2-oxohexahydro-1*H*-thieno[3,4-d]imidazol-4-yl)pentaneamido)heptyloxy)benzylcarbamate (121). To a solution of biotin (0.033 g, 0.134 mmol) and HBTU (0.044 g, 0.116 mmol) in dry DMF (3 ml), EtN(*i*Pr)$_2$ (250 µL, 0.142 mmol) was added and the resulting mixture was stirred for 10 min. Then compound **119** (0.054 g, 0.089 mmol) was added as a solution in DMF (1 mL) and the mixture was stirred under argon overnight. The mixture was diluted with H_2O (10 mL) and extracted with EtOAc (3 × 5 ml). The combined organic extracts were dried with $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography (dichloromethane/MeOH 19:1) to give amide **121** as a colorless powder (0.051 g, 69%). Further purification by preparative HPLC (Method A) afforded colorless powder after lyophilization (0.036 g). mp = 176°C; $[\alpha]_D^{20}$ = +45.7 (c = 1.0, $CHCl_3$, 20°C); R_f = 0.25 (dichloromethane/MeOH 9:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.41 (d, *J* = 8.4 Hz, 1H), 8.03 (dd, *J* = 22.3, 8.4 Hz, 2H), 7.80 (t, *J* = 7.8 Hz, 1H), 7.61 – 7.54 (m, 2H), 7.50 – 7.41 (m, 2H), 6.80 (s, 2H), 5.83 (s, 1H), 5.67 (s, 1H), 5.25 (d, *J* = 6.0 Hz, 2H), 4.57 (dd, *J* = 12.6, 7.2 Hz, 2H), 4.37 (dt, *J* = 12.2, 6.7 Hz, 2H), 3.71 (dt, *J* = 13.7, 6.8 Hz, 4H), 3.18 (dd, *J* = 13.6, 6.5 Hz, 7H), 2.98 – 2.85 (m, 4H), 2.79 (dd, *J* = 12.6, 5.8 Hz, 3H), 2.35 (t, *J* = 7.4 Hz, 1H), 1.98 – 1.81 (m, 5H), 1.68 (dddd, *J* = 31.3, 23.5, 11.0, 4.9 Hz, 8H), 1.46 (s, 5H), 1.44 (s, 5H), 0.89 (dd, *J* = 8.6, 4.6 Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 179.2, 178.6, 177.5, 162.3, 157.6, 155.4, 148.2, 132.1, 130.3, 129.8, 129.6, 128.7, 126.5, 122.4, 114.3, 73.5, 67.3, 64.8, 54.1, 52.9, 49.7, 54.0, 40.7, 38.1, 36.4, 35.7, 32.3, 28.2, 27.7,

26.9, 26.7, 25.8, 24,4, 22.1 ppm; LC-MS (ESI) t_R = 9.75 min, *m/z* = 830 $[M+H]^+$; HRMS (ESI) *m/z* calculated for $C_{45}H_{59}N_5O_8S$ 830.4084, found 830.4153 $[M+H]^+$.

(3a*S*,9*S*,10a*S*,10b*R*)-6,6-dimethyl-1,3-dioxo-2-phenyl-2,3,3a,4,6,8,9,10,10a,10b-decahydro-1*H*-oxepino[4,3-e]isoindol-9-yl-4-(7-(5-((4*S*)-2-oxohexahydro-1*H*-thieno[3,4-d]imidazol-4-yl)pentanamido)heptyloxy)benzylcarbamate (122). Compound **122** was prepared as described above from amine **120** (0.048 g, 0.080 mmol). Colorless powder (0.042 g, 63%). Compound **122** was further purified by preparative HPLC (Method A) to give a colorless powder after lyophilization (0.028 g). mp = 176 °C; $[\alpha]_D^{20}$ = -45.2 (c = 1.0, $CHCl_3$, 20°C); R_f = 0.25 (dichloromethane/MeOH 9:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.52 – 7.45 (m, 2H), 7.40 (ddd, *J* = 7.4, 3.8, 1.2 Hz, 1H), 7.30 (d, *J* = 7.5 Hz, 2H), 7.16 (d, *J* = 8.4 Hz, 2H), 6.82 (d, *J* = 8.6 Hz, 2H), 5.87 (dd, *J* = 6.6, 2.9 Hz, 1H), 5.68 (s, 2H), 5.01 (s, 1H), 4.88 (s, 1H), 4.85 – 4.73 (m, 1H), 4.53 – 4.44 (m, 1H), 4.31 (dd, *J* = 7.5, 4.6 Hz, 1H), 4.25 (s, 2H), 3.92 (t, *J* = 6.5 Hz, 2H), 3.60 (d, *J* = 12.2 Hz, 1H), 3.34 – 3.01 (m, 7H), 2.90 (dd, *J* = 12.8, 4.9 Hz, 1H), 2.71 (d, *J* = 12.8 Hz, 2H), 2.60 – 2.47 (m, 1H), 2.25 – 2.02 (m, 3H), 1.91 – 1.55 (m, 12H), 1.56 – 1.41 (m, 6H), 1.36 (s, 6H), 1.29 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 179.2, 178.6, 177.5, 162.3, 157.6, 155.4, 148.2, 132.1, 130.3, 129.8, 129.6, 128.7, 126.5, 122.4, 114.3, 73.5, 67.3, 64.8, 54.1, 52.9, 49.7, 54.0, 40.7, 38.1, 36.4, 35.7, 32.3, 28.2, 27.7, 26.9, 26.7, 25.8, 24,4, 22.1 ppm; LC-MS (ESI) t_R = 9.75 min, *m/z* = 830 $[M+H]^+$; HRMS (ESI) *m/z* calculated for $C_{45}H_{59}N_5O_8S$ 830.4084, found 830.4153 $[M+H]^+$.

4-(Aminomethyl)phenol hydrobromide (126). 4-Methoxy-benzylamine (10 ml, 77.1 mmol) was added slowly to stirred aq. HBr (48%, 30 mL) at 0 °C. The mixture was allowed to warm to room temperature and heated to reflux. The reaction was cooled down to room temperature and concentrated to dryness. The solid residue was suspended in cold acetonitrile (20 ml) collected by

filtration and dried under vacuum to give phenol **126** (15.3 g, 97%) as a pink solid. mp > 300 °C; ^{1}H NMR (400 MHz, D_2O): δ = 7.30 (d, *J* = 8.7 Hz, 2H), 6.99 – 6.87 (m, 2H), 4.64 (s, 3H), 4.02 (s, 2H), ppm; ^{13}C NMR (100 MHz, D_2O): δ = 159.60, 130.75, 125.37, 114.77, 55.60 ppm.

***tert*-Butyl-4-hydroxybenzylcarbamate (127).** To a solution of amine hydrobromide **126** (10.0 g, 49.0 mmol) in MeOH (100 mL), $NaHCO_3$ (16.5 g, 196.0 mmol) and $(Boc)_2O$ (11.6 g, 52.9 mmol) were added and the mixture was stirred at room temperature for 24 h under Ar. The mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel chromatography (cyclohexane/ethyl acetate 4:1) to give carbamate **127** as a yellowish wax (9.5 g, 87%). mp: 105–107 °C; R_f = 0.45 (cyclohexane/ethyl acetate 9:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.10 (d, *J* = 7.8 Hz, 2H), 6.77 (d, *J* = 8.5 Hz, 2H), 6.25 (br, 1H), 4.85 (br, 1H), 4.21 (d, *J* = 4.1 Hz, 2H), 1.46 (s, 9H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 156.22, 155.55, 130.03, 128.81, 115.51, 79.87, 44.20, 28.40 ppm; LC-MS (ESI) t_R = 7.41 min, *m/z* = 223 $[M+H]^+$; HRMS (ESI) *m/z* calculated for $C_{12}H_{17}NO_3$ 224.1208, found 224.1282 $[M+H]^+$.

***tert*-Butyl-4-(7-bromoheptyloxy)benzylcarbamate (128).** To a solution of phenol **127** (4.5 g, 20.2 mmol) in acetone (500 mL), K_2CO_3 (5.6 g, 40.4 mmol) and 1,7-dibromoheptane (6.90 mL, 40.4 mmol) was added and the mixture was heated to reflux for 20 h. The reaction was filtered hot and the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (cyclohexane/ethyl acetate 4:1) to give bromide **128** as a waxy yellowish solid (5.42 g, 67%). mp: 67–68 °C; R_f = 0.40 (cyclohexane/ethyl acetate 95:5); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.20 (d, *J* = 8.5 Hz, 2H), 6.88 – 6.80 (m, 2H), 4.77 (br, 1H), 4.24 (d, *J* = 5.3 Hz, 2H), 3.95 (t, *J* = 6.5 Hz, 2H), 3.42 (t, *J* = 6.8 Hz, 2H), 1.92 – 1.83 (m, 2H), 1.78 (dq, *J* = 13.1, 6.5 Hz, 2H), 1.53 – 1.44 (m, 13H), 1.44 – 1.34 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.4,

155.8, 130.8, 128.8, 114.6, 79.29, 67.9, 44.2, 33.9, 29.1, 28.5, 28.49, 28.41, 28.06, 25.9 ppm; LC-MS (ESI) t_R = 7.68 min, *m/z* = 400 $[M+H]^+$; HRMS (ESI) *m/z* calculated for $C_{19}H_{30}BrNO_3$ 400.1409, found 400.1514 $[M+H]^+$.

***tert*-Butyl-4-(7-azidoheptyloxy)benzylcarbamate (129).** To a solution of bromide **128** (5.00 g, 12.5 mmol) in DMF (100 mL), NaN_3 (4.06 g, 62.4 mmol) was added and the solution was stirred at 90°C overnight. The mixture was diluted with H_2O (300 mL) and extracted with EtOAc (3 × 100 mL). The combined organic extracts were dried with $MgSO_4$, concentrated, and purified by flash chromatography (cyclohexane/ethyl acetate 4:1) to give **129** as a colorless waxy solid (4.51 g, quantitative yield). mp: 71–73 °C; R_f = 0.42 (cyclohexane/ethyl acetate 96:4); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.18 (d, *J* = 8.5 Hz, 2H), 6.87 – 6.83 (m, 2H), 4.79 (br, 1H), 4.24 (d, *J* = 5.3 Hz, 2H), 3.94 (t, *J* = 6.5 Hz, 2H), 3.27 (t, *J* = 6.8 Hz, 2H), 1.82 – 1.66 (m, 2H), 1.62 (dq, *J* = 13.1, 6.5 Hz, 2H), 1.52 – 1.30 (m, 15H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.4, 155.8, 130.8, 128.8, 114.6, 79.29, 67.9, 51.39, 44.2, 29.1, 28.9, 28.8, 28.7, 26.6, 25.9 ppm; LC-MS (ESI) t_R = 11.07 min, *m/z* = 363 $[M+H]^+$; HRMS (ESI) *m/z* calculated for $C_{19}H_{30}N_4O_3$ 363.2318, found 363.2391 $[M+H]^+$.

