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Article

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Boron-Pleuromutilins as Anti-*Wolbachia* Agents with Potential for Treatment of Onchocerciasis and Lymphatic Filariasis.

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Abstract

A series of pleuromutilins modified by introduction of a boron-containing heterocycle on C(14) of the polycyclic core are described. These analogs were found to be potent anti-*Wolbachia* antibiotics, and as such, may be useful in the treatment of filarial infections caused by *Onchocherca volvulus,* resulting in Onchocerciasis or river blindness, or *Wuchereria bancrofti* and *Brugia malayi* and related parasitic nematodes resulting in lymphatic filariasis. These two important Neglected Tropical Diseases (NTDs) disproportionately impact patients in the developing world. The lead preclinical candidate compound containing a 7-fluoro-6-oxybenzoxaborole (**15**, AN11251) was shown to have good *in vitro* anti-*Wolbachia* activity and physicochemical and pharmacokinetic properties providing high exposure in plasma. The lead was effective in reducing the *Wolbachia* load in filarial worms following oral administration to mice.

Introduction

Parasitic nematodes of the family Filarioidea including *Onchocerca volvulus, Wuchereria bancrofti* and *Brugia malayi* are responsible for significant disease burden in developing countries around the world.[1] Specifically, *O. volvulus* is the parasite responsible for River Blindness, and *W. bancrofti, B. malayi* and *B. timori* cause lymphatic filariasis (elephantiasis).[2] Lymphatic filariasis is one of the the leading causes of global disability, and accounts for at least 2.8 million disability-adjusted life years (DALYs). Treatment/control and elimination programs for these infections have been in place for many years, but fall short of full effectiveness due to the demanding treatment paradigm required to break disease transmission.[3-5] More specifically, the long life span of adult worms (up to 15 years) requires annual to bi-annual mass drug administration (MDA) of therapeutics such as ivermectin which only kills the juvenile microfilariae released by adult worms and sterilizes adult worms but is not macrofilaricidal.[5] Very recently, the results of a triple-drug treatment (ivermectin, albendazole and diethylcarbamazine) clinical trial demonstrated that this combination may require less frequent administration (perhaps once every three years), but only a marginal improvement of macrofilarical effects over a dual-drug therapy were noted.[6] Consequently, new approaches to kill the adult worms (macrofilaricides) are required in order for elimination time-frames of both

diseases to be radically reduced. A short course of treatment (7 days or fewer) would likely be required for ease of implementation in the field.

A unique feature of these parasitic worms is the presence of an obligate symbiotic bacteria of the *Wolbachia* genus.[7] It has been known for some time that classical antibacterial agents such as doxycycline can kill the bacteria present in the worms, which results in a reduced life span of the adult worm itself.[8-10] Unfortunately, doxycycline presents challenges as a drug for mass administration, including the requirement for long treatment periods (4–6 weeks) and contraindications in pregnancy and in children.[11] An anti-*Wolbachia* approach to the treatment of filarial infections has a number of patient benefits especially as the co-endemic eyeworm *Loa loa* does not harbor the endosymbiont and is therefore unaffected by treatment. It has been observed that concurrent killing of *Loa loa* microfilariae by directly acting drugs in patients with >30,000 microfilariae per mL can have serious side effects including neurologic effects, coma and death.[12] In addition, recent work has suggested that depleting *Wolbachia* in worms can also diminish the number of microfilariae that are able to develop in the insect vector, thus providing transmission blocking activity.[13] The underlying parasitology suggests that the discovery and development of new anti-*Wolbachia* drugs remains an attractive option for improving our ability to reduce the global burden of River Blindness and Elephantiasis and accelerate disease elimination goals.

As part of our ongoing effort to capitalize on the unique properties of benzoxaboroles in modifying pharmacologic, physicochemical and pharmacokinetic properties of existing drug scaffolds, we prepared analogs of the antibiotic class known as pleuromutilins.[14, 15] This class of ribosomal protein synthesis inhibitors predominantly targets Gram-positive bacteria and had been extensively explored since the 1950s, but had not previously been shown to have activity against *Wolbachia*. The pleuromutilins have been shown to inhibit protein synthesis through binding to the peptidyl-transfer center (PTC) of the ribosome, with a crystal structure of tiamulin (**4**) with the 50S subunit of *Deinoccus radiodurans* providing the most direct evidence of this mechanism of action.[16] Recently, Nabriva Therapeutics has been developing lefamulin (**2**), which is currently in clinical trials for community-acquired bacterial pneumonia, demonstrating the potential of this class of antibiotic.[17] Furthermore, the starting material for our explorations,

pleuromutilin (**1**), is readily available and inexpensive with synthetic modification, particularly of the hydroxyacetate at C(14), being synthetically straightforward. In addition to boronated analogs of the pleuromutilin core, we also obtained or prepared several clinically relevant non-boron analogs as summarized in Figure 1.[18-21]

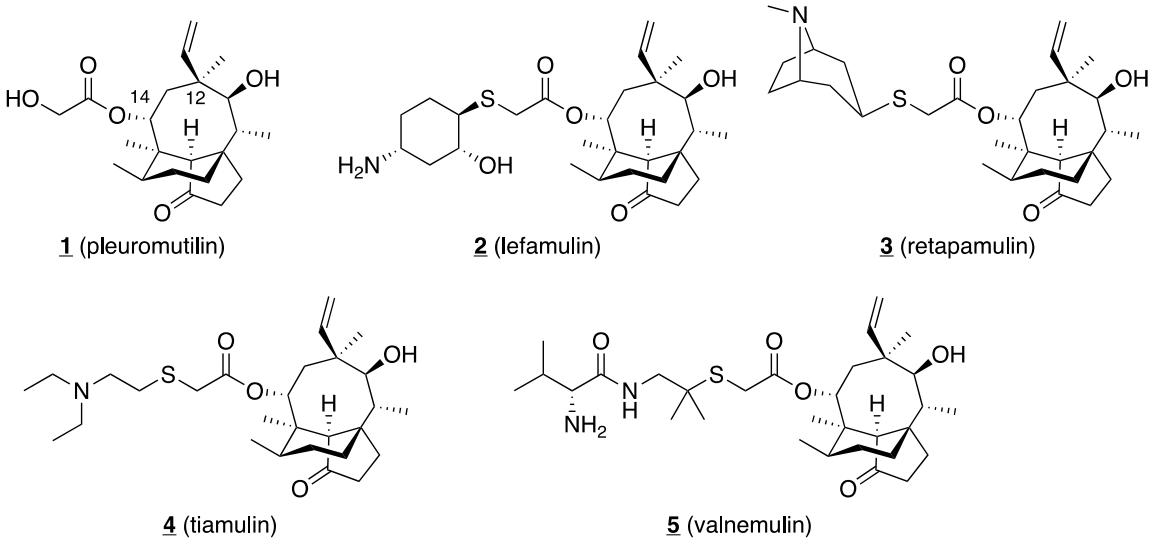
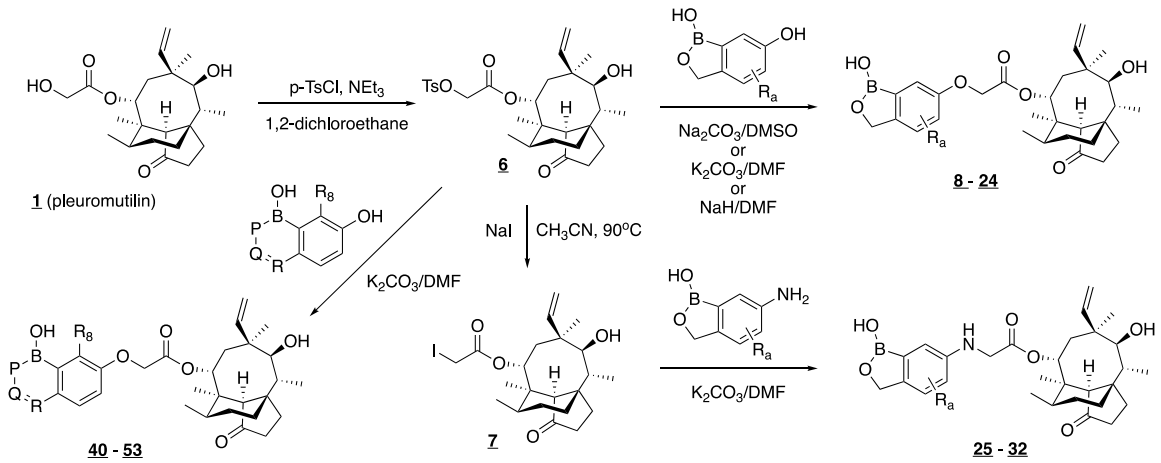


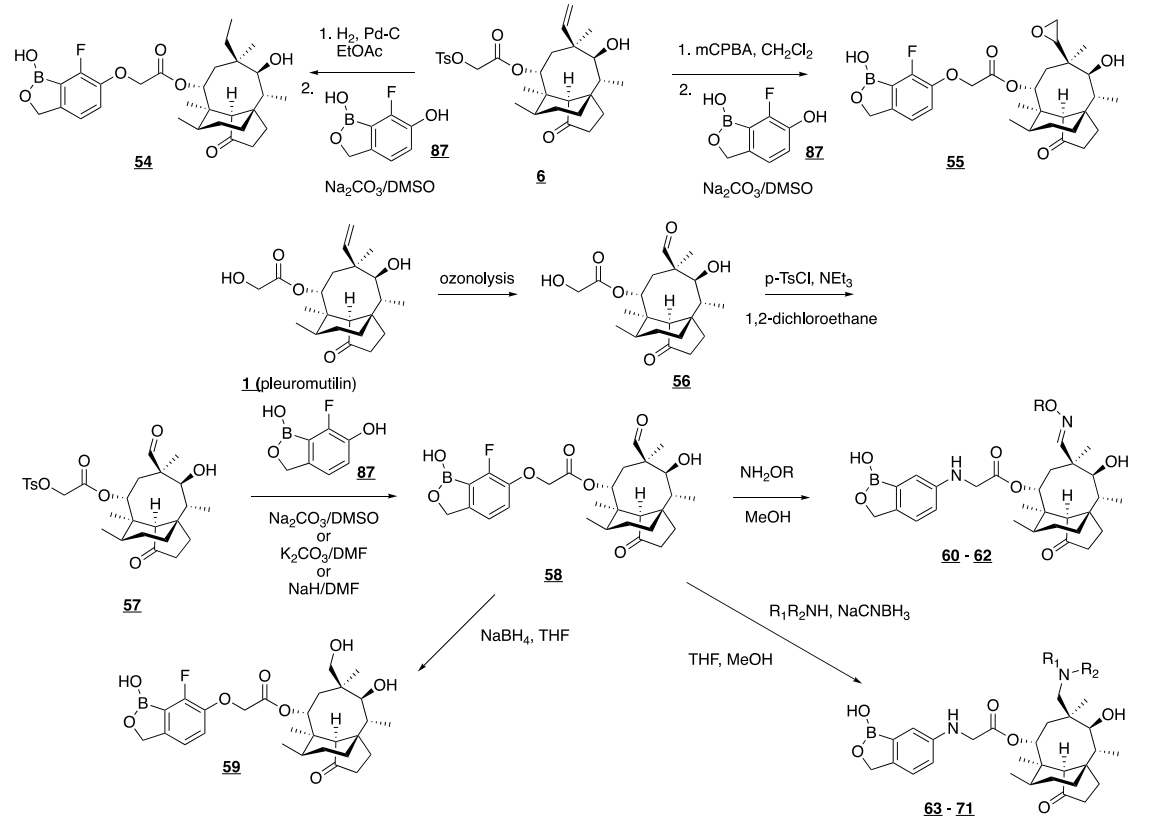
Figure 1. Pleuromutilin and clinically relevant derivatives.

Results

The boronpleuromutilins modified at the C(14) position of the pleuromutilin core were easily prepared via an S_N2 displacement reaction of the pleuromutilin tosylate or iodide (Scheme 1). These intermediates were synthesized from the commercially available pleuromutilin. For compounds wherein the vinyl group at C(12) was modified, ozonolysis of the pleuromutilin tosylate with a reductive workup provided the C(12) aldehyde (Scheme 2). Displacement of the C(14) tosylate with 7-fluoro-6-hydroxybenzoxaborole afforded the boronpleuromutilin, which in turn was condensed with hydroxyl amines to provide the oximes, or with amines and sodium cyanoborohydride to provide the amines. Hydrogenation of the C(12) vinyl group of the boronpleuormutilin afforded the corresponding C(12) ethyl analog.



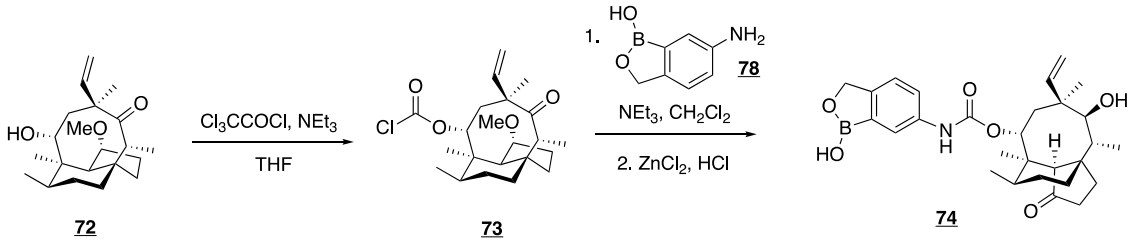
Scheme 1. General route to C(14)-modified boronpleuromutilins.



Scheme 2. Route to C(12)-modified boronpleuromutilins.

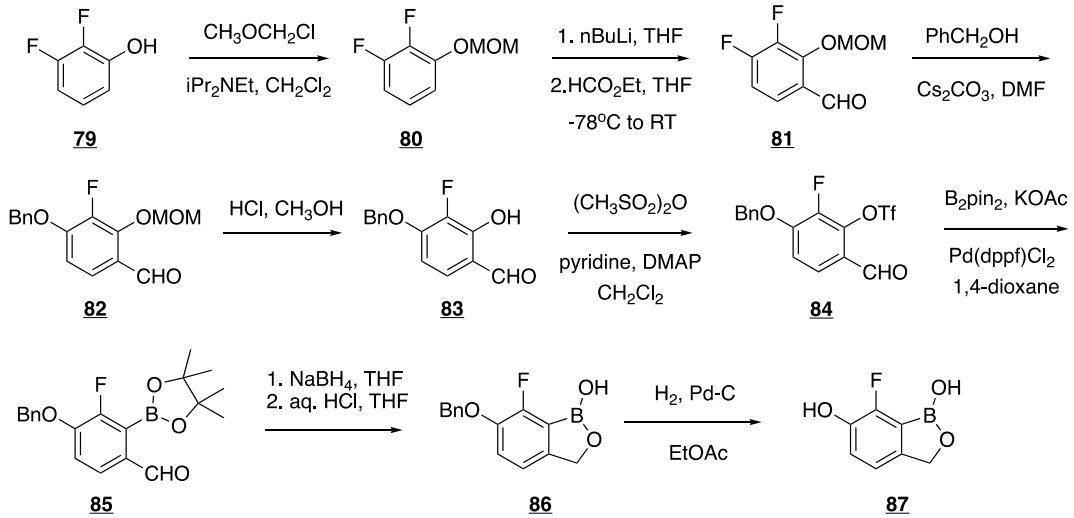
The boronpleuromutilins with a C(14)-carbamate linker were prepared in two steps from 4-*epi*-pleuromutilin (**72**)[22] in a manner analogous to that described in the literature (Scheme 3).[23] For example, following conversion of **72** to the carbamoyl chloride **73**, treatment with 6-aminobenzoxaborole **78** followed by a zinc chloride mediated 1,5-

hydride shift gave the C(14)-carbamate **74**. Compounds **75** – **77** were also prepared in a similar manner.



Scheme 3. Route to C(14) boronpleuromutilin carbamates.

The boron containing heterocycles were prepared by a variety of synthetic sequences that we have described in previous publications.[24] As an example, 7-fluoro-6-hydroxybenzoxaborole was prepared by the route shown in Scheme 4. Protection of 3,4-difluorophenol (**79**) as the methoxymethyl ether was followed by ortho-metallation and trapping of the intermediate aryllithium with ethyl formate to provide the benzaldehyde derivative **81**. Nucleophilic displacement of the para fluorine with benzyl alcohol under basic conditions provided **82**. Deprotection of the methoxymethyl ether followed by conversion to triflate **84** permitted introduction of the boron via palladium mediated borylation. Reduction of the aldehyde **85** with sodium borohydride followed by treatment with aqueous hydrochloric acid resulted in formation of the benzoxaborole **86**. Finally, hydrogenolysis of the benzyl ether gave the desired 7-fluoro-6-hydroxybenzoxaborole intermediate **87**.

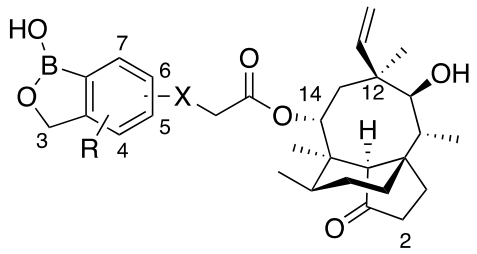


Scheme 4. Synthesis of 7-fluoro-6-hydroxybenzoxaborole.

We screened the pleuromutilin drug candidates in two primary *in vitro* assays that differed in the strain of *Wolbachia* used, the insect cell line used to host the bacteria, and in the method of detection.[25, 26] We used data from both assays to help ensure robust selection of compounds for progression to subsequent *in vitro* ADME and *in vivo* PK experiments. While the absolute potency of individual compounds varied between the two assays in some cases, the SAR trends observed in these assays were generally similar. Initial screening revealed that several of the clinically relevant pleuromutilins, e.g., retapamulin (**3**) and valnemulin (**5**), showed good activity in *in vitro* assays; significantly less activity was observed for lefamulin (**2**), tiamulin (**4**), or for the unadorned pleuromutilin core (**1**, Table 1). More interestingly, attachment of a benzoxaborole ring with either an oxygen (**8**), nitrogen (**25**) or sulfur (**33**) linker at the 6-position to the C(14) sidechain provided compounds (hereafter referred to as boronpleuromutilins) of similar *in vitro* potency to valnemulin and retapamulin. Introduction of a methylene spacer between the pleuromutilin core and the benzoxaborole ring as in **35** significantly reduced potency. Attachment of the benzoxaborole ring via the 5- position also provided compounds (**37** – **39**) with significantly reduced *in vitro* activity.

We next turned our attention to substitution of the benzoxaborole core, where we found that introduction of a fluoro substituent at the 4- (**13**), 5- (**14**) or 7- (**15**) positions was generally tolerated with the C(7) analog **15** exhibiting very potent activity. Other small substituents at C(7) such as methyl (**12**), chloro (**16**) or methoxy (**17**) were also tolerated, but did not improve activity beyond the fluoro analog. As the pleuromutilins are intrinsically very lipophilic, we also examined introduction of an aminomethyl substituent onto the benzoxaborole core (**19** – **23**, **26** – **29**), but these analogs were generally less active than the corresponding neutral compounds. Finally, we found that addition of two methyl groups at C(3) of the benzoxaborole (**24**, **30**), a strategy that had improved pharmacokinetic properties in other series,[27] significantly reduced potency.