(4-(7-Azidoheptyloxy)phenyl)methanamine hydrochloride (130). To a solution of carbamate **129** (4.00 g, 11.1 mmol) in anhydrous dichloromethane (15 mL), HCl (2M in dioxane, 2.76 mL, 5.52 mmol) was added and the mixture was stirred for 20 min. at room temperature. More HCl was added (2M in dioxane, 2.76 mL, 5.52 mmol). After 20 min. of stirring the majority of the solvent was removed under reduced pressure and the precipitated residue was collected by filtration to give amine hydrochloride **130** as a colorless solid (2.81 g, 85%). mp: 287–290°C; ^{1}H NMR (400 MHz, CD_3OD): δ = 7.51 – 7.35 (d, *J* = 8.5 Hz, 2H), 7.09 – 6.93 (d, *J* = 8.3 Hz, 2H), 4.87 (br, 3H), 4.09 (s, 2H), 4.04 – 4.01 (t, *J* = 6.3 Hz, 2H), 3.35 – 3.30 (t, *J* = 6.8 Hz, 2H), 1.85 – 1.78 (m, 2H), 1.66 – 1.53

(m, 2H), 1.51 –1.43 (m, 6H) ppm; ^{13}C NMR (100 MHz, CD_3OD): δ = 161.3, 131.7, 126.2, 116.1, 69.1, 52.5, 44.0, 30.2, 30.0, 29.9, 27.8, 27.09 ppm; LC-MS (ESI) t_R = 7.68 min, *m/z* = 263 [M+H-Cl]$^+$; HRMS (EI) *m/z* calculated for $C_{14}H_{23}ClN_4O$ 298.1560, found 263.1866 [M+H-Cl]$^+$.

{3-[2-(2-{3-[5-(2-Oxo-hexahydro-thieno[3,4-d]imidazol-6-yl)-pentanoylamino]-propoxy}-ethoxy)-ethoxy]-propyl}-carbamic acid 6,6-dimethyl-1,3-dioxo-2-phenyl-2,3,3a,4,6,8,9,10,10a, 10b-decahydro-1*H*-7-oxa-2-aza-cyclohepta[e]inden-9-yl-ester (125). To a solution of **124** (89 mg, 0.184 mmol) and ***rac*-107** (27 mg, 0.062 mmol) in THF/DMF (4:1, 0.5 mL) was added K_2CO_3 (60 mg, 0.434 mmol). The suspension was stirred at room temperature overnight, then filtered and concentrated. The residue was purified by silica gel chromatography (dichloromethane/MeOH 100:0→90:10) to provide **125** as a colorless solid (30 mg, 61%). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ 1:1) δ = 7.74 (s, 1H), 7.44 – 7.36 (m, 2H), 7.36 – 7.30 (m, 1H), 7.16 – 7.08 (m, 2H), 7.04 (s, 2H), 5.75 (dd, *J*=1.4, 7.1, 1H), 4.82 – 4.69 (m, 1H), 4.44 (dd, *J*=4.7, 7.3, 1H), 4.25 (dd, *J*=4.7, 7.5, 1H), 3.62 – 3.44 (m, 11H), 3.35 – 3.21 (m, 5H), 3.21 – 3.06 (m, 5H), 2.86 (dd, *J*=5.0, 12.8, 1H), 2.77 (dd, *J*=7.2, 15.9, 1H), 2.67 (d, *J*=12.8, 1H), 2.46 (dd, *J*=7.8, 15.9, 1H), 2.20 (dd, *J*=5.7, 11.9, 1H), 2.13 (td, *J*=2.5, 7.2, 2H), 1.77 – 1.44 (m, 9H), 1.39 (dd, *J*=7.8, 15.2, 2H), 1.27 (s, 3H), 1.24 – 1.19 (m, 1H), 1.10 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3/CD_3OD$: 1/1) δ = 179.7, 178.5, 173.9, 164.1, 156.0, 148.3, 132.0, 129.3 (2xC), 128.9, 126.4 (2xC), 121.0, 78.0, 71.8, 70.5, 70.5, 70.2, 70.1, 69.7, 69.3, 65.2, 62.0, 60.2, 55.6, 46.6, 40.5, 38.8, 38.7, 38.4, 37.5, 36.0, 31.6, 29.6, 29.0, 28.5, 28.3, 27.3, 25.6, 23.8, 23.3 ppm; LC-MS (ESI) t_R = 5.72 min, *m/z* = 814 [M+H]$^+$; HRMS (ESI) *m/z* calculated for $C_{41}H_{60}N_5O_{10}S$ 814.4055, found 814.4078 [M+H]$^+$.

Cytotoxicity assay. The human embryonic kidney cell line HEK293 was obtained from DSMZ (No. ACC 305). The human endothelial cell line HeLa was obtained from DSMZ (No. ACC 57) and

the human cell line HepG2 of hepatic origin was obtained from DSMZ (No. ATCC HB-8065). Cells were seeded in a concentration of $2x10^4$ cells per well in clear flat bottom 96 well plates. Cells were grown for 1 day in a total volume of 100 µl already containing the appropriate concentration of small molecules or DMSO as control using either DMEM containing 10% FCS, non essential amino acids, pyruvic acid and 4.5 g/L glucose or RMPI 1640 containing 10% FCS in the case of HepG2 cells. All cells were grown at 37°C and 5% CO_2. Measurements were done at 440 nm after applying 10 µL of WST reagent (Roche, Germany) by using a spectrophotometer. Between the measurements the cells were incubated at 37°C under linear shaking. Every concentration was measured in quadruplicate and normalised to DMSO treated cells.

Affinity purification via pulldown. All purifications were done using HEK293 cell lysate. The initial pulldowns were executed using a racemic probe as active molecule (**125**) and a PEG-biotin molecule (**123**) as control. The results were verified using an enantiopure probe **121** and also enantiopure control molecule **122**. The HEK293 cells were grown in a 75 cm^2 dish to 80% confluency, washed twice with PBS and lysed by applying lysis buffer containing 50 mM PIPES (pH = 7.4), 50 mM NaCl, 5 mM $MgCl_2$, 5 mM EGTA, 0.1% NP40, 0.1% Triton X-100, 0.1% Tween20, 0.1% ß-mercaptoethanol and protease inhibitor mix (Roche, Germany). The cells were scraped off and transferred to an ice chilled eppendorf tube, incubated for 15 min at 4°C and pressed though a 0.55 mm cannula. The cell lysate was kept at -20°C until used. 400 µl of a streptavidin-iron-bead suspension (NEB, USA) were used for each sample of the pulldown experiment. The suspension was cleared using a magnetic rack, washed once with PBS and afterwards incubated for 30 min at 22°C and 400 rpm with 400 µL PBS containing 10 µM of the probe. The magnetic beads were washed once with PBS and incubated with 400 µL cell lysate at 4°C for 1 h and 300 rpm. To identify the specific interaction partners the beads were washed twice using lysis buffer which did

not contain proteinase inhibitor or ß-mercaptoethanol, and twice using PBS. The washed beads were either further processed for mass spectrometry or for western blotting.

Mass spectrometric analysis of the bound proteins. The beads were resuspended in protein loading buffer containing 62.5 mM Tris-HCl (pH = 6.8), 2% SDS, 10% glycerol, 5% ß-mercapto-ethanol and bromophenol blue as indicator dye, denatured and loaded on a 12.5% denaturing PAGE gel. After electrophoresis the gel was subjected to zinc staining. To this end the gel was fixed using 50% aqueous methanol and 5% acetic acid in water for 20 min and washed with water twice for 15 min each. The gel was incubated in 0.2 M imidazole solution and 0.1% SDS solution for 15 min and stained using 0.2 M zinc sulfate for 30–60 s. The stained gel was cut into small pieces which were placed in 1.5 mL eppendorf tubes. For de-staining, the gel pieces were incubated for 30 min at 37°C and 300 rpm in 25 mM ammonium hydrogen carbonate containing 25% acetonitrile, and for 30 min at 37°C and 300 rpm in 25 mM ammonium hydrogen carbonate solution containing 50% acetonitrile. These two steps were repeated once and the gel pieces were incubated for 45 min at 37°C and 300 rpm in 25 mM ammonium hydrogen carbonate containing 50 mM DTT (100 µL). The solution was exchanged against 25 mM ammonium hydrogen carbonate containing 55 mM iodoacetamide (100 µL) and the gel pieces were kept for 1 hour at room temperature and 300 rpm in the dark. The pieces were washed twice for 15 min with 25 mM ammonium hydrogen carbonate solution containing 50% acetonitrile (200 µL) and dehydrated by adding acetonitrile (60 µL) for 10 min. The acetonitrile was removed in vacuo and the gel pieces were dried on air. Bound proteins were digested by the addition of 25 mM ammonium hydrogen carbonate solution containing 0.01 µg/ml trypsine (Roche, Germany, 30 µL) and 25 mM ammonium hydrogen carbonate (50 µL) for 12 h at 30 °C. The samples were incubated at 0°C for 30 min in a ultrasonic bath. The pieces were incubated with acetonitrile (200 µL) and the supernatant was transferred into a new 1.5 mL

Eppendorf tube and evaporated using a speedvac. Dried peptides were solubilised in 0.1% aq. TFA and analysed by Nano-LC-MS/MS (Dionex, Germany and Thermo Scientific, USA).

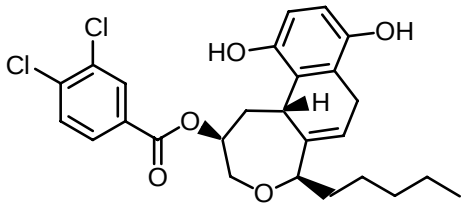
Competitive western blot for the analysis of the reversible binding of the proteins. Affinity resin was incubated with a solution of compound in PBS buffer (40 µL) at 30°C for 30 min and 300 rpm. The suspension was cleared using a magnetic rack and the supernatant and the beads loaded with protein were resuspended each in protein loading buffer containing 62.5 mM Tris-HCl (pH = 6.8), 2% SDS, 10% glycerol, 5% ß-mercaptoethanol and bromophenol blue, denatured and loaded on a 12.5% PAGE gel. The PAGE-gel was transferred on a nitrocellulose membrane using the semi dry blot technique. The membrane was blocked using Odyssey blocking buffer (LICOR, USA) for 1 h and incubated for 12 h at 4°C with the primary mouse anti-VANGL1 antibody (abcam, GB) diluted 1:300 in Odyssey blocking buffer (LICOR, USA). The membrane was washed with TBS-T (3 ×) and the antibody was visualised using an infrared anti-mouse antibody (LICOR, USA) in Odyssey blocking buffer (LICOR, USA) in a concentration of 1:20.000, followed by scanning with an infrared scanner (LICOR, USA).

Western blot analysis of total amount of ß-catenin protein. For western blot analysis of ß-catenin, 500,000 cells were seeded into a 9.6 cm^2 tissue culture flask 12 h before compound application. The tested compound **35** was diluted in tissue culture medium (1 mL) and applied for 4 h. The cells were rinsed using 4°C cold PBS buffer and lysed by addition of lysis buffer consisting of 50 mM Tris-HCl (pH = 7.4), 150 mM NaCl, 1 mM EDTA, 0.25% sodium-desoxycholate, 1% NP-40 and protease inhibitor cocktail (Roche, Germany). The tissue culture flasks were incubated for 10 min at 4 °C. Remaining cells were scraped off, transferred into a 1.5 mL reaction vessel and sonicated on ice twice for 10 s at 40 W each. The lysate was centrifuged for 10 min at 4°C and

15.000 g and the supernatant was transferred into a new reaction vessel. Protein concentration was measured using the Bradford protocol (5) and equal amounts were denatured at 95°C for 10 min after addition of protein loading buffer containing 62.5 mM Tris-HCl (pH = 6.8), 2% SDS, 10% glycerol, 5% ß-mercaptoethanol and bromphenolblue. The samples were loaded on a 12.5% PAGE-gel and separated using 20 mA per gel. The PAGE-gel was transferred on a nitrocellulose membrane using the semi dry blot technique. The membrane was blocked using 2% SlimFast Schoko (Allpharm Vertriebs GmbH, Messel, Germany) in TBS-T for 1 hour. The membrane was washed tree times using TBS-T and incubated over night at 4°C in 2% SlimFast Schoko in TBS-T with the appropriate amount of primary antibody. Afterwards the membrane was washed again tree times using TBS-T and incubated for 1 hour at room temperature in 2% SlimFast Schoko in TBS-T with the appropriate amount of secondary antibody. Finally the membrane was washed again tree times using TBS-T and incubated for 1 min in, Super Signal West Pico Luminol Solution“ (Thermo Scientific, Waltham, USA). After this incubation the HRP-catalysed light reaction was detected using X-ray film (Kodak, Germany).