Table 1. Pleuromutilin Derivatives with Benzoxaboroles Linked via C(14)

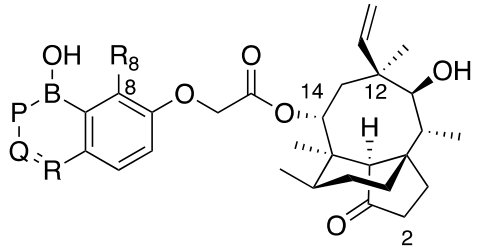


ID	Link atom	X	R	*Wolbachia* infected C6/36 cells (*w*Alb) EC_{50} (nM)	*Wolbachia* infected LDW1 cells (*w*Mel) EC_{50} (nM)
1 (pleuromutilin)	NA[a]	OH	NA	>1000	6868
2 (lefamulin)	NA	NA	NA	205	220
3 (retapamulin)	NA	NA	NA	NT[b]	91
4 (tiamulin)	NA	NA	NA	317	606
5 (valnemulin)	NA	NA	NA	NT	6.1
8	6	O	H	6.3	1.3
9	6	O	3-Me (R,S)	215	24
10	6	O	4-Me	100	28
11	6	O	5-Me	11	2.8
12	6	O	7-Me	52	2.9
13	6	O	4-F	158	19
14	6	O	5-F	8.4	NT
15	6	O	7-F	15	1.5
16	6	O	7-Cl	32	14
17	6	O	7-OMe	NT	3.7
18	6	O	5,7-F_2	NT	1.2
19	6	O	3-CH_2NH_2	104	19
20	6	O	4-CH_2NH_2	64	18
21	6	O	7-Cl, 3-CH_2NH_2	197	49
22	6	O	7-F, 3-CH_2NH_2	>1000	317
23	6	O	7-Cl, 4-CH_2NH_2	50	9
24	6	O	3,3-Me_2	>10000	148
25	6	NH	H	5.0	4.1
26	6	NH	3-CH_2NH_2	229	17
27	6	NH	4-CH_2NH_2	409	44
28	6	NH	5-CH_2NH_2	302	1182
29	6	NH	7-CH_2NH_2	257	173
30	6	NH	3,3-Me_2	>1000	198

31	6	NH	5-F	23	NT
32	6	NH	7-F	106	13
33	6	S	H	123	17
34	6	S	7-F	NT	15
35	6	$-CH_2NH-$	H	>1000	243
36	6	$-CH_2NH-$	3,3-Me_2	>10000	1418
37	5	O	H	>10000	NT
38	5	O	5-F	298	39
39	5	O	7-F	149	NT

[a]NA = not applicable, [b]NT = not tested.

Table 2. Pleuromutilins with Other Boron Heterocycles Linked via C(14)



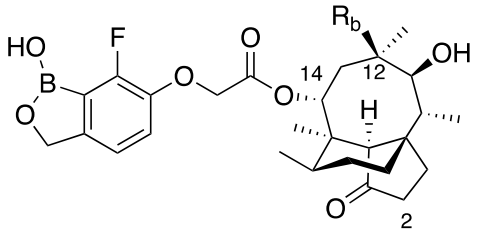
ID	P	Q-R	R_8	*Wolbachia* infected C6/36 cells (*w*Alb) EC_{50} (nM)	*Wolbachia* infected LDW1 cells (*w*Mel) EC_{50} (nM)
40	CH_3SO_2N	N=CH	H	9	0.8
41	CH_3N	N=CH	H	14.8	1.5
42	$CH_3C(=O)N$	N=CH	H	4	0.2
43	Boc-N	N=CH	H	0.7	0.2
44	HN	N=CH	H	3.5	2.7
45	$CH_3OC(=O)N$	N=CH	H	1.5	0.3
46	O	N=CH	H	14.2	5.1
47	CH_3SO_2N	N=CH	8-F	240	6.3
48	CH_3N	N=CH	8-F	172	11
49	$CH_3C(=O)N$	N=CH	8-F	107	NT[a]
50	$CH_3OC(=O)N$	N=CH	8-F	164	1.3
51	O	N=CH	8-F	329	27
52	$CH_3C(=O)N$	N=CH	8-CH_3	26.1	NT
53	O	CH_2CH_2	H	>1000	NT

[a]NT = not tested.

Initial results with other boron containing heterocycles linked via C(14) of the pleuromutilin core were encouraging, as a number of these analogs were very potent in the *in vitro Wolbachia* assay (Table 2). In particular, the N-methanesulfonyl (**40**), N-acetyl (**42**) and N-methylcarbamoyl (**45**) derivatives of the diazaborine scaffold exhibited low nanomolar to picomolar potency. Unlike the benzoxaboroles, however, inclusion of a fluorine atom adjacent to the B-OH did not improve potency; in fact, these derivatives (**47** – **51**) were significantly less potent. The benzoxaborine **53**, wherein the five membered ring was expanded by one carbon to a six membered ring, was completely inactive.

We also explored modification of the C(12) vinyl substituent (Table 3). Work in the classical pleuromutilin literature had shown that this double bond could be reduced to provide an ethyl group (**54**), converted to the epoxide (**55**) or oxidized to an aldehyde (**58**) that could be functionalized in a variety of ways.[28] In particular, we sought to introduce small amines at C(12) to modify physicochemical and pharmacokinetic properties. Given the interesting properties of **15** (vide supra), this work was performed with the 7-fluoro-6-oxobenzoxaborole linked via to C(14) of the pleuromutilin core.

Table 3. Boronpleuromutilins Modified at C(12)



ID	Rb	*Wolbachia* infected C6/36 cells (*w*Alb) EC_{50} (nM)	*Wolbachia* infected LDW1 cells (*w*Mel) EC_{50} (nM)
54	CH_3CH_2-	38	2.7
55	epoxide	148	13
58	OHC-	278	33
59	$HOCH_2$-	>1000	242
60	HON=CH-	>1000	71

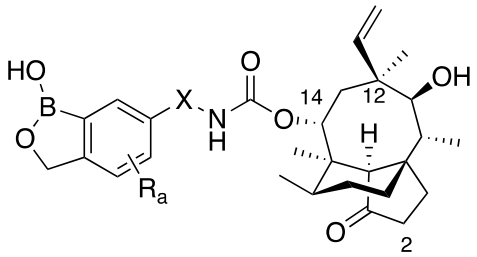
61	$CH_3ON=CH$-	197	39
62	iso-$C_3H_7ON=CH$-	NT[a]	101
63	H_2NCH_2-	>1000	6892
64	CH_3NHCH_2-	>1000	1718
65	$C_2H_5NHCH_2$-	474	1399
66	n-$C_3H_7NHCH_2$-	357	478
67	n-$C_4H_9NHCH_2$	372	243
68	cyclo-$C_3H_5NHCH_2$	115	44
69	$(CH_3)_2NCH_2$-	328	439
70	CH_3ONHCH_2-	NT	133
71	$CH_3C(=O)NHCH_2$-	>1000	29

[a]NT = not tested.

Disappointingly, only the ethyl (**54**) derivative exhibited potency close to the C(12) vinyl analog **15**; oximes (**60** – **62**) and amines (**63** – **70**) were significantly less potent. Some potency was regained by acylation of the amine (**71**), but this was not perceived as being of sufficient advantage to pursue further.

As a final area of exploration, we prepared several boronpleuromutilins where the C(14)-hydroxyacetate linker was replaced by a shorter carbamate (Table 4). This strategy had previously been explored in the pleuromutilins as a means to improve oral bioavailability.[23] The carbamates prepared (**74** – **77**) exhibited moderate to good *in vitro* potency.

Table 4. Boronpleuromutilin Carbamates



ID	X	Ra	*Wolbachia* infected C6/36 cells (*w*Alb) EC_{50} (nM)	*Wolbachia* infected LDW1 cells (*w*Mel) EC_{50} (nM)
74	bond	H	101	7.5
75	bond	5-F	>1000	14
76	CH2	H	12	3.5

77	CH2	7-F	113	4.9

Concurrent with our evaluation of the *in vitro* potency of the boronpleuromutilins, we also selected a number of active analogs for characterization in both *in vitro* ADME (Absorption, Distribution, Metabolism, Excretion) and *in vivo* pharmacokinetics (PK) experiments. It has been suggested that one of the main limitations of the pleuromutilins is high metabolic instability leading to poor pharmacokinetics.[29, 30] In fact, the majority of the clinically relevant pleuromutilins are of limited oral bioavailability, which has hampered their utility for human applications. A notable exception is the recently described lefamulin (**2**), which is currently in Phase 3 clinical trials as an oral treatment for a variety of bacterial infections.[31, 32] In order to calibrate our expectations for the boronpleuromutilins, we screened several of the clinically relevant analogs in our *in vitro* ADME assays as well (Table 5). As anticipated, tiamulin and retapamulin were found to be rapidly metabolized by mouse liver S9 fraction, whereas lefamulin was slowly metabolized. These classical pleuromutilins exhibited a range of binding to mouse plasma proteins, with free fraction ($f_{unbound}$) from 0.05 – 0.26. Somewhat surprisingly, we found that lefamulin was not particularly permeable in our MDR1-MDCK assay, though the high stringency of this assay may underestimate the permeability of this compound through the gut. We had chosen the MDR1-MDCK assay to assess permeability over the related Caco2 assay due to throughput and the opportunity to more directly assess the impact of P-glycoprotein mediated efflux in this cell line. In addition, it has also been reported that the MDR1-MDCK is an acceptable assay for evaluation of the permeability of compounds through the intestinal mucosa of humans.[33]

The neutral boronpleuromutilins evaluated (**8** – **12**, **14**, **25**, **33**) were generally metabolized quickly by mouse S9 (Cl_{int} > 50 μL/min/mg), were highly protein bound ($f_{unbound}$ < 0.03) and were predicted to have good permeability based on the MDR1-MDCK assay (P_{app} > 5 × 10^{-6} cm/sec). In contrast, boronpleuromutilins bearing an aminomethyl subsitituent (**19** – **21**, **23**, **26**) were less rapidly metabolized (Cl_{int} < 10 μL/min/mg) much less protein bound ($f_{unbound}$ > 0.1), but were predicted to be of low permeability (P_{app} < 1 × 10^{-6} cm/sec). Later compounds incorporating boron-containing heterocycles other than the benzoxaborole (**40** – **42**, **44**- **46**, **52**, **53**) or modification of C(12) (**55**, **59**) did not significantly change the overall *in vitro* ADME profile. One

compound that stood out as having an attractive balance of properties was the 7-fluorobenzoxaborole derivative **15**, which exhibited modest clearance (Cl_{int} = 33 μL/min/mg) and protein binding ($f_{unbound}$ = 0.03), and good permeability (P_{app} = 14.1 × 10^{-6} cm/sec).

Table 5. In vitro ADME properties of Boronpleuromutilins.

ID	mouse S9 Clint (μL/min/mg)	Mouse protein binding ($f_{unbound}$ @ 2 μM)	MDR1-MDCK P_{app}[a]
2	4	0.218	0.2
3	41	0.154	6.7
4	131	0.26[b]	22.1
5	NT	0.05[b]	1.4
8	145	0.003	15.1
9	168	0.002	NT[c]
10	139	0.005	NT
11	77	< 0.001	NT
12	136	0.012	NT
14	117	0.003	NT
15	33	0.034	14.1
16	44	0.008	NT
19	NT	0.116	0.3
20	7	0.110	0.1
21	NT	0.119	0.5
23	NT	0.048	0.1
25	102	0.008	36.5
26	2	0.306	0.1
32	NT	0.005	22.6
33	142	0.008	9.0
40	226	0.058	6.6
41	NT	0.007	NT
42	NT	0.005	NT
44	80	0.008	2.3
45	NT	0.037	NT
46	NT	< 0.001	8.8
52	217	0.006	NT
53	NT	< 0.001	NT
55	NT	NT	8.7
59	53	0.033	3.7

[a]Permeability measured in a Madin Darby Canine Kidney cell monolayer transfected with the multi-drug resistance 1 (mdr1) gene encoding p-glycoprotein MDR1. [b]Protein binding measured at 1 μM. [c]NT = not tested.

Based on these *in vitro* profiles and due to resource constraints, we selected representative compounds from the various classes (e.g. neutral, aminomethyl), different linkers (e.g. O, N) and boron heterocycles (e.g. benzoxaboroles, diazaborines) for progression to *in vivo* PK experiments as summarized in Table 6. When the *in vivo* pharmacokinetics in mice of these representative compounds were measured, we observed that the PK parameters between them were variable following both IV and oral administration, consistent with the *in vitro* properties described above. Several observations were made from these studies. First, and as expected, clearance following intravenous dosing was generally quite high, with only compound **15** exhibiting low to modest clearance. Secondly, incorporation of an aminomethyl group on the benzoxaborole core (**19**) significantly reduced oral bioavailability, as did replacement of the benzoxaborole with a diazaborine (**45**, **52**). Finally, oral exposure and bioavailability was generally good for the 7-fluorobenzoxaborole derivatives tested (**15**, **32** and **77**).

Table 6. *In vivo* pharmacokinetic properties of lead boronpleuromutilins in BALB/c mice.

Cmpd	IV at 5 mg/kg, Vehicle 55/25/20 PEG/PG/H_2O				PO at 10 mg/kg, Vehicle 55/25/20 PEG/PG/H_2O		
	Cmax (μg/mL)	CL (mL/hr/kg)	Vss (mL/kg)	$AUC_{0\text{-last}}$ (hr*μg/mL)	Cmax (μg/mL)	$AUC_{0\text{-last}}$ (hr*μg/mL)	%F
8	4.13	2296	2716	2.18	0.673	1.54	35
15	3.21	505	4186	8.85	1.60	12.1	61
19	2.48	2120	10435	2.26	0.003	0.02	<1
32	5.91	1080	2231	4.60	1.66	11.0	~100
45	7.40	1642	854	3.03	0.262	0.425	7
52	3.22	3273	4658	1.52	0.227	0.166	6
77	3.91	1338	2561	3.71	1.09	8.51	~100

Given the relatively low clearance and high exposure exhibited by **15**, coupled with its good biological potency, additional PK experiments were performed with this compound. These confirmed the attractive PK profile following oral administration to mice of **15**, as systemic exposures at doses from 10 – 50 mg/kg were good, with C_{max} values increasing dose-dependently while AUC values remained relatively constant, suggesting dissolution-limited absorption of compound from the gut (Table 7).

Table 7. *In vivo* pharmacokinetic properties of 7-fluorobenzoxaborole analog 15 in BALB/c mice.

15 IV				
5 mg/kg (Mean, n=3)				
Vehicle	55/25/20 PEG/PG/H_2O			
C_{max} (µg/mL) @ 5 min	3.21 ± 1.3			
CL (mL/h/kg)	505			
V_{ss} (mL/kg)	4186			
AUC_{last} (h*µg/mL)	8.85			
$AUC_{0\text{-}inf}$ (h*µg/mL)	9.91			
Terminal $t_{1/2}$ (h)	9.10			
15 PO				
	10 mg/kg Solution (Mean, n=3)	10 mg/kg Suspension (Mean, n=3)	25 mg/kg Suspension (Mean, n=3)	50 mg/kg Suspension (Mean, n=3)
Vehicle	55/25/20 PEG/PG/H_2O	1% CMC, 0.1% Tween 80 in H_2O	1% CMC, 0.1% Tween 80 in H_2O	1% CMC, 0.1% Tween 80 in H_2O
C_{max} (µg/mL)	1.60	2.81	4.25	6.38
T_{max} (h)	0.083	0.50	0.25	0.50
AUC_{last} (h*µg/mL)	12.1	20.7	23.7	23.0
$AUC_{0\text{-}inf}$ (h*µg/mL)	14.0	22.8	24.2	NC[a]
Terminal $t_{1/2}$ (h)	8.41	7.51	4.60	NC
Bioavailability (%)	61.1	NC	NC	NC

[a]NC = not calculated.

Based on this combination of *in vitro* potency and *in vivo* PK data, we next examined the ability of **15** to deplete the *Wolbachia* symbiont from filarial worms *in vivo*. In all in vivo efficacy assays, depletion of *Wolbachia* was determined through comparison to the *Wolbachia* load found in worms present in animals that were treated with only the dosing vehicle as described in the Experimental section. For our initial *in vivo* screen, groups of three CB.17 SCID mice were inoculated with 50 L3-stage *B. malayi* larvae via the peritoneal cavity and treated from point of infection with **15** for 7 or 14 days with oral doses of 25 mg/kg, BID. At point of necropsy, on day 14, fourth stage (L4) *B.malayi* larvae were isolated from the peritoneal cavity. Larvae were pooled per group and intra-worm *Wolbachia* titres were quantified from groups of 10 larvae using qPCR. Included in the same experiment were a vehicle control group and two groups of doxycycline-treated animals as positive controls. We were encouraged to find that **15** reduced the

Wolbachia load in the *B. malayi* larvae by 75.4% and 98.8% in the 7- and 14-day treatment groups, respectively. In this experiment, doxycycline reduced *Wolbachia* load by 76.3% and 96.7% following 7- and 14-day treatment at 50 mg/kg, QD, respectively (Table 8).

Table 8. Summary of Efficacy of 15 and doxycycline in reduction of *Wolbachia* in a larval *B. malayi* SCID mouse model.

Dosing Duration	7 days	14 days
doxycycline,50 mg/kg QD	76.3%	96.7%
15, 25 mg/kg, BID	75.4%	98.8%

Values represent percent (%) median reduction of Wolbachia versus the vehicle control, as measured by qPCR of Wolbachia surface protein single copy gene (wsp) per B. malayi L4 larva (n=10 per group) obtained from necropsies undertaken two weeks after first dose.

Encouraged by the profile of **15**, and in preparation for more extensive *in vivo* characterization of this molecule, we conducted a number of preliminary *in vitro* and *in vivo* studies to assess the safety of this molecule. In a non-GLP Ames assay (Bioreliance, Rockville, MD), **15** was determined to be non-mutagenic. Furthermore, **15** was found to be negative in its potential to induce micronuclei in human peripheral blood lymphocytes (Bioreliance, Rockville, MD). In a panel of over 50 mammalian receptors, enzymes and ion channels (Eurofins Cerep, France), **15** was found to be without any significant effect at 10 μM, and it was also shown to have no significant (<10%) effect on the hERG potassium channel expressed in HEK cells at a concentration of 30 μM (ChanTest, Cleveland, OH). Finally in a preliminary safety study (Anacor, Palo Alto, CA), no adverse effects were observed in BALB/c mice treated with **15** at doses up to 200 mg/kg/day for 7 days.

We next progressed **15** to chronic *in vivo* infection models of adult filarial parasitism (the target life cycle stage for an anti-*Wolbachia* indication) ; one using *B. malayi* and one using *Litomosoides sigmodontis*. We initially chose to use the *B. malayi* SCID mouse model[34] as this worm species is a causative agent of LF in humans.[2] In this *B. malayi* assay, 100 L3-stage larvae, were inoculated via the peritoneal route and adult infections were allowed to develop. At six-weeks post-infection, the mice were treated with **15** (25

mg/kg, BID) for 7, 14 or 28 days. At the end of the experiment (12 weeks post infection), worms were recovered from the mice and *Wolbachia* load was determined by qPCR. In this experiment, vehicle and minocycline (25 mg/kg, BID × 28 days) groups were included as negative and positive controls, respectively. Minocycline was chosen as the positive control for this study based on concurrent results in a *Litomosoides sigmondontis* model (*vide infra*) that suggested this tetracycline antibiotic exhibited superior efficacy to doxycycline.[35] Somewhat disappointingly, efficacy of **15** was not nearly as good in the adult model, with a 45.5% reduction of *Wolbachia* load observed for the 28 day treatment group. By comparison, minocycline provided 98.2% reduction in this experiment (Table 9). We hypothesize that the failure of **15** to show efficacy at the dose chosen in the *B. malayi* adult worm model is likely due to the increased washout period between treatment start and necropsy in comparison to the *B. malayi* L3 larval model, leading to a rebound of the *Wolbachia* with a suboptimal treatment regimen of **15**.

Table 9. Summary of Efficacy of 15 and minocycline against *Wolbachia* in the adult *B. malayi* SCID mouse model.

	Dosing Duration		
	28 days	14 days	7 days
15			
25 mg/kg, BID	45.5	16.6	13.4
minocycline			
25 mg/kg, BID	98.2		

Values represent percent (%) median reduction of Wolbachia versus the vehicle control, as measured by qPCR of Wolbachia surface protein single copy gene (wsp) per adult female B. malayi (n=10 per group) obtained from necropsies undertaken six weeks after first dose.

In a second efficacy model,[35, 36] we used mice infected with the rodent filarial nematode *Litomosoides sigmodontis*, another helminth species known to host *Wolbachia*, and treated these animals with **15** at a dose of 50 mg/kg, BID for 14 or 28 days. The *L. sigmodontis* model was chosen as an additional, confirmatory filarial model as it uses immunocompetent wild-type mice as host and initial studies demonstrated the efficacy of tetracycline against *Wolbachia*-containing filariae first in this model.[37] In addition, the infection could be maintained in mice using a natural infection protocol.[35, 38-40] Upon completion of dosing, animals were maintained for a total of 64–77 days (to maintain a consistent total time following the final dose), at which time worms were recovered from

the peritoneum and the thoracic cavity. *Wolbachia* load was determined by qPCR. Doxycycline was included as a positive control in this experiment. We were encouraged to find that **15** was able to reduce *Wolbachia* burden in *L. sigmodontis* by >99% at this dose and duration (Table 10). The stronger reduction of the *Wolbachia* load in the *L. sigmodontis* mouse model in comparison to the adult *B. malayi* mouse model is probably mostly due to the increased dose used for treatment with **15**, although *L. sigmodontis* were previously reported to have an increased susceptibility for anti-*Wolbachia* drugs compared to *B. malayi* filariae.[41]

Table 10. Efficacy of 15 and doxycycline against *Wolbachia* in the adult *L. sigmodontis* mouse model.