Characterization data of selected library compounds

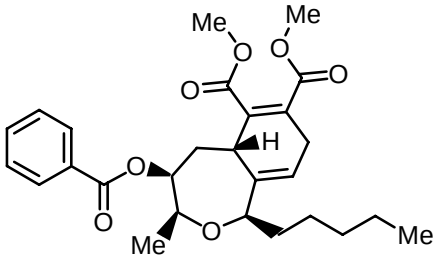
Compound 10



$[\alpha]_D^{20}$: + 5.6 (c = 2, $CHCl_3$); R_f = 0.50 (cyclohexane/ethyl acetate 4:1); Yield: 13 mg (30 % after 5 steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.07-7.92 (m, 1H), 7.79-7.71 (m, 1H), 7.53-7.45 (m, 1H),

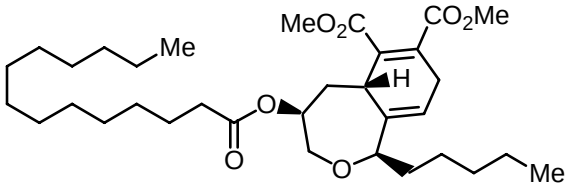
6.90-6.83 (dd, J = 10.2 Hz, 18.4 Hz, 1H), 6.75-6.75 (d, J = 1.9 Hz, 1H), 6.53-6.52 (d, J = 1.2 Hz, 1H), 5.74-5.73 (dd, J = 1.6 Hz, 5.8 Hz, 1H), 5.30-5.20 (m, 1H), 4.92-4.88 (dd, J = 4.4 Hz, 8.5 Hz, 1H), 4.33-4.28 (dd, J = 6.4 Hz, 12.1 Hz, 1H), 4.24-4.13 (m, 1H), 4.03-3.95 (m, 2H), 3.77-3.73 (m, 1H), 3.63-3.55 (m, 1H), 3.52-3.46 (m, 1H), 2.38-2.19 (m, 1H), 2.03-1.86 (m, 1H), 1.65-1.59 (m, 2H), 1.35-1.24 (m, 6H), 0.88-0.84 (t, J = 6.9 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 186.8, 185.9, 164.1, 163.6, 149.7, 143.5, 141.3, 140.8, 136.4, 131.7, 130.7, 128.9, 120.8, 113.3, 84.61, 72.5, 69.1, 54.0, 39.9, 35.8, 31.9, 31.8, 29.5, 28.6, 25.9, 25.6, 22.8, 14.2 ppm; HR-MS (FAB, 70 eV): *m/z* calcd for $C_{26}H_{30}Cl_2O_5$: 492.1314, found: 492.1300 $[M+2H]^+$.

Compound 11



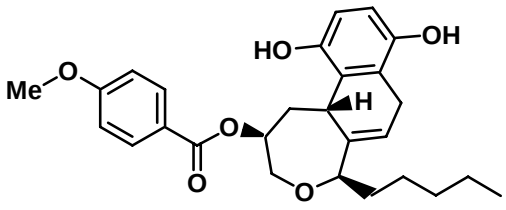
$[\alpha]_D^{20}$: +13.0 (c = 1, $CHCl_3$); R_f = 0.50 (cyclohexane/ethyl acetate 4:1); Yield: 10 mg (26% after 5 steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.63-7.58 (m, 3H), 7.56-7.46 (m, 2H), 5.67-5.66 (t, J = 3.4 Hz, 1H), 5.20-5.16 (m, 1H), 3.94-3.88 (m, 1H), 3.79-3.78 (m, 1H), 3.75 (s, 3H), 3.61-3.55 (m, 1H), 3.49 (s, 3H), 3.15-3.07 (m, 1H), 3.02-2.95 (m, 1H); 2.24-2.19 (dd, J = 4.0 Hz, 14.2 Hz, 1H), 2.01-1.94 (m, 1H), 1.72-1.69 (m, 2H), 1.57-1.55 (m, 1H), 1.37-1.32 (m, 9H), 0.92-0.88 (t, J = 6.6 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.3, 168.5, 168.3, 165.9, 139.2, 137.5, 134.0, 133.4, 132.3, 130.5, 130.4, 129.8, 129.6, 128.7, 128.6, 115.6, 83.1, 79.9, 78.8, 52.5, 52.3, 36.3, 35.8, 31.9, 28.0, 26.1, 22.8, 20.9, 14.3 ppm; HR-MS (FAB, 70 eV): *m/z* calcd for $C_{27}H_{35}O_7$: 471.2305, found: 471.2338 $[M+H]^+$.

Compound 12



$[\alpha]_D^{20}$: -10.9 (*c* = 1, $CHCl_3$); R_f = 0.50 (cyclohexane/ethyl acetate 9:1); Yield: 32.5 mg (28% after 5 steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.71-5.70 (t, *J* = 1.7 Hz, 1H), 4.95-4.92 (m, 1H), 4.01-3.94 (m, 1H), 3.79 (s, 3H), 3.76 (s, 3H), 3.61-3.57 (m, 1H), 3.42-3.37 (dd, *J* = 9.2 Hz, 12.1 Hz, 1H), 3.35-3.32 (m, 1H), 3.05-3.03 (m, 2H), 2.30-2.25 (m, 1H), 2.22-2.18 (t, *J* = 7.5 Hz, 1H), 1.72-1.69 (m, 1H), 1.62-1.53 (m, 4H), 1.33-1.22 (m, 16H), 0.90-0.85 (m, 6H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.8, 168.3, 167.8, 141.7, 138.1, 134.1, 119.8, 79.7, 71.5, 65.5, 52.7, 52.5, 38.5, 36.4, 34.6, 32.1, 31.9, 30.6, 9.8, 29.6, 29.5, 29.4, 29.3, 28.5, 25.6, 25.1, 22.9, 22.8, 14.3, 14.2 ppm; HR-MS (FAB, 70 eV): *m/z* calcd for $C_{33}H_{54}O_7$: 562.3870, found: 562.3800 $[M]^+$.

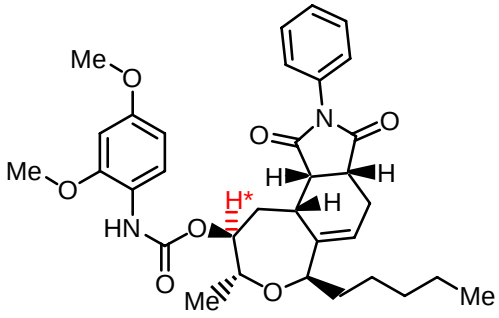
Compound 14



$[\alpha]_D^{20}$: -62.5 (*c* = 2, $CHCl_3$); R_f = 0.4 (cyclohexane/ethyl acetate 4: 1); Yield: 15 mg (17% after 5 steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.04-8.00 (m, 1H), 7.92-7.87 (m, 2H), 7.48-7.46 (m, 1H), 6.89-6.84 (m, 2H), 5.36-5.32 (t, *J* = 8.0 Hz, 1H), 4.89-4.86 (dd, *J* = 4.4 Hz, 8.5 Hz, 1H), 4.19-4.16 (m, 1H), 4.09-4.08 (m, 1H), 3.97-3.92 (dd, *J* = 3.3 Hz, 14.0 Hz, 1H), 3.84 (s, 3H), 3.80-3.76 (m, 1H), 2.06-2.03 (m, 1H), 1.87-1.81 (m, 1H), 1.60-1.57 (m, 2H), 1.51-1.28 (m, 6H), 0.92-0.88 (t, *J* = 6.9 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 187.7, 184.9, 165.8, 163.6, 149.8, 141.3, 136.6,

132.7, 131.9, 131.7, 131.4, 126.2, 122.9, 113.7, 83.7, 72.1, 55.6, 36.5, 31.9, 28.8, 25.8, 22.8, 14.3 ppm; HR-MS (FAB, 70 eV): m/z calcd for $C_{27}H_{33}O_6$: 453.2199, found: 453.2232 $[M+H]^+$.

Compound 22

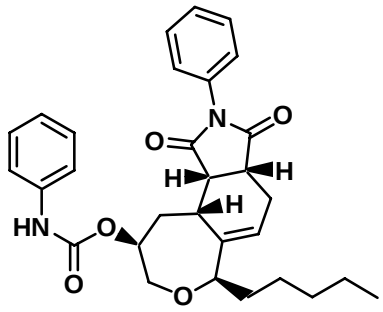


(d.r. = 8:1)*

* = inseparable mixture, ratio determined by ^{1}H NMR spectroscopy

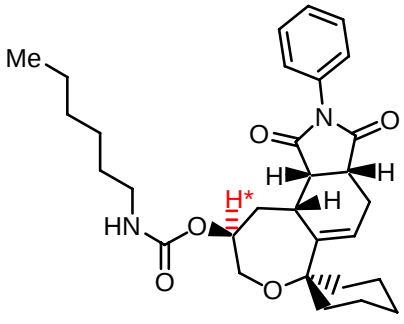
R_f = 0.30 (cyclohexane/ethyl acetate 4:1); Yield: 14.4 mg (50% after 5 steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.97-7.95 (m, 1H), 7.46-7.37 (m, 3H), 7.16-7.14 (m, 3H), 6.47-6.45 (m, 2H), 5.85-5.84 (t, *J* = 2 Hz, 1H), 4.91-4.90 (m, 1H), 4.20-4.09 (m, 2H), 3.83 (s, 3H), 3.78 (s, 3H), 3.31-3.29 (m, 1H), 3.19-3.15 (dd, *J* = 4.6 Hz, 8.9 Hz, 1H), 2.37-2.25 (m, 2H), 1.60-1.58 (m, 1H), 1.33-1.31 (d, *J* = 6.8 Hz, 3H), 1.28-1.25 (m, 8H), 0.89-0.86 (t, *J* = 6.7 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.0, 177.7, 164.9, 149.2, 145.0, 142.6, 132.0, 129.4, 126.6, 121.0, 104.1, 98.9, 76.2, 74.9, 71.1, 55.8, 45.6, 40.8, 32.1, 29.5, 27.1, 26.1, 22.8, 14.3 ppm; HR-MS (FAB, 70 eV): *m/z* calcd for $C_{33}H_{41}N_2O_7$: 577.2836, found: 577.2869 $[M+H]^+$.

Compound 25



$[\alpha]_D^{20}$: -19.8 (*c* = 1, $CHCl_3$); R_f = 0.40 (cyclohexane/ethyl acetate 4:1); Yield: 34 mg (32% after 5 steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.47-7.43 (m, 2H), 7.39-7.35 (m, 3H), 7.30-7.27 (m, 3H), 7.23-7.19 (m, 2H), 7.07-7.03 (m, 1H), 5.83-5.80 (t, *J* = 4.9 Hz, 1H), 4.17-4.09 (m, 1H), 3.91-3.88 (t, *J* = 6.4 Hz, 1H), 3.31-3.27 (m, 1H), 3.21-3.17 (m, 1H), 2.73-2.68 (m, 1H), 2.56-2.53 (m, 1H), 2.24-2.23 (m, 1H), 1.67-1.65 (m, 1H), 1.57-1.52 (m, 1H), 1.30-1.23 (m, 8H), 0.90-0.87 (t, *J* = 6.6 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.1, 177.7, 152.9, 144.3, 143.8, 138.0, 131.9, 129.4, 128.9, 126.5, 123.7, 122.3, 120.7, 82.15, 72.4, 71.1, 45.0, 39.8, 33.3, 33.0, 32.0, 31.9, 29.5, 27.1, 25.9, 23.7, 22.7, 14.3 ppm; HR-MS (FAB, 70 eV): *m/z* calcd for $C_{30}H_{35}N_2O_5$: 503.2468, found: 503.2401 $[M+H]^+$.

Compound 27

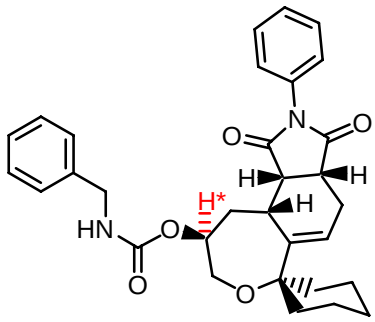


(d.r. = 8:1)*

* = inseparable mixture, ratio determined by ^{1}H NMR spectroscopy

R_f = 0.30 (cyclohexane/ethyl acetate 4:1); Yield: 78 mg (32% after 6 steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.50-7.45 (m, 2H), 7.41-7.39 (m, 1H), 7.31-7.27 (m, 2H), 5.82-5.78 (m, 1H), 4.77-4.73 (m, 1H), 3.61-3.58 (m, 1H), 3.26-3.19 (m, 2H), 3.16-3.09 (m, 2H), 2.80-2.74 (m, 1H), 2.53-2.44 (m, 1H), 2.07-1.82 (m, 2H), 1.71-1.57 (m, 6H), 1.48-1.43 (m, 4H), 1.30-1.25 (m, 8H), 0.89-0.86 (t, J = 6.0 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.4, 177.4, 156.2, 155.5, 149.4, 148.7, 132.0, 129.4, 129.3, 128.7, 126.6, 126.5, 121.5, 120.6, 78.5, 72.1, 69.7, 64.4, 54.1, 53.7, 44.9, 44.7, 41.2, 38.7, 31.9, 31.6, 31.4, 26.6, 26.0, 22.7, 21.9, 21.6, 14.2 ppm; HR-MS (FAB, 70 eV): *m/z* calcd for $C_{30}H_{41}N_2O_5$: 509.2937, found: 509.2971 $[M+H]^+$.

Compound 28



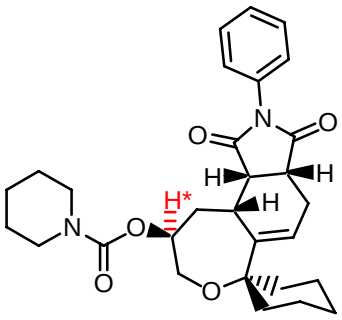
(d.r. = 7.5:1)*

* = inseparable mixture, ratio determined by ^{1}H NMR spectroscopy

R_f = 0.3 (cyclohexane/ethyl acetate 4:1); Yield: 99 mg (40% after 6 steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.48-7.45 (m, 3H), 7.41-7.39 (m, 1H), 7.31-7.27 (m, 3H), 7.23-7.17 (m, 3H), 7.13 (bs, 1H), 5.84-5.81 (dd, J = 2.7 Hz, 6.8 Hz, 1H), 4.81-4.76 (m, 1H), 4.31-4.28 (m, 2H), 3.83-3.63 (m, 1H), 3.26-3.16 (m, 1H), 3.14-3.09 (dd, J = 6.6 Hz, 9.9 Hz, 1H), 2.79-2.72 (m, 1H), 2.52-2.45 (m 1H), 2.07-2.00 (m, 1H), 1.93-1.86 (m, 2H), 1.71-1.58 (, 6H), 1.28-1.14 (m, 6H) ppm; ^{13}C NMR (100

MHz, $CDCl_3$): δ = 179.4, 177.4, 155.6, 148.6, 140.8, 134.6, 131.9, 130.2, 129.4, 128.8, 127.7, 126.5, 125.7, 121.6, 79.5, 71.4, 69.7, 54.1, 47.6, 44.9, 43.8, 41.2, 38.7, 35.7, 31.9, 29.5, 27.1, 25.9, 21.9, 21.6 ppm; HR-MS (FAB, 70 eV): *m/z* calcd for $C_{31}H_{34}N_2O_5$: 514.2468, found: 514.2445 $[M]^+$.

Compound 29

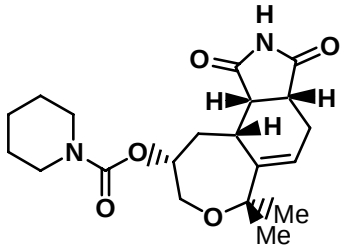


(d.r. = 8:1)*

* = inseparable mixture, ratio determined by ^1H NMR spectroscopy

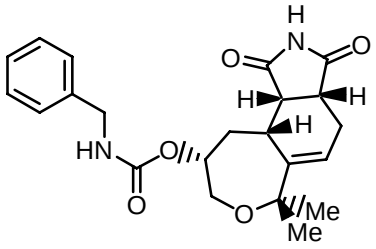
R_f = 0.4 (cyclohexane/ethyl acetate 4:1); Yield: 75 mg (32% after 6 steps); ^1H NMR (400 MHz, $CDCl_3$): δ = 7.46-7.43 (m, 2H), 7.40-7.35 (m, 1H), 7.29-7.26 (m, 2H), 5.81-5.78 (dd, *J* = 2.6 Hz, 6.9 Hz, 1H, major isomer), 5.77-5.75 (dd, *J* = 2.4 Hz, 7.2 Hz, 1H, minor isomer), 4.79-4.72 (m, 1H), 3.78-3.71 (m, 1H), 3.59-3.56 (m, 1H), 3.41-3.31 (m, 4H), 3.24-3.07 (m 2H), 2.79-2.72 (m, 1H), 2.53-2.43 (m, 1H), 2.05-2.01 (m, 1H), 1.91-1.87 (m, 1H), 1.71-1.42 (m, 16H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.5, 177.6, 177.4, 155.0, 154.5, 149.4, 148.8, 132.0, 129.4, 129.3, 128.8, 128.7, 126.6, 126.5, 121.4, 120.6, 71.1, 69.7, 64.5, 63.5, 54.1, 53.6, 45.0, 44.8, 38.7, 36.6, 33.4, 31.9, 29.5, 27.1, 26.0, 24.5, 21.9, 21.6 ppm; HR-MS (FAB, 70 eV): *m/z* calcd for $C_{29}H_{35}N_2O_5$: 491.2624, found: 491.2600 $[M-H]^+$.

Compound 34



$[\alpha]_D^{20}$: +10.1 (c = 1, $CHCl_3$); R_f = 0.3 (cyclohexane/ethyl acetate 7:1); Yield: 53 mg (22% after 6 steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.79-5.77 (dd, J = 2.8 Hz, 6.9 Hz, 1H), 4.75-4.68 (m, 1H), 3.55-3.51 (m, 1H), 3.29-3.23 (m, 4H), 3.11-3.04 (dd, J = 9.6 Hz, 17.7 Hz, 2H), 3.00-2.97 (dd, J = 6.6 Hz, 9.8 Hz, 1H), 2.66-2.56 (m, 1H), 2.42-2.35 (m, 1H), 2.02-1.98 (m, 1H), 1.52-1.50 (m, 4H), 1.47-1.44 (m, 4H), 1.30 (s, 3H), 1.20 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 180.7, 178.7, 154.5, 148.1, 121.4, 78.2, 72.5, 65.4, 54.1, 46.1, 44.9, 39.8, 32.7, 31.9, 31.1, 29.5, 27.5, 27.1, 24.5, 22.0, 22.2 ppm; HR-MS (FAB, 70 eV): m/z calcd for $C_{20}H_{27}N_2O_5$: 375.1998, found: 375.1920 $[M-H]^+$.

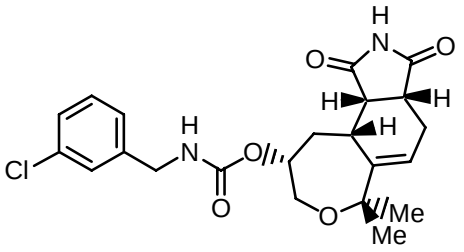
Compound 35



$[\alpha]_D^{20}$: +42.8 (c = 1, $CHCl_3$); R_f = 0.3 (cyclohexane/ethyl acetate 3:2); Yield: 49 mg (20% after 6 steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.90 (bs, 1H), 7.29-7.19 (m, 5H), 5.78-5.75 (dd, J = 2.8 Hz, 6.7 Hz, 1H), 4.77-4.69 (m, 1H), 4.35-4.23 (m, 2H), 3.56-3.53 (m, 1H), 3.24-3.19 (m, 1H), 3.07-2.95 (m, 3H), 2.57-2.55 (m, 1H), 2.36-2.32 (m, 1H), 2.02-1.98 (m, 2H), 1.29 (s, 3H), 1.22 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 180.77, 178.8, 156.3, 155.6, 147.8, 138.6, 128.8, 127.7, 121.7,

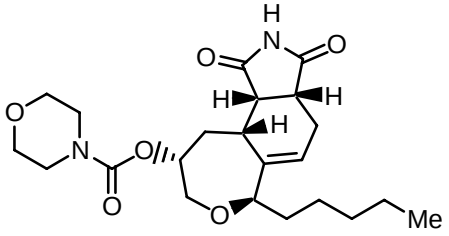
72.6, 69.8, 65.2, 54.1, 46.1, 45.2, 39.7, 31.9, 29.5, 27.4 ppm; HR-MS (FAB, 70 eV): *m/z* calculated for $C_{22}H_{25}N_2O_5$: 397.1842, found: 397.1826 $[M\text{-}H]^+$.

Compound 36



$[\alpha]_D^{20}$: +2.5 (*c* = 1, $CHCl_3$); R_f = 0.3 (cyclohexane/ethyl acetate 3:2); Yield: 44 mg (16% after 6 steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.94 (bs, 1H), 7.31-7.15 (m, 4H), 5.85-5.83 (dd, *J* = 2.8 Hz, 6.7 Hz, 1H), 4.83-4.75 (m, 1H), 4.35-4.32 (m, 2H), 3.62-3.59 (m, 1H), 3.32-3.27 (m, 1H), 3.13-3.03 (m, 3H), 2.63-2.56 (m, 1H), 2.43-2.41 (m, 1H), 2.07-2.02 (m, 2H), 1.36 (s, 3H), 1.28 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 180.7, 178.8, 155.7, 147.7, 140.7, 134.6, 130.1, 127.8, 125.8, 121.7, 72.8, 69.8, 65.1, 54.1, 46.1, 44.6, 39.7, 31.9, 29.5, 27.4, 27.1 ppm; HR-MS (FAB, 70 eV): *m/z* calcd for $C_{22}H_{24}ClN_2O_5$: 431.1452, found: 431.1400 $[M\text{-}H]^+$.