	Dosing Duration	
	28 days	14 days
15		
50 mg/kg, BID	99.2	99.7
Doxycycline		
40 mg/kg, BID	99.9	99.9

Values represent percent (%) reduction of Wolbachia versus the untreated control, as measured by qPCR FtsZ/actin per mouse and were obtained from necropsies performed one month after first dose.

Discussion and Conclusions

Previous work from our laboratories has demonstrated that boron-containing heterocycles can be useful in the treatment of various diseases, including infectious diseases of the developing world such as malaria,[42, 43] human African trypanosomiasis[27, 44] and tuberculosis.[45, 46] We have extended our work to look at modification of known classes of antibacterial agents as an approach to treat River Blindness via the bacterial symbiont (*Wolbachia*) present in the worms that cause this disease. Clear clinical "proof of concept" for an anti-*Wolbachia* approach has been demonstrated with the tetracycline antibiotic doxycycline.[9] The utility of doxycycline is limited, however, by the requirement that patients must be treated for 4–6 weeks in order to achieve significant reduction of *Wolbachia* in the *O. volvulus* parasite.[8-10] More recently, it has been demonstrated that combination of doxycycline (200 mg/day for 3 weeks) with albendazole (800 mg/day for 3 days) may provide a shorter treatment course.[47] While moving closer to the desired short course (7 day or fewer) treatment paradigm, this

combination therapy is still not ideal and does not address tetracycline contraindicated groups (children ≤8 and pregnant women).

Our work started by evaluating representatives of various classes of antibiotics including several clinically relevant pleuromutilins. This initial screening revealed that some activity was present in this class of compounds, and we prepared several analogs containing our benzoxaborole core. The synthesis of these boronpleuromutilins was facilitated by the ready availability of the tosylate derivative of the hydroxyacetate ester at C(14) of the pleuromutilin core. Simple nucleophilic displacement reactions of this to afford C(14)-functionalized boronpleuromutilins with nitrogen, oxygen or sulfur linker atoms proceeded in good yield. Early structure-activity relationships (SAR) revealed that direct attachment of the benzoxaborole ring to the pleuromutilin core provided compounds with good in vitro potency. In contrast, inclusion of a linker group such as present in the clinically relevant pleuromutilins between the core and benzoxaborole ring were of poor activity. Based on our previous work in the benzoxaboroles, we focused our attention on compounds linked through the 6-position, as these were both synthetically accessible and generally had been observed to have good ADME properties. Inclusion of a variety of substituents such as halogen, small alkyl or aminoalkyl at positions 3, 4, 5 and 7 of the 6-O linked benzoxaboroles gave compounds of similar in vitro activity and allowed us to explore the effect of these substituents on ADME and PK. We were particularly pleased to find that the 7-fluoro analog (**15**) exhibited a good balance of potency, physicochemical and ADME properties. In particular, we were encouraged by the modest metabolic stability in mouse microsomes (Cl_{int} = 33 μL/min/mg) and good permeability through an MDR1-MDCK monolayer (P_{app} = 14.1 × 10^{-6} cm/sec). We were somewhat concerned about the high protein binding of **15** ($f_{unbound}$ = 0.03), though this was predicted based on the calculated high lipophilicity (logD 4.23) of this compound. While other compounds, particularly those with an aminomethyl substituent on the benzoxaborole core (e.g. **20**) exhibited better metabolic stability (Cl_{int} = 6 μL/min/mg), they were compromised by very low permeability (P_{app} < 0.1 × 10^{-6} cm/sec).

When **15** was dosed to mice by the intravenous route (5 mg/kg), we were encouraged by the relatively low clearance (Cl_{int} = 505 mL/hr/kg), good exposure ($AUC_{0\text{-}24h}$ = 8.85 hr/μg/mL). Following oral administration (10 mg/kg), **15** exhibited good exposure ($AUC_{0\text{-}24h}$ = 14.0 hr/μg/mL) and bioavailability (F = 61%). Additional PK studies at higher oral doses demonstrated that exposure increased with dose, though not dose proportionally, to very high levels ($AUC_{0\text{-}24h}$ = 58.7 hr/μg/mL at 400 mg/kg). In addition to the parent compound, we also tracked the major (and active) metabolite of **15** (**15a**, IC_{50} = 193 nM) where hydroxylation at C(2) of the pleuromutilin core had occurred, as this represented about 10–15% of the parent dose at most time points. Metabolism of pleuromutilins at this position of the core is well precedented.[48, 49]

Based on these observations, **15** was selected for evaluation in several *in vivo* models that had been established for evaluation of anti-*Wolbachia* compounds in mice. In the first study, we found that **15**, when dosed orally at 25 mg/kg, BID, to SCID mice infected with larval stage *B. malayi*, efficacy (defined as >99% reduction in *Wolbachia* load in worms) was achieved following 14 days of dosing; 7 days dosing did not achieve full efficacy. In a second *B. malayi* model employing worms that had been allowed to mature to the adult stage, we were disappointed to find that **15** did not demonstrate measureable efficacy when dosed at 25 mg/kg, BID for 28 days. More encouraging data was obtained in the *L. sigmodontis* BALB/c mouse model, where **15** was found to show excellent efficacy (>99% *Wolbachia* depletion) when dosed at 50 mg/kg, BID for 14 or 28 days. Additional *in vivo* studies to address this hypothesis are ongoing and will be reported in due course.

In conclusion, exploration of a series of pleuromutilin derivatives incorporating a novel boron-containing heterocycle linked to C(14) of pleuromutilin core as anti-*Wolbachia* agents has resulted in the identification of **15** as a potential pre-clinical candidate. SAR developed in this program has demonstrated that optimal activity was obtained by direct linkage of the benzoxaborole to the pleuromutilin core via C(6) of the benzoxaborole, and that small substituents on the benzoxaborole ring system had an impact on potency and pharmacokinetic properties. Good-to-excellent exposure following oral administration of **15** to mice was achieved, and this translated into high levels of *Wolbachia* depletion in several *in vivo* models. We were encouraged by the efficacy of **15** in these models, prompting more extensive evaluation of this compound, both alone and in combination

with other known anti-*Wolbachia* drugs. The outcome of these studies will be reported in due course.

Experimental Section

General

All chemicals were purchased from commercial suppliers and used as received. 1H NMR spectra were recorded on a Bruker Avance 400 spectrometer. Chemical shifts are expressed in δ ppm referenced to an internal tetramethylsilane (δ = 0 ppm) standard. Abbreviations used in describing peak signals are br = broad, s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet. All final compounds were purified to have purity higher than 95% by reverse phase high-performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC), normal phase flash chromatography or crystallization. Preparative HPLC was accomplished using a Luna C18 250 × 30 mm, 10 μm column. The purity was assessed by reverse phase HPLC with a gradient of 5 – 95% acetonitrile in water (with or without acid modifier) and monitored by diode array ultraviolet detector at 220 and 254 nm. Low resolution mass spectra were recorded on a liquid chromatography-mass spectrometer in electrospray positive (ESI +) or negative (ESI -) modes.

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-(tosyloxy)acetate. (6).

p-Toluenesulfonyl chloride (19.1 g, 0.1 mol) in 1,2-dichloroethane (100 mL) was slowly added to a mixture of (3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-hydroxyacetate (35.4 g, 0.1 mol), triethylamine (12.0 g, 0.1 mol) and pyridine (1 mL) in 1, 2-dichloroethane (100 mL). The mixture was stirred at 10–15 °C for 20h, washed with water (3 × 100 mL), then concentrated to dryness. Purification was achieved by recrystallization from dichloromethane/petroleum ether (1:100) to afford (3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-(tosyloxy)acetate **6** as a white solid (45.0 g, yield 90.0%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.80 (d, J = 8.0 Hz, 1H), 7.47 (d, J = 8.0 Hz,

1H), 6.05 (dd, *J* = 17.8, 11.2 Hz, 1H), 5.53 (d, *J* = 8.4 Hz, 1H), 5.09-4.96 (m, 2 H), 4.81-4.59 (m, 2H) , 3.40 (d, *J* = 5.6 Hz, 1H) , 2.41 (s, 2H), 2.39 (br. s., 1H), 2.24-1.95 (m, 5H), 1.75-1.41 (m, 4H), 1.30 (s, 4H), 1.27-1.18 (m, 4H), 1.03 (s, 3H), 0.99-0.92 (m, 2H), 0.81 (d, *J* = 7.2 Hz, 3H), 0.50 (d, *J* = 7.2 Hz, 3H).

Preparation of (3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-iodoacetate (7).

To a solution of **6** (51.5 g, 96.7 mmol, 1.0 eq.) in acetonitrile (600.0 mL) was added sodium iodide (87.0 g, 580.1 mmol, 6.0 eq.). The mixture was stirred at 90 °C for 16 hours at which time HPLC indicated the reaction was completed. The reaction mixture was concentrated under reduced pressure to remove acetonitrile. The residue was diluted with H_2O (500 mL) and extracted with dichloromethane (3 × 500 mL). The combined organic layers were concentrated under reduced pressure to give a residue. The residue was washed with petroleum ether (200 mL). (3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-iodoacetate **7** (40.0 g, 81.9 mmol, 84.7% yield) was obtained as a yellow solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 6.11 (dd, *J* = 11.2, 17.9 Hz, 1H), 5.52 (d, *J* = 7.9 Hz, 1H), 5.13-5.01 (m, 2H), 4.55 (d, *J* = 5.7 Hz, 1H), 3.82-3.75 (m, 1H), 3.73-3.66 (m, 1H), 3.43 (t, *J* = 5.3 Hz, 1H), 2.45-2.39 (m, 1H), 2.25-2.00 (m, 4H), 1.72-1.57 (m, 2H), 1.53-1.21 (m, 9H), 1.11-0.97 (m, 4H), 0.83 (d, *J* = 6.6 Hz, 3H), 0.64 (d, *J* = 7.1 Hz, 3H).