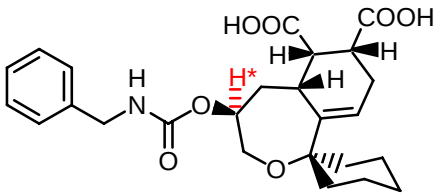
Compound 45



$[\alpha]_D^{20}$ **:** +20.0 (*c* = 2, $CHCl_3$); R_f = 0.2 (cyclohexane/ethyl acetate 4:1); Yield**:** 22 mg (16% after 6 steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.54 (bs, 1H), 5.76-5.74 (t, *J* = 4.8 Hz, 1H), 4.75-4.68 (m, 1H), 3.93-3.90 (dd, *J* = 3.0 Hz, 11.6 Hz, 1H), 3.86-3.83 (t, *J* = 6.4 Hz, 1H), 3.67-3.62 (m, 4H), 3.47-

3.46 (m, 4H), 3.36-3.31 (t, J = 11.0 Hz, 1H), 3.18-3.12 (m, 1H), 3.08-3.05 (dd, J = 5.9 Hz, 9.5 Hz, 1H), 2.62-2.54 (m, 1H), 2.45-2.41 (m, 1H), 2.13-2.09 (m, 1H), 1.90-1.81 (m, 1H), 1.58-1.52 (m, 1H), 1.48-1.37 (m, 2H), 1.27-1.24 (m, 6H), 0.88-0.85 (t, J = 6.8 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 180.1, 178.5, 154.4, 143.9, 135.3, 122.7, 82.4, 73.4, 71.3, 66.7, 46.2, 40.6, 34.2, 33.1, 32.4, 31.9, 25.7, 22.9, 14.2 ppm; HR-MS (FAB, 70 eV): *m/z* calcd for $C_{22}H_{33}N_2O_6$ = 421.2260, found = 421.2294 $[M+H]^+$.

Compound 62

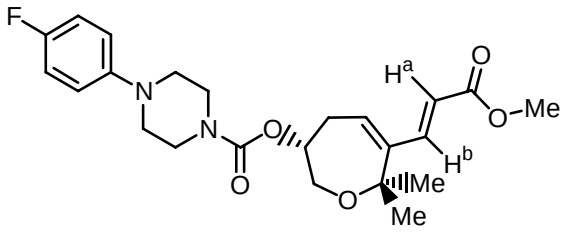


(d.r. = 4:1)*

* = inseparable mixture, ratio determined by ^{1}H NMR spectroscopy

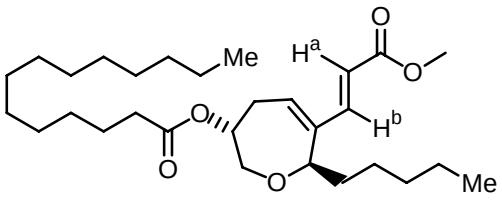
R_f = 0.4 (ethyl acetate/methanol 9:1); Yield: 45 mg (20% after 7 steps); ^{1}H NMR (400 MHz, DMSO-d^6): δ = 7.75-7.72 (t, J = 6.1 Hz, 1H), 7.36-7.24 (m, 5H), 5.48-5.47 (t, J = 3.5 Hz, 1H), 4.48-4.46 (m, 1H), 4.20-4.17 (t, J = 5.3 Hz, 2H), 3.74-3.70 (m, 1H), 3.55-3.50 (m, 1H), 3.39-3.30 (m, 5H), 2.78-2.76 (m, 2H), 1.67-1.35 (m, 10H) ppm; ^{3}C NMR (100 MHz, DMSO-d^6): δ = 177.2, 156.6, 148.2, 140.6, 129.2, 129.1, 127.8, 121.6, 79.8, 72.5, 65.7, 51.5, 44.6, 43.2, 37.7, 36.8, 33.0, 32.6, 30.5, 27.3, 27.1, 26.7, 26.6, 22.3, 22.2 ppm; HR-MS (FAB, 70 eV): *m/z* calcd for $C_{25}H_{31}NO_7$: 457.2101, found: 457.2155 $[M]^+$.

Compound 80



$[\alpha]_D^{20}$: -1.5 (c = 1, $CHCl_3$); R_f = 0.5 (cyclohexane/ethyl acetate 2:1); Yield: 40 mg (38% after 5 steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.15-7.11 (d, $J_{Ha\text{-}Hb}$ = 15.6 Hz, 1H), 6.98-6.93 (m, 2H), 6.87-6.84 (m, 2H), 6.01-5.97 (d, $J_{Ha\text{-}Hb}$ = 15.6 Hz, 1H), 5.95-5.91 (m, 1H), 5.04-4.99 (m, 1H), 4.06-4.01 (dd, J = 5.7 Hz, 13.8 Hz, 1H), 3.73 (s, 3H), 3.70-3.66 (m, 1H), 3.61-3.58 (m, 4H), 3.10-2.98 (m, 4H), 2.78-2.73 (m, 1H), 2.56-2.15 (m, 1H), 1.40 (s, 3H), 1.35 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.3, 158.9, 156.6, 154.9, 148.1, 145.3, 125.7, 119.3, 118.9, 118.8, 115.9, 115.7, 80.9, 74.4, 65.8, 54.1, 51.8, 50.6, 31.9, 29.5, 28.7, 27.6, 26.1 ppm; ^{19}F NMR (338.6 MHz, $CDCl_3$): -123.8 ppm; HR-MS (FAB, 70 eV): m/z calcd for $C_{23}H_{29}FN_2O_5$: 432.2061, found: 432.2011 $[M]^+$.

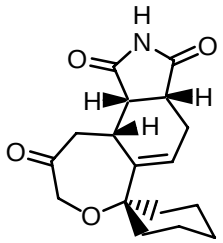
Compound 84



$[\alpha]_D^{20}$: -16.3 (c = 1, $CHCl_3$); R_f = 0.5 (cyclohexane/ethyl acetate 9:1); Yield: 25 mg (75% after 5 steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.14-7.09 (d, $J_{Ha\text{-}Hb}$ = 16.2 Hz, 1H), 6.08-6.04 (dd, J = 5.8 Hz, 9.2 Hz, 1H), 5.72-5.68 (d, $J_{Ha\text{-}Hb}$ = 16.4 Hz, 1H), 5.05-5.01 (m, 1H), 4.41-4.38 (m, 1H), 3.90-3.86 (d, J = 14.0 Hz, 1H), 3.74 (s, 3H), 3.00-2.97 (m, 1H), 2.40-2.33 (m, 1H), 2.30-2.26 (t, J = 7.5 Hz, 2H), 1.70-1.67 (m, 2H), 1.61-1.57 (m, 2H), 1.52-1.46 (m, 2H), 1.28-1.24 (m, 24H), 0.89-0.85 (m, 6H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.6, 167.5, 145.9, 141.1, 134.1, 116.6, 82.1,

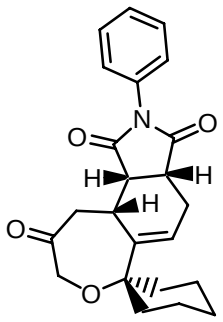
73.2, 70.9, 51.8, 34.6, 34.5, 32.1, 32.0, 29.9, 29.8, 29.7, 29.5, 29.4, 29.3, 28.1, 25.2, 25.1, 22.9, 22.8, 14.3, 14.2 ppm; HR-MS (FAB, 70 eV): *m/z* calcd for $C_{29}H_{51}O_5$: 479.3658, found: 479.3692 $[M+H]^+$.

Compound 90



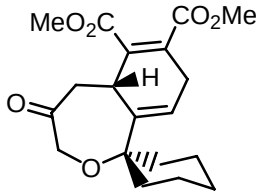
$[\alpha]_D^{20}$: +2.0 (*c* = 2, $CHCl_3$); R_f = 0.4 (cyclohexane/ethyl acetate 3:2); Yield: 22 mg (15% after 5 steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.44 (bs, 1H), 5.86-5.83 (dd, *J* = 2.9 Hz, 6.8 Hz, 1H), 4.02-3.98 (d, *J* = 17.9 Hz, 1H), 3.91-3.87 (d, *J* = 17.9 Hz, 1H), 3.78-3.72 (m, 1H), 3.10-3.07 (m, 2H), 2.79-2.73 (dd, *J* = 7.0 Hz, 16.4 Hz, 1H), 2.67-2.60 (m, 1H), 2.38-2.28 (m, 1H), 2.25-2.21 (m, 1H), 1.99-1.95 (m, 1H), 1.87-1.84 (m, 1H), 1.72-1.65 (m, 2H), 1.58-1.48 (m, 4H), 1.29-1.17 (m, 4H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 210.9, 179.8, 177.9, 147.5, 122.7, 79.2, 70.5, 54.0, 45.2, 41.4, 39.6, 35.6, 29.5, 25.8, 22.4, 21.6, 21.5 ppm; HR-MS (FAB, 70 eV): *m/z* calcd for $C_{17}H_{22}NO_4$: 304.1471, found: 304.1456 $[M+H]^+$.

Compound 91



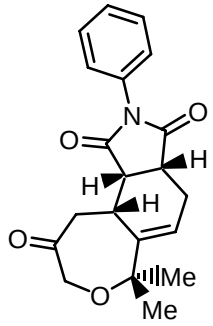
$[\alpha]_D^{20}$: +4.0 (c = 2, $CHCl_3$); R_f = 0.4 (cyclohexane/ethyl acetate 9:1); Yield: 19 mg (10% after 5 steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.49-7.45 (m, 2H), 7.41-7.40 (m, 1H), 7.29-7.25 (m, 2H), 5.90-5.88 (dd, J = 2.8 Hz, 6.9 Hz, 1H), 4.04-3.99 (d, J = 17.9 Hz, 1H), 3.93-3.89 (d, J = 18.0 Hz, 1H), 3.23-3.19 (m, 2H), 2.83-2.77 (dd, J = 7.0 Hz, 16.4 Hz, 1H), 2.75-2.71 (m, 1H), 2.40-2.31 (m, 1H), 2.03-2.00 (m, 1H), 1.89-1.86 (m, 1H), 1.71-1.51 (m, 6H), 1.32-1.20 (m, 4H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 210.6, 178.7, 176.9, 147.6, 131.8, 129.5, 129.0, 126.6, 122.9, 79.3, 70.6, 44.0, 41.4, 38.4, 35.7, 29.9, 25.9, 22.8, 21.6 ppm; HR-MS (FAB, 70 eV): *m/z* calcd for $C_{23}H_{25}NO_4$ = 379.1784, found = 379.1700 $[M]^+$.

Compound 92



$[\alpha]_D^{20}$: +6.0 (c = 1, $CHCl_3$); R_f = 0.4 (cyclohexane/ethyl acetate 4:1); Yield: 26 mg (15% after 5 steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.75-5.73 (t, J = 3.7 Hz, 1H), 4.09-4.03 (m, 1H), 3.99-3.95 (m, 1H), 3.90 (s, 3H), 3.79 (s, 3H), 2.23-2.16 (m, 1H), 2.08-2.02 (m, 2H), 1.75-1.61 (m, 6H), 1.27-1.20 (m, 4H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 211.9, 168.4, 167.5, 149.1, 143.2, 136.7, 134.8, 129.5, 128.9, 120.8, 79.2, 70.2, 52.9, 52.8, 47.2, 37.4, 35.6, 29.9, 28.9, 27.1, 25.9, 21.9, 21.4 ppm; HR-MS (FAB, 70 eV): *m/z* calcd for $C_{19}H_{24}O_6$: 348.1573, found: 348.1526 $[M]^+$.

Compound 93



$[\alpha]_D^{20}$: +14.0 (c = 1, $CHCl_3$); R_f = 0.5 (cyclohexane/ethyl acetate 8:1); Yield: 38 mg (12% after 5 steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.48-7.45 (m, 2H), 7.41-7.39 (m, 1H), 7.28-7.26 (m, 2H), 5.92-5.89 (dd, J = 2.8 Hz, 6.7 Hz, 1H), 3.99-3.98 (d, J = 2.3 Hz, 2H), 3.86-3.79 (m, 1H), 3.23-3.20 (dd, J = 4.4 Hz, 2H), 2.82-2.77 (dd, J = 6.8 Hz, 16.4 Hz, 1H), 2.75-2.69 (m, 1H), 2.44-2.33 (m, 2H), 1.40 (s, 3H), 1.38 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 210.3, 178.7, 176.9, 147.0, 131.8, 129.5, 129.5, 129.0, 126.6, 126.3, 122.9, 78.8, 71.4, 54.0, 43.9, 41.3, 38.3, 29.6, 29.5, 27.4, 22.9, 22.7 ppm; HR-MS (FAB, 70 eV): *m/z* calcd for $C_{20}H_{22}NO_4$: 340.1471, found: 340.1400 $[M+H]^+$.

Supporting References

1. Brown HC, Jadhav PK (1983) Asymmetric Carbon-Carbon Bond Formation Via Beta-Allyldiisopinocampheylborane. Simple Synthesis of Secondary Homoallylic Alcohols With Excellent Enantiomeric Purity. *J Am Chem Soc* 105: 2092-2093.
2. Chen B, Dodge ME, Tang W, Lu J, Ma Z, Fan C-W, Wei S, Hao W, Kilgore J, Williams NS, Roth MG, Amatruda JF, Chen C, Lum L (2009) Development of small molecules targeting the Wnt pathway for the treatment of colon cancer: a high-throughput screening approach. *Nat Chem Biol* 2: 100-107.
3. Lepourcelet M, Chen YN, France DS, Wang H, Crews P, Petersen F, Bruseo C, Wood AW, & Shivdasani RA (2004) Small-molecule antagonists of the oncogenic Tcf/beta-catenin protein complex. *Cancer Cell* 5: 91-102.
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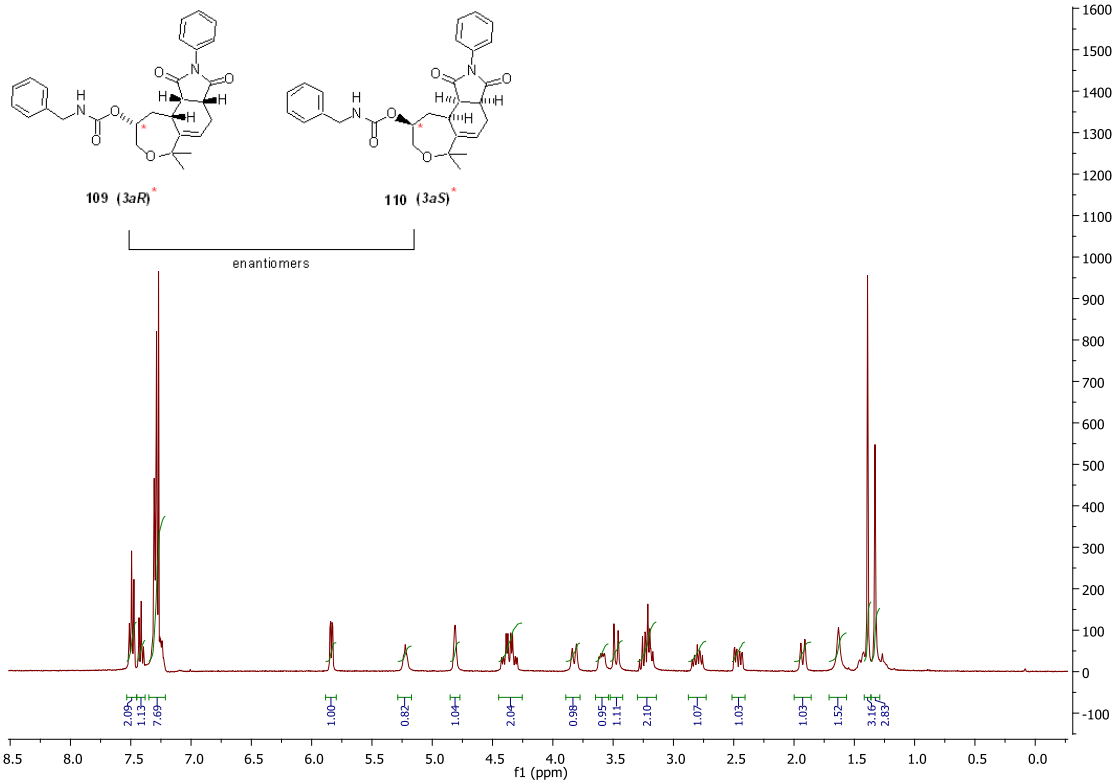
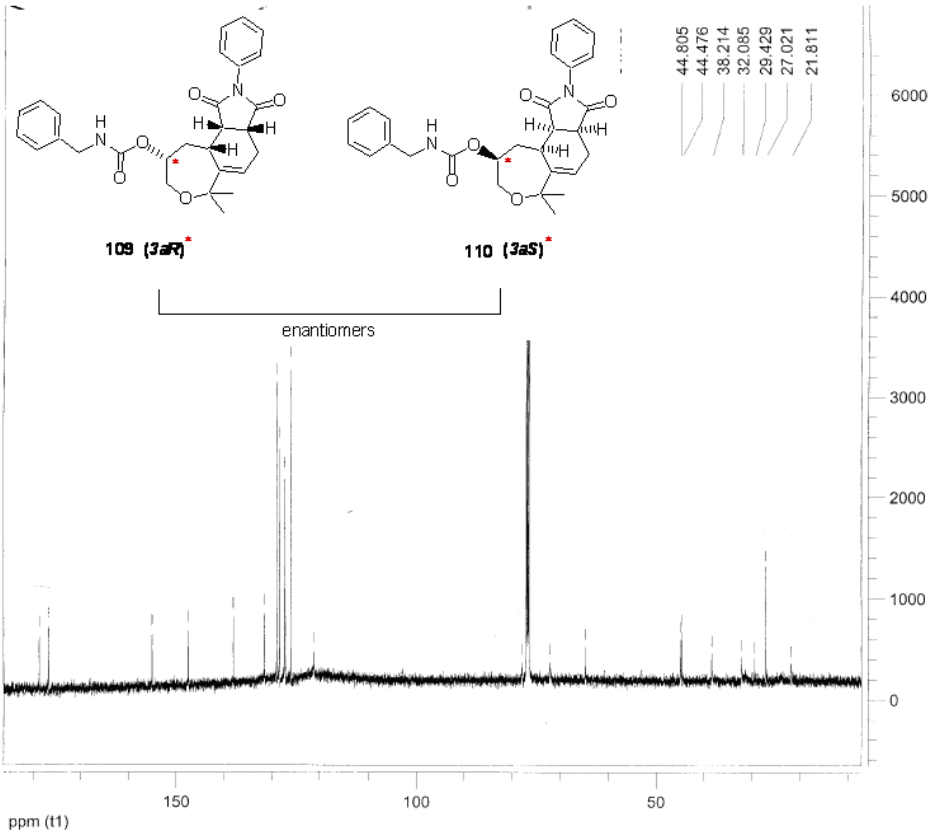
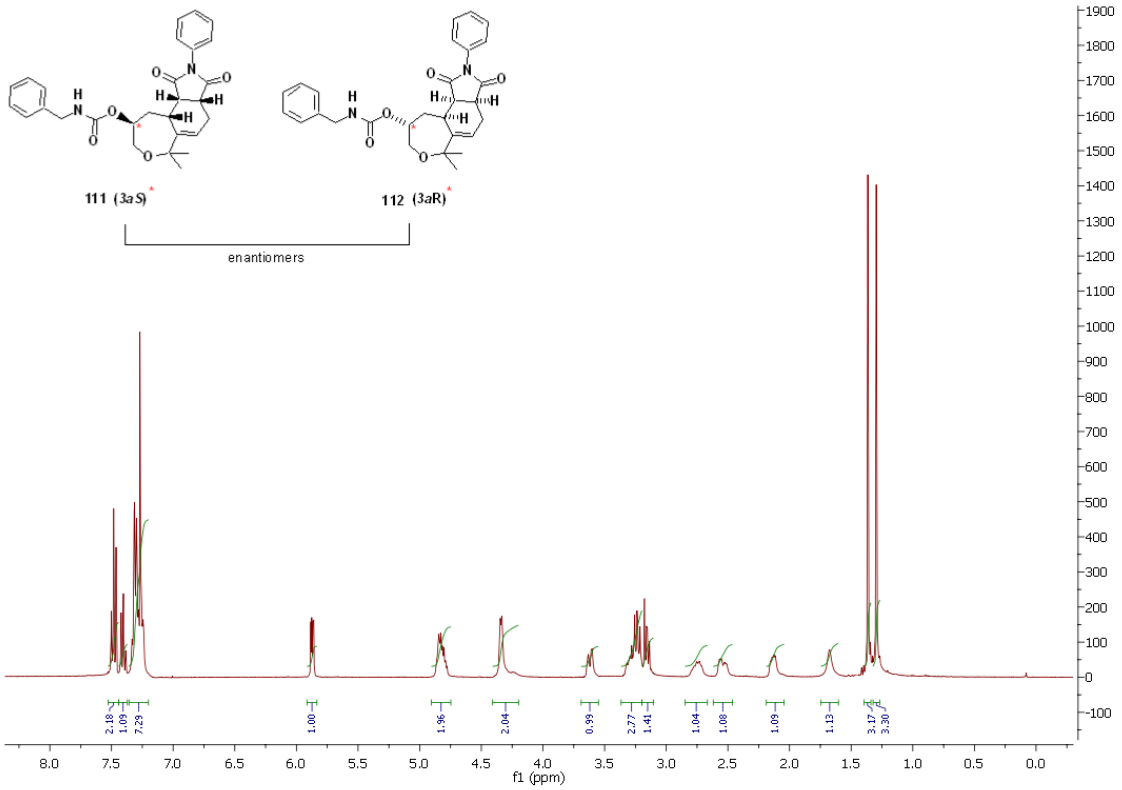
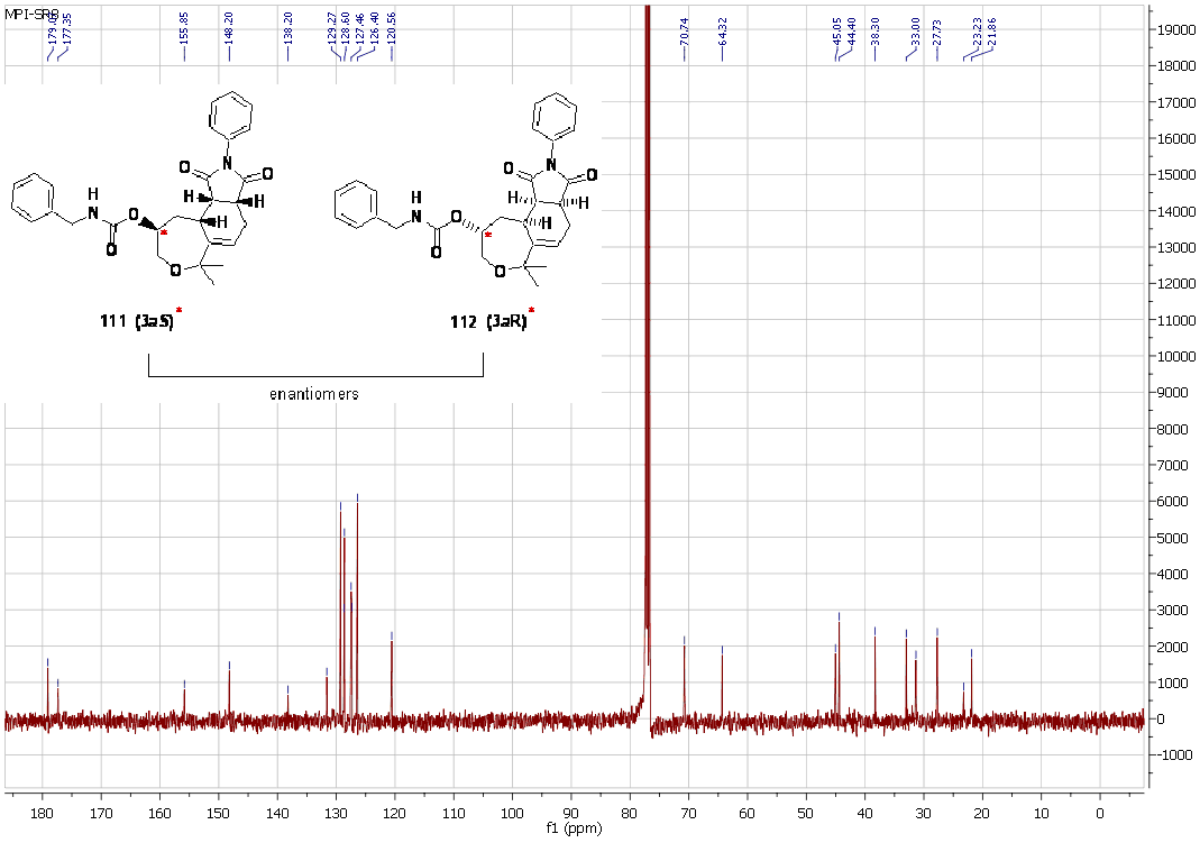
5. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248-254.