Method A. Use of Pleuromutilin tosylate 6. Preparation of (3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (8)

A solution of **6** (1.8 g, 3.3 mmol), benzo[c][1,2] oxaborole-1,6(3H)-diol (0.5 g, 3.3 mmol) and K_2CO_3 (0.7 g, 5.0 mmol) in 20 mL of DMF was heated to 50 ˚C overnight, at which time LC/MS indicated the reaction was completed. Water was added and the mixture was adjust pH<4 with 2N HCl. The solid was filtered and the crude product was purified by prep HPLC to give (3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy -4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl2-((1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy) acetate **8** (1.0 g, yield 57.0 %). ^{1}H

NMR (DMSO-d_6, 400 MHz) δ 7.30 (d, J = 8.4 Hz, 1H), 7.19 (d, J = 2.0 Hz, 1H), 7.04 (dd, J = 8.4, 2.4 Hz, 1H) 6.10 (dd, J = 17.6, 11.2 Hz, 1H), 5.59 (d, J = 8.4 Hz, 1H), 5.11-4.97 (m, 2H), 4.91 (s, 2H), 4.76-4.63 (m, 2H), 3.41 (d, J = 5.6 Hz, 1H), 2.40 (br. s., 1H), 2.26-1.99 (m, 4H), 1.72-1.43 (m, 4H) , 1.38 (d, J = 13.2 Hz, 1H) , 1.34 (s, 3H), 1.29-1.15 (m, 4H), 1.03 (s, 3H), 1.01 (br. s., 1H), 0.81 (d, J = 6.8 Hz, 3H), 0.63 (d, J = 6.8 Hz, 3H).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((1-hydroxy-3-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (9).

Prepared from **6** by Method A. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.00 (s, 1H), 7.28 (d, J = 9.2 Hz, 1H), 7.15 (d, J = 2.4 Hz, 1H), 7.02 (d, J = 8.0 Hz, 1H), 6.09 (dd, J = 17.6, 11.4 Hz, 1H), 5.59 (d, J = 8.0 Hz, 1H), 5.15-4.98 (m, 3H), 4.72-4.62 (m, 2H), 4.51 (d, J = 6.0 Hz, 1H), 3.40 (m, 1H), 2.40 (s, 1H), 2.26-1.98 (m, 4H), 1.67-1.26 (m, 14H), 1.07-1.01 (m, 3H), 0.81 (d, J = 6.8 Hz, 3H), 0.63 (d, J = 6.8 Hz, 3H). MS (ESI): mass calcd. for $C_{30}H_{41}BO_7$ 524.3, m/z found 523.2 $[M-1]^-$. HPLC: 93.3% (220 nm), 100% (254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((1-hydroxy-4-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (10).

Prepared from **6** by Method A. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.02 (d, J = 1.76 Hz, 1H), 6.83 (d, J = 1.8 Hz, 1H), 6.10 (dd, J = 17.6, 11.2 Hz, 1H), 5.60 (d, J = 8.4 Hz, 1H), 5.12-4.96 (m, 2H), 4.87 (s, 2H), 4.75-4.60 (m, 2H), 2.41 (br. s., 1H), 2.17 (s, 3H), 2.00-2.13 (m, 5H), 1.72-1.55 (m, 2H), 1.54-1.39 (m, 2H), 1.35 (s, 3H), 1.32-1.20 (m, 3H), 1.04 (m, 4H), 0.82 (d, J = 6.4 Hz, 3H), 0.64 (d, J = 7.2 Hz, 3H). MS: 523 $(M-1)^-$

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((1-hydroxy-5-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (11)

Prepared from **6** by Method A. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.93 (br. s., 1 H), 7.19 (s, 1 H), 7.09 (s, 1 H), 6.08 (dd, J = 17.6, 11.2 Hz, 1 H), 5.59 (d, J = 8.0 Hz, 1H), 5.12-4.95 (m, 2H), 4.87 (s, 2H), 4.71 (s, 2H), 3.40 (d, J = 4.8 Hz, 1H), 2.40 (s, 1H), 2.25 (s, 3H), 2.20-1.96 (m, 3H), 1.74-1.55 (m, 2H), 1.50-1.45 (m, 1H), 1.37 (s, 3H), 1.45-1.17

(m, 6H), 1.10-0.94 (m, 4H), 0.82 (d, J = 6.4 Hz, 3H), 0.62 (d, J = 6.4 Hz, 3H). MS: 523 $(M-1)^-$

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (12).

Prepared from **6** by Method A. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.09 (d, J = 8.0 Hz, 1H), 6.95 (d, J = 8.0 Hz, 1H), 6.11 (dd, J = 17.6, 11.2 Hz, 1H), 5.61 (d, J = 8.0 Hz, 1H), 5.09-4.98 (m, 2H), 4.87 (s, 2H), 4.72 (d, J = 2.0 Hz, 2H), 3.41 (d, J =5.6 Hz, 1H), 2.36 (s, 1H), 2.33 (s, 3H), 2.20-2.10 (m, 1H), 2.10-1.95 (m, 4H), 1.72-1.55 (m, 2H), 1.54-1.36 (m, 2H), 1.34 (s, 3H), 1.31-1.15 (m, 3H), 1.03 (s, 4H), 0.82 (d, J = 6.8 Hz, 3H), 0.63 (d, J = 6.8 Hz, 3H). MS: 523 $(M-1)^-$.

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((4-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (13).

Prepared from **6** by Method A. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.29-9.24 (br. s.,1H), 7.08 (s, 1H), 6.93 (d, J = 11.2 Hz, 1H), 6.15-6.05 (m, 1H), 5.60-5.56 (d, J = 8.8 Hz, 1H), 5.05-4.99 (m, 4H), 4.77-4.75 (d, J = 8.0 Hz, 2H), 2.41 (s, 1H), 2.41-2.04 (m, 4H), 1.63-1.41 (m, 4H), 1.33-1.28 (m, 7H), 1.04-0.85 (m, 4H), 0.81 (d, J = 6.4 Hz, 3H), 0.63 (d, J = 6.4 Hz, 3H). MS (ESI): mass calcd. for $C_{29}H_{38}BFO_7$ 528.27, m/z found 527.2 $[M-1]^-$. HPLC: 96.0% (220 nm), 69.6% (254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((5-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (14)

Prepared from **6** by Method A. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.10 (s, 1H), 7.35-7.26 (m, 2H), 6.08 (dd, J = 18.0, 11.2 Hz, 1H), 5.58 (d, J = 7.6 Hz, 1H), 5.09-4.96 (m, 2H), 4.90 (s, 2H), 4.85-4.74 (m, 2H), 4.52 (d, J = 6.0 Hz, 1H), 3.43-3.38 (m, 1H), 2.41 (s, 1H), 2.25-1.96 (m, 6H), 1.73-1.20 (m, 9H), 1.03 (s, 3H), 0.81 (d, J = 6.8 Hz, 3H), 0.61 (d, J = 6.8 Hz, 3H). MS: 527 $(M-1)^-$.

Method B. Use of Pleuromutilin tosylate 7. Preparation of (3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (15)

To a solution of 7-fluorobenzo[c][1,2]oxaborole-1,6(3H)-diol **80** (5.0 g, 29.77 mmol, 1.0 eq) and **7** (18.9 g, 38.71 mmol, 1.3 eq) in DMSO (60.00 mL) was added Na_2CO_3 (9.5 g, 89.32 mmol, 3.0 eq). The mixture was stirred at 35 °C for 14 hours under a nitrogen atmosphere, after which time HPLC indicated that starting material was consumed completely. The reaction mixture was quenched by addition H_2O (200 mL) at 0°C, and then was adjusted to pH = 7 and then filtered to give crude product. Combined four batches together, and the crude product was purified by prep HPLC, then removed the acetonitrile, resulting aqueous phase was extracted by dichloromethane (3 × 1500 mL). The combined organic layers were concentrated under reduced pressure to give the product as a light yellow solid. This product was dissolved in dichloromethane, then MTBE and petroleum ether was added until the product precipitated. The suspension was filtered and the filtrate was concentrated under reduced pressure to give additional product as a white solid. (3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate **15** (15.0 g, 28.2 mmol, 24% yield, 99.0% purity) was obtained as a white solid. ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 9.26 (br. s., 1H), 7.25-7.17 (m, 1H), 7.09 (d, *J* = 7.9 Hz, 1H), 6.11 (dd, *J* = 11.2, 17.9 Hz, 1H), 5.60 (d, *J* = 7.9 Hz, 1H), 5.10-4.98 (m, 2H), 4.92 (s, 2H), 4.86-4.74 (m, 2H), 4.52 (br. s., 1H), 3.41 (br. s., 1H), 2.41 (br. s., 1H), 2.25-2.01 (m, 4H), 1.70-1.57 (m, 2H), 1.48-1.20 (m,8H), 1.15-0.91 (m, 4H), 0.82 (d, *J* = 6.6 Hz, 3H), 0.62 (d, *J* = 7.1 Hz, 3H). MS (ESI): mass calcd. for C29H38BFO7 528.27, m/z found 527.3[M-H]-. HPLC: 99.0% (220 nm), 100.0% (254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((7-chloro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (16).

Prepared from **6** by Method A. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.06 (s, 1H), 7.18 (d, J = 8 Hz, 1H), 7.07 (d, J = 8.4 Hz, 1H), 6.04 (dd, J = 17.6, 11.2 Hz, 1H), 5.53 (d, J = 7.2 Hz, 1H), 5.02 (m, 2H), 4.84 (s, 2H), 4.79 (s, 2H), 4.49 (d, J = 6.4 Hz, 1H), 3.35 (m, 1H), 2.35 (s, 1H), 2.11-1.96 (m, 4H), 1.61-1.17 (m, 10 H), 0.98-0.93 (m, 4 H), 0.74 (d, J = 6.8 Hz, 3H), 0.58 (d, J = 6.8 Hz, 3H). MS (ESI): mass calcd. For $C_{29}H_{38}BO_7Cl$ 544.87, m/z found 543.2 $[M-H]^-$. HPLC: 100% (220 nm), 100% (254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((1-hydroxy-7-methoxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (17).

Prepared from **6** by Method A. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.20 - 8.94 (m, 1H), 7.03 (d, J = 8.4 Hz, 1H), 6.91 (d, J = 8.4 Hz, 1H), 6.12 (dd, J = 11.5, 17.6 Hz, 1H), 5.61 (d, J = 8.4 Hz, 1H), 5.10-5.00 (m, 2H), 4.89 (s, 2H), 4.74-4.60 (m, 2H), 3.93 (s, 3H), 3.42 (d, J = 6.2 Hz, 1H), 2.41 (br. s., 1H), 2.24-1.99 (m, 4H), 1.71-1.20 (m, 11H), 1.12-0.94 (m, 4H), 0.82 (d, J = 7.1 Hz, 3H), 0.63 (d, J = 6.6 Hz, 3H). MS (ESI): mass calcd. for $C_{30}H_{41}BO_8$ 540.3, m/z found 539.3 $[M-1]^-$. HPLC: 100% (220 nm), 100% (254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((5,7-difluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (18).

Prepared from **6** by Method A. ^{1}H NMR (DMSO-d_6, 400 MHz) δ7.16 (d, J = 10.1 Hz, 1H), 6.05 (dd, J = 11.5, 17.6 Hz, 1H), 5.57 (d, J = 7.9 Hz, 1H), 4.97 (d, J = 6.6 Hz, 1H), 4.93 (s, 1H), 4.89 (s, 2H), 4.71 (d, J = 19.0 Hz, 2H), 3.36 (d, J = 6.2 Hz, 1H), 2.34 (br. s., 1H), 2.20-2.09 (m, 1H), 2.08-1.94 (m, 3H), 1.67-1.51 (m, 3H), 1.41 (d, J = 17.2 Hz, 1H), 1.36-1.14 (m, 6H), 1.03-0.90 (m, 4H), 0.78 (d, J = 7.1 Hz, 3H), 0.53 (d, J = 6.6 Hz, 3H). MS (ESI): mass calcd. For $C_{29}H_{37}BF_2NO_7$ 546.3, m/z found 545.2$[M-H]^-$. HPLC: 97.3% (220 nm), 94.0% (254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((3-(aminomethyl)-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate hydrochloride (19).

Prepared from **7** by Method B. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.47 (br. s., 1H), 8.13 (br. s., 3H), 7.44 (d, *J* = 8.4 Hz, 1H), 7.28 (d, *J* = 2.2 Hz, 1H), 7.09 (td, *J* = 2.4, 8.5 Hz, 1H), 6.10 (dd, *J* = 11.2, 17.9 Hz, 1H), 5.60 (d, *J* = 8.4 Hz, 1H), 5.28 (dd, *J* = 2.6, 9.3 Hz, 1H), 5.12-4.96 (m, 2H), 4.80-4.64 (m, 2H), 4.62-4.48 (m, 1H), 3.42 (d, *J* = 5.7 Hz, 2H), 2.78-2.63 (m, 1H), 2.42 (br. s., 1H), 2.26-2.00 (m, 4H), 1.73-1.18 (m, 7H), 1.05 (s, 2H), 0.89-0.78 (m, 5H), 0.63 (d, *J* = 7.2 Hz, 3H). MS (ESI): mass calcd. for $C_{30}H_{42}BNO_7$ 539.31, m/z found 540.1[M+H]$^+$. HPLC: 99.2% (220 nm), 100.0% (254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((4-(aminomethyl)-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate hydrochloride (20).

Prepared from **7** by Method B. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.19 (s, 1H), 8.30 (br. s., 3H), 7.26-7.17 (m, 2H), 6.11 (dd, *J* = 11.2, 17.9 Hz, 1H), 5.62 (d, *J* = 8.8 Hz, 1H), 5.15-4.95 (m, 4H), 4.79-4.65 (m, 2H), 4.55 (br. s.,1H), 3.93 (d, *J* = 5.3 Hz, 2H), 3.43 (br. s., 2H), 2.44 (d, *J* = 7.9 Hz, 1H), 2.26-2.01 (m, 4H), 1.73-1.19 (m, 10H), 1.12-0.94 (m, 4H), 0.82 (d, *J* = 7.1 Hz, 3H), 0.65 (d, *J* = 7.1 Hz, 3H). MS (ESI): mass calcd. for $C_{30}H_{43}BClNO_7$ 575.3, m/z found 540.4 [M+H]$^+$. HPLC: 96.1% (220 nm), 91.0% (weak absorption at 254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((3-(aminomethyl)-7-chloro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate hydrochloride (21).

Prepared from **7** by Method B. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.31 (s, 1H), 8.13 (s, 3H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.18 (t, *J* = 8.4 Hz, 1H), 6.15-6.08 (m, 1H), 5.60 (d, *J*=7.2 Hz, 1H), 5.29 (d, *J* = 8.0 Hz, 1H), 5.14-4.57 (m, 5H), 3.47-3.38 (m, 3H), 2.87 (b, 1H), 2.42 (s, 1H), 2.23-2.02 (m, 4H), 1.67-1.05 (m, 14H), 0.82 (d, *J* = 6.8 Hz, 3H), 0.63 (d, *J* = 6.8 Hz, 3H). HPLC purity: 100% (220 nm); MS (ESI): mass calcd. for $C_{30}H_{41}BClNO_7$ 573.27, m/z found 574.2 [M+H]$^+$.

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((3-(aminomethyl)-7-

fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate hydrochloride (22).

Prepared from 7 by Method B. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.51 (brs., 1H), 8.14 (brs, 3H), 7.27-7.22 (m, 2H), 6.11 (dd, J = 10.8, 18.0 Hz, 1H), 5.60 (d, J = 7.6 Hz, 1H), 5.30 (d, J = 8.8 Hz, 1H), 5.06 (d, J = 18.0 Hz, 1H), 5.03 (d, J = 10.8 Hz, 1H), 4.86-4.82 (m, 2H), 2.84-2.82 (m, 2H), 2.41 (s, 1H), 2.18-2.05 (m, 4H), 1.64-1.20 (m, 9H), 1.09-0.94 (m, 4H), 0.83-0.79 (d, J = 6.8 Hz, 3H), 0.63 (d, J = 6.8 Hz, 3H). MS (ESI): mass calcd. for $C_{30}H_{42}BClFNO_7$ 593.3, m/z found 558.0$(M+H)^+$. HPLC: 99.0% (220 nm), 96.3% (254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((4-(aminomethyl)-7-chloro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (23).

Prepared from 7 by Method B. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.04 (s, 1H), 7.17 (s, 1H), 6.16-6.13 (m, 1H), 5.61 (d, J=8.0 Hz, 1H), 5.11-5.01 (m, 2H), 4.97 (s, 2H), 4.822 (d, J= 4.0 Hz, 2H), 4.54 (d, J = 6.0 Hz, 1H), 4.46-4.44 (m, 2H), 3.62 (s, 2H), 2.43 (s, 1H), 2.23-2.04 (m, 4H), 1.68-0.97 (m, 14H), 0.81 (d, J = 7.2 Hz, 3H), 0.66 (d, J = 6.8 Hz, 3H). HPLC purity: 100% (214 nm); MS (ESI): mass calcd. For $C_{30}H_{41}BClNO_7$ 573.27, m/z found 573.8 $[M+H]^+$.

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((1-hydroxy-3,3-dimethyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (24).

Prepared from 7 by Method B. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.92 (s, 1 H), 7.29 (d, J = 8.4 Hz, 1H), 7.11 (d, J = 2.4 Hz, 1H), 7.00 (dd, J = 8.4, 2.4 Hz, 1H), 6.09 (dd, J = 17.6, 11.2 Hz, 1H), 5.59 (d, J = 8.4 Hz, 1H), 5.08-4.95 (m, 2H), 4.75-4.61 (m, 2H), 4.51 (d, J = 6.0 Hz, 1H), 3.45-3.38 (m, 1H), 2.40 (s, 1H), 2.25-1.98 (m, 5H), 1.73-1.55 (m, 3H), 1.52-1.15 (m, 12H), 1.08-0.98 (m, 4H), 0.81 (d, J = 7.0 Hz, 3H), 0.62 (d, J = 6.8 Hz, 3H). MS (ESI): mass calcd. for $C_{31}H_{43}BO7_3$ 538.3, m/z found 537.2 $[M-1]^-$. HPLC: 99.3% (220 nm), 100% (254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl (1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)glycinate (25).

Prepared from 7 by Method B. ^{1}H NMR (DMSO-d_6, 400MHz) δ 7.09 (d, J = 8.4 Hz, 1H), 6.83 (br. s., 1H), 6.72 (d, J = 8.4 Hz, 1H), 6.12-5.96 (m, 1H), 5.53 (d, J = 8.0 Hz, 1H), 5.07-4.90 (m, 2H), 4.87-4.77 (m, 1H), 3.78 (d, J = 5.2 Hz, 2H), 3.37 (d, J = 5.2 Hz, 1H), 2.33 (br. s., 1H), 2.22-2.12 (m, 1H), 2.10-1.91 (m, 3H), 1.70-1.52 (m, 3H), 1.43 (br. s., 2H), 1.37-1.15 (m, 8H), 1.06-0.89 (m, 5H), 0.79 (d, J = 6.4 Hz, 3H), 0.61 (d, J = 6.4 Hz, 3H). MS (ESI): mass calcd. for $C_{29}H_{41}BClNO_6$ 545.27, m/z found 508.3[M-H]-. HPLC: 100.0% (220 nm), 100.0% (254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl (3-(aminomethyl)-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)glycinate (26).

Prepared from 7 and 6-amino-1-hydroxy-3-nitromethyl-1,3-dihydrobenzo[c][1,2]oxaborole by Method B and reduction of the nitromethyl intermediate by hydrogenation. ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.12 (br. s., 2H), 7.20 (d, J = 7.9 Hz, 1H), 6.91 (br. s., 1H), 6.73 (dd, J = 1.6, 8.4 Hz, 1H), 6.08 (dd, J = 11.2, 17.6 Hz, 1H), 5.55 (d, J = 8.0 Hz, 1H), 5.19 (d, J = 7.6 Hz, 1H), 5.04 (d, J = 19.2 Hz, 1H), 4.95 (d, J = 11.2 Hz, 1H), 3.80 (d, J = 13.2 Hz, 2H), 3.41 (d, J = 5.6 Hz, 2H), 2.39 (br. s., 1H), 2.22-2.14 (m, 1H), 2.12-1.98 (m, 3H), 1.69-1.57 (m, 2H), 1.54-1.39 (m, 2H), 1.38-1.32 (m, 5H), 1.31-1.18 (m, 3H), 1.08-0.94 (m, 4H), 0.81 (d, J = 6.4 Hz, 3H), 0.64 (d, J = 6.4 Hz, 3H). MS (ESI): mass calcd. For $C_{30}H_{44}BClN_2O_6$ 574.9, m/z found 539.5$[M+H]^+$. HPLC: 98.9% (220 nm), 100.0% (254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl (4-(aminomethyl)-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)glycinate (27).