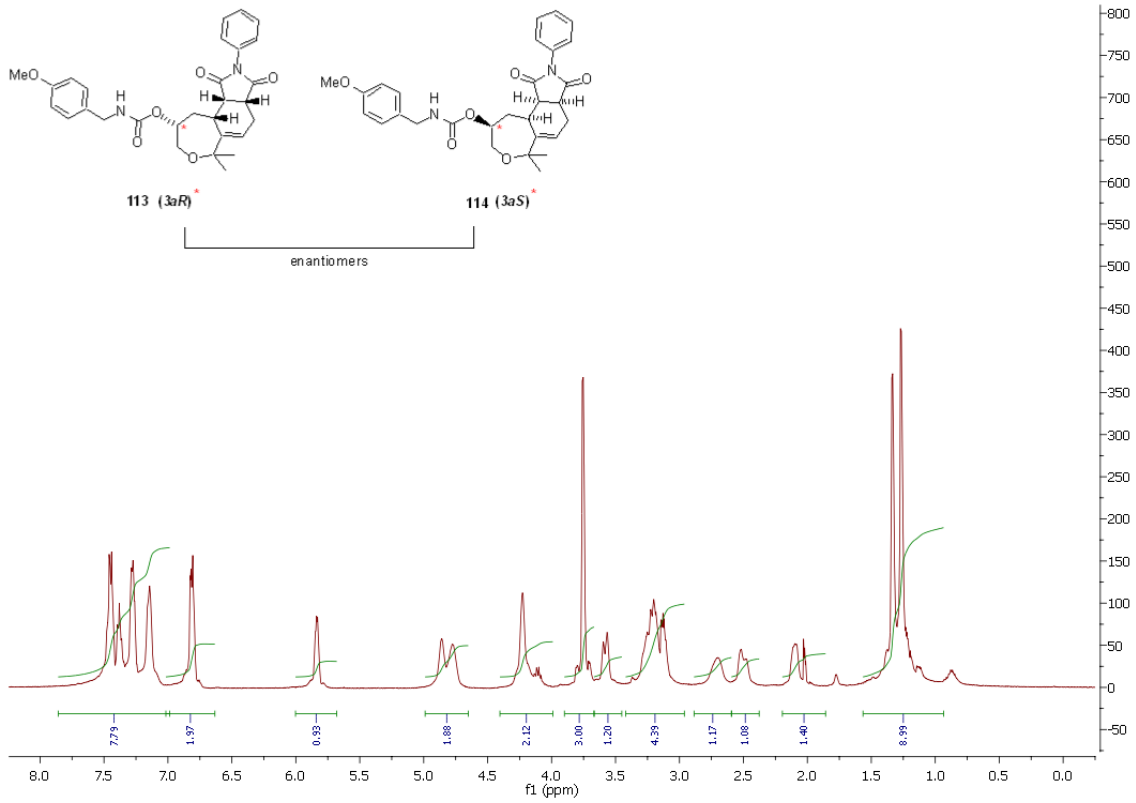
Copies of NMR spectra: 6 pages

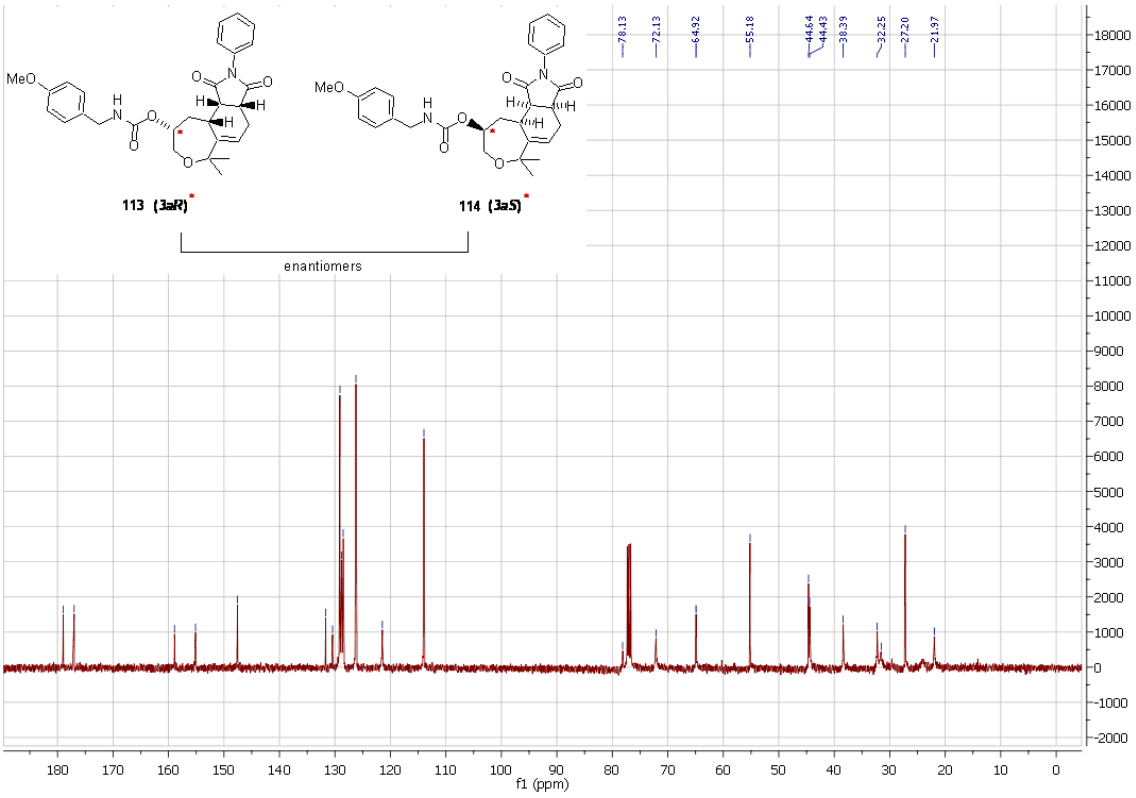

109 (3aR)*
110 (3aS)*
enantiomers
f1 (ppm)


44.805
44.476
38.214
32.085
29.429
27.021
21.811
109 (3aR)*
110 (3aS)*
enantiomers
ppm (t1)


111 (3aS)*
112 (3aR)*
enantiomers
f1 (ppm)


MPI-SR9
111 (3aS)*
112 (3aR)*
enantiomers
f1 (ppm)


MeO
113 (3aR)*
114 (3aS)*
enantiomers
7.79
1.97
0.93
1.88
2.12
3.00
1.20
4.39
1.17
1.08
1.40
8.99
f1 (ppm)