Prepared from 7 and 6-amino-4-(t-butyloxycarbonyl)aminomethyl-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole by Method B and deprotection of the (t-butyloxycarbonyl)aminomethyl intermediate with trifluoracetic acid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.84 (s, 1H), 6.71(m, 2H), 6.09(m, 1H), 5.92(m, 1H), 5.54(m, 1H),

4.89-5.07(m, 4H), 4..52(d, 1H), 3.78(m, 2H), 3.58(s, 4H), 3.41(m, 1H), 2.41(m, 1H), 2.02-2.09 (m, 5H), 1.34-1.67 (m, 7H), 1.24-1.28 (m, 4H), 1.01 (s, 4H). HPLC purity: 98.4% (214 nm), 100% (254 nm); MS (ESI): mass calcd. for $C_{30}H_{43}BN_2O_6$ 538.32, m/z found 539.2 $[M+H]^+$.

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl (5-(aminomethyl)-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)glycinate (28).

Prepared from **7** and 6-amino-5-(t-butyloxycarbonyl)aminomethyl-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole by Method B and deprotection of the (t-butyloxycarbonyl)aminomethyl intermediate with trifluoracetic acid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.98 (s, 1H), 7.22 (s, 1H), 6.82 (s, 1H), 6.00-6.08 (dd, 1H), 5.56 (s, 1H), 5.53-5.57 (d, 1H), 4.88-5.02 (m, 3H), 4.43-4.54 (m, 1H), 3.85-4.04 (m, 2H), 3.04 (s, 1H), 2.07-2.50 (m, 7H), 1.31-1.66 (m, 13H), 1.05-1.24 (m, 4H), 0.50-0.60 (m, 3H), 0.22-0.37 (m, 3H). HPLC purity: 100% (214 nm), 100% (254 nm); MS (ESI): mass calcd. for $C_{30}H_{43}BN_2O_6$ 538.32, m/z found 539.3 $[M+H]^+$.

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl (7-(aminomethyl)-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)glycinate (29).

Prepared from **7** and 6-amino-7-(t-butyloxycarbonyl)aminomethyl-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole by Method B and deprotection of the (t-butyloxycarbonyl)aminomethyl intermediate with trifluoracetic acid. ^{1}H NMR (500 MHz, added one drop of con. HCl, DMSO-d_6) δ 8.2 (s, 3H), 7.19 (d, J = 8.0 Hz, 1H), 6.70 (d, J = 8.5 Hz, 1H), 6.08 (m, 1H), 5.55 (d, J = 8.5 Hz, 2H), 4.89(s, 4H), 4.21 (m, 2H), 3.91 (m, 2H), 3.41 (m, 1H), 2.41 (s, 1H), 2.00-2.22 (m, 4H), 1.58-1.66 (m, 2H), 1.48 (m, 1H), 1.24-1.39 (m, 7H), 0.97 -1.03 (m, 4H), 0.80 (d, J =7.5 Hz, 3H), 0.64 (d, J = 7.0 Hz, 3H). HPLC purity: 100% (214 nm), 100% (254 nm); MS (ESI): mass calcd. for $C_{30}H_{43}BN_2O_6$ 538.32, m/z found 539.4 $[M+H]^+$.

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl (1-hydroxy-3,3-dimethyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)glycinate (30).

Prepared from **7** by Method B. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.72 (s, 1H), 7.06 (d, *J* = 8.4 Hz, 1H), 6.75-6.65 (m, 2H), 6.11-6.03 (m, 2H), 5.55 (d, *J* = 8.4 Hz, 1H), 5.04-4.93 (m, 2H), 4.50 (d, *J* = 6 Hz, 1H), 3.83-3.75 (m, 2H), 3.40 (t, *J* = 7.8 Hz, 1H), 2.39 (s, 1H), 1.48-1.21 (m, 21H), 0.80 (d, *J* = 6.8 Hz, 3H), 0.63 (d, *J* = 8.8 Hz, 3H). MS (ESI): mass calcd. For $C_{31}H_{44}BNO_6$ 537.33, m/z found 538.4 $[M+H]^+$. HPLC: 98.8% (220 nm), 99.5% (254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl (5-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)glycinate (31).

Prepared from **7** by Method B. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.88 (s, 1H), 7.11-7.09 (d, *J* = 8.0 Hz, 1H), 6.88-6.86 (d, *J* = 8.0 Hz, 1H), 6.09-6.04 (m, 1H), 5.75 (s, 1H), 5.54-5.42(d, *J* = 8.0 Hz, 1H), 5.05-5.01 (m, 1H), 4.95-4.93 (m, 1H), 4.83 (s, 2H), 3.86-3.83 (m, 2H), 2.37-2.17 (m, 1H), 2.14-2.08 (m, 2H), 2.06-2.01 (m, 3H),1.66-1.62 (m, 3H),1.59-1.32 (m, 5H), 1.24-1.21 (m, 4H), 1.00 (s, 3H), 0.81-0.79 (d, *J* = 8.0 Hz, 3H), 0.62-0.61 (d, *J* = 4.0 Hz, 3H).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl (7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)glycinate (32).

Prepared from **7** by Method B. ^{1}H NMR **(**DMSO-d_6, 400 MHz**)** δ 9.10 (s, 1H), 6.94 (d, *J* = 7.9 Hz, 1H), 6.73 (t, *J* = 8.2 Hz, 1H), 6.09 (dd, *J* = 11.0, 17.6 Hz, 1H), 5.63 (br. s., 1H), 5.55 (d, *J* = 7.9 Hz, 1H), 5.08-4.96 (m, 2H), 4.86 (s, 2H), 4.49 (d, *J* = 6.2 Hz, 1H), 3.91-3.81 (m, 2H), 3.38 (s, 1H), 2.38 (br. s., 1H), 2.23-2.12 (m, 1H), 2.11-1.97 (m, 3H), 1.69-1.56 (m, 2H), 1.52-1.42 (m, 2H), 1.32 (s, 3H), 1.29-1.16 (m, 3H), 1.05-0.96 (m, 3H), 0.80 (d, *J* = 7.1 Hz, 3H), 0.63 (d, *J* = 6.6 Hz, 3H). MS (ESI): mass calcd. For $C_{29}H_{39}BFNO_6$ 527.4, m/z found 526.3$[M-H]^-$. HPLC: 96.7% (220 nm), 100.00% (254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)thio)acetate (33).

Prepared from **7** by Method B. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.18 (s, 1H), 7.72 (s, 1H), 7.49 (dd, J = 1.2, 8.0 Hz, 1H), 7.35 (d, J = 8.0 Hz, 1H), 6.03-5.94 (m, 1H), 5.47 (d, J = 8.8 Hz, 1H), 4.94 (s, 2H), 4.90 (d, J = 4.4 Hz, 1H), 4.47 (d, J = 6.0 Hz, 1H), 3.79 (q, J = 16.0 Hz, 2H), 3.37 (s, 1H), 2.37-2.31 (m, 1H), 2.22-1.87 (m, 4H), 1.68-1.55 (m, 2H), 1.50-1.14 (m, 7H), 1.08-0.93 (m, 6H), 0.79 (d, J = 6.4 Hz, 3H), 0.55 (d, J = 6.4 Hz, 3H). MS (ESI): mass calcd. for $C_{29}H_{39}BO_6S$ 526.26, m/z found 549.3 $[M+Na]^+$. HPLC: 96.5% (220 nm), 96.8% (254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)thio)acetate (34)

Prepared from **7** by Method B. ^{1}H NMR (DMSO-d_6, 400 MHz) □ 9.34 (s, 1H), 7.61 (t, J = 7.2 Hz, 1H), 7.22 (d, J = 8.0 Hz, 1H), 6.04-5.94 (m, 1H), 5.46 (d, J = 8.0 Hz, 1H), 5.00-4.88 (m, 4H), 4.50 (d, J = 6.0 Hz, 1H), 3.88-3.71 (m, 2H), 3.41-3.36 (m, 2H), 2.38-2.35 (m, 1H), 2.24-1.87 (m, 4H), 1.69-1.55 (m, 2H), 1.50-1.40 (m, 1H), 1.29 (s, 5H), 1.06-0.93 (m, 5H), 0.80 (d, J = 6.8 Hz, 3H), 0.55 (d, J = 6.8 Hz, 3H). MS (ESI): mass calcd. for $C_{29}H_{38}BFO_6S$ 544.25, m/z found 543.2 $[M-H]^-$. HPLC: 100% (220 nm), 100% (254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl ((1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)methyl)glycinate (35).

Prepared from **7** by Method B. ^{1}H NMR (400M Hz, DMSO-d_6) δ 0.60 (d, J = 7.2 Hz, 3 H), 0.78 (d, J = 6.8 Hz, 3 H), 0.9 7 (br. s, 1 H), 1.02 (d, 3H), 1.24 (d, 3 H), 1.25 (s, 1H), 1.4, (m, 3H), 1.61-1.9 (m, 3H), 2.02 (m, 2H), 2.07 (m, 2 H), 2.4 (s, 2 H), 3.8 (m, 2H), 4.1 (m, 2H), 4.4 (br, s, 1H), 5.0 -5.1 (m, 4H), 5.59 (d, J = 8.4 Hz, 1 H), 6.06 (dd, J =17.8, 11.2 Hz, 1 H), 7.5 (m , 2 H), 7.75 (s, 1H), 9.3 (br, s, 1 H), 9.5 (br, s, 1 H). MS (ESI): mass calcd. For $C_{30}H_{42}BNO_6$ 523.31, m/z found 524.3 $[M+H]^+$. HPLC: 99.0% (220 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl ((1-hydroxy-3,3-dimethyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)methyl)glycinate (36).

Prepared from **7** by Method B. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.96 (s, 1H), 7.57 (s, 1H), 7.38-7.32 (m, 2H), 6.24-6.17 (m, 1H), 5.62 (d, *J* = 8.0 Hz, 1H), 5.12-5.06 (m, 2H), 4.53 (d, *J* = 6.0 Hz, 1H), 3.71-3.68 (m, 2H), 3.43-3.17 (m, 3H), 2.42 (s, 2H), 2.16-2.08 (m, 4H), 1.70-1.03 (m, 21H), 0.83 (d, *J* = 6.8 Hz, 3H), 0.62 (d, *J* = 6.8 Hz, 3H). HPLC purity: 100% (214 nm), 