MeO
113 (3aR)*
114 (3aS)*
enantiomers
78.13
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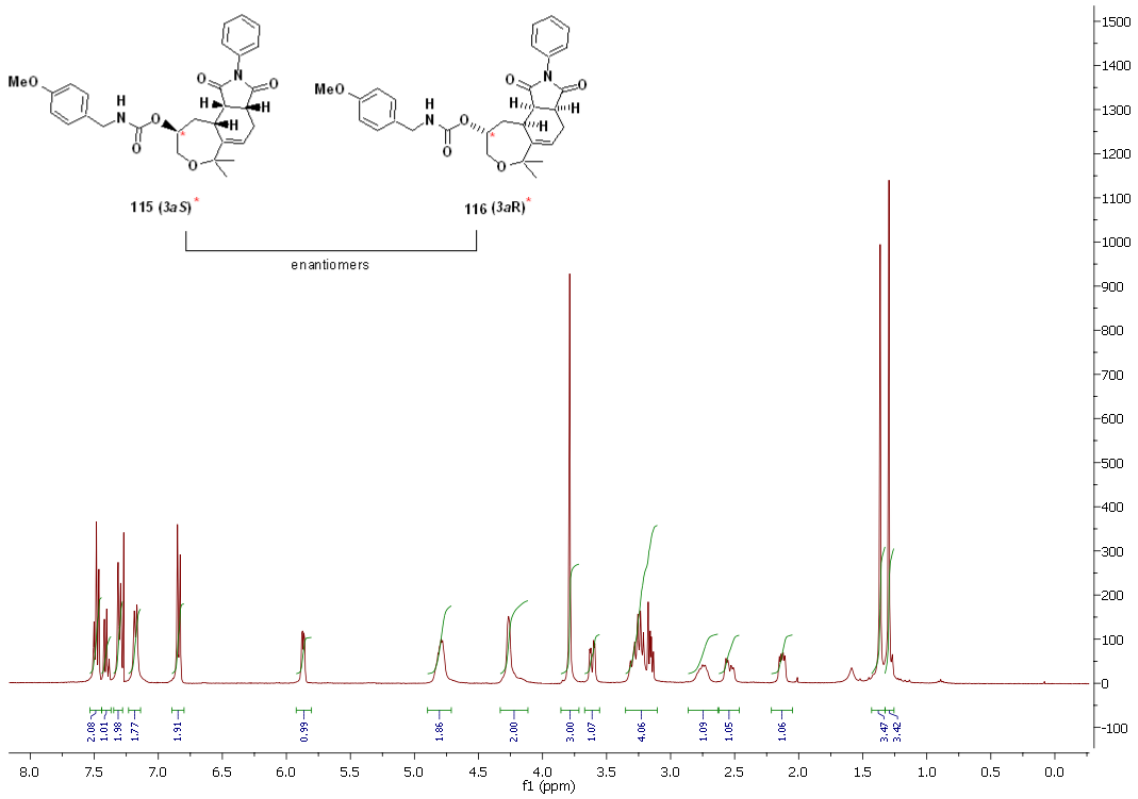
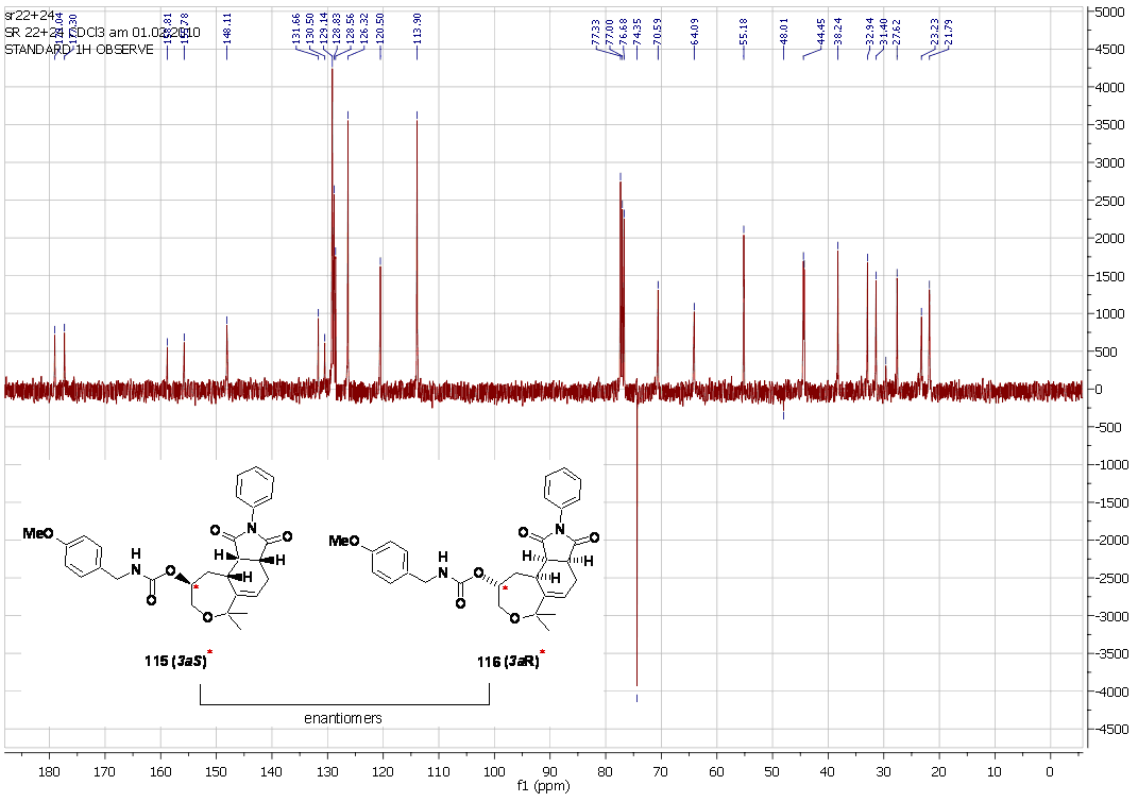
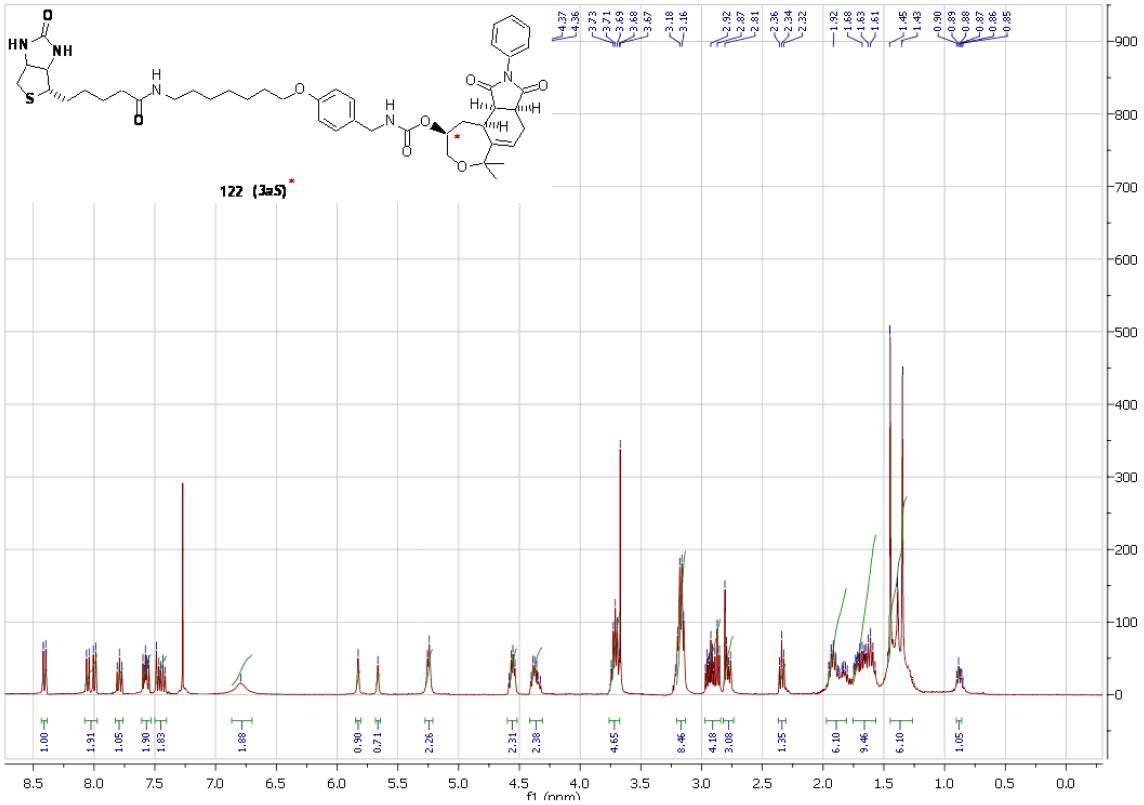
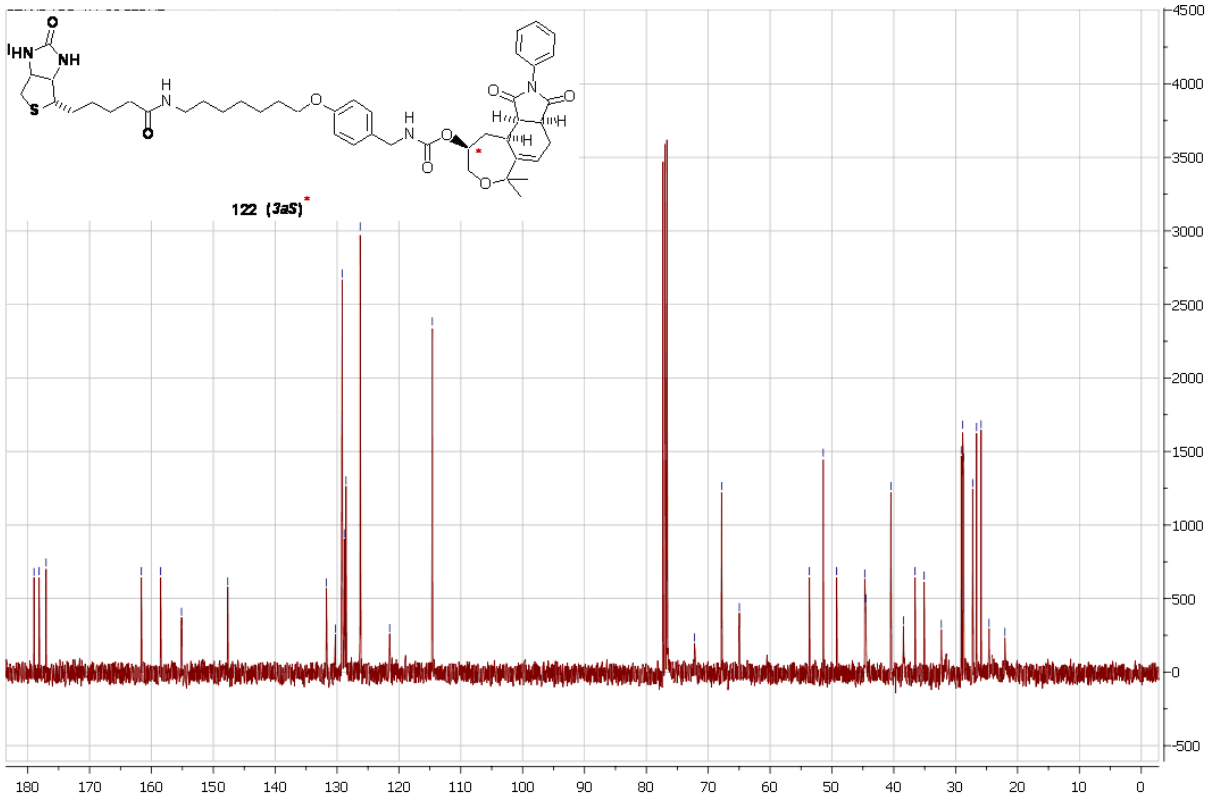
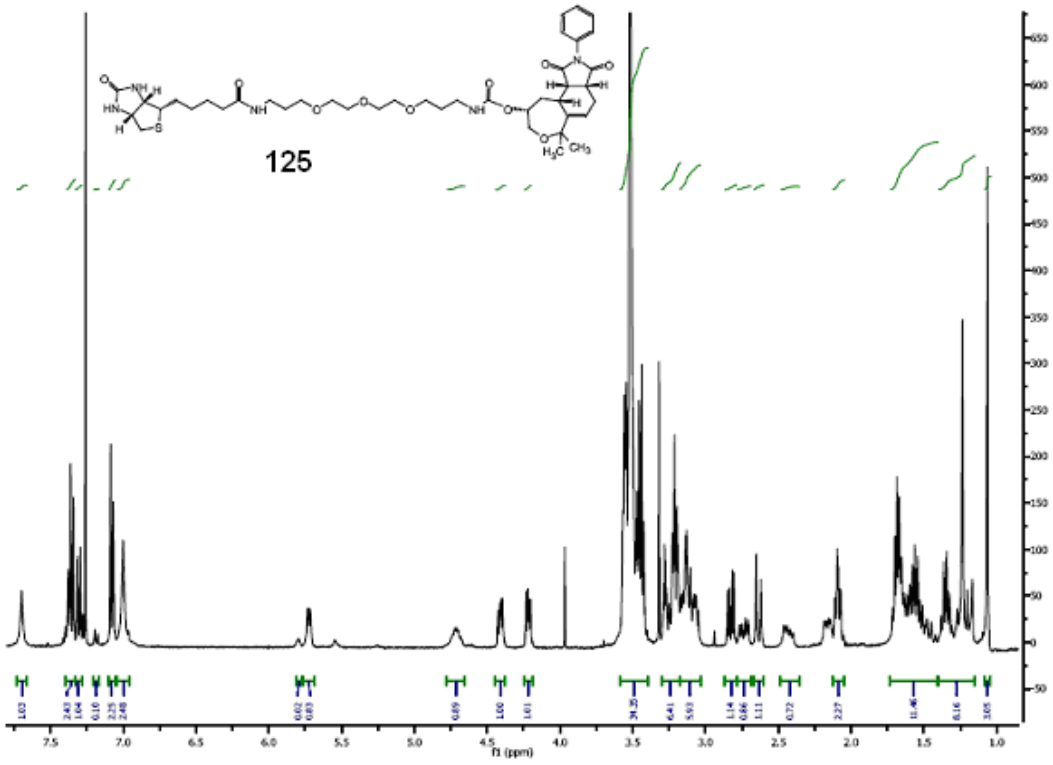
64.92
55.18
44.64
44.43
38.39
32.25
27.20
21.97
f1 (ppm)


MeO
115 (3aS)*
116 (3aR)*
enantiomers
f1 (ppm)


sr22+24
SR 22+24 13CDCl3 am 01.02.2010
STANDARD 1H OBSERVE
MeO
115 (3aS)*
116 (3aR)*
enantiomers
f1 (ppm)


122 (3aS)*
4.37
4.36
3.73
3.71
3.69
3.68
3.67
3.18
3.16
2.92
2.87
2.81
2.36
2.34
2.32
1.92
1.68
1.63
1.61
1.45
1.43
0.90
0.89
0.88
0.87
0.86
0.85
1.00
1.91
1.05
1.90
1.83
1.88
0.90
0.71
2.26
2.31
2.38
4.65
8.46
4.18
3.08
1.35
6.10
9.46
6.10
1.05
f1 (ppm)


122 (3aS)*


125
f1 (ppm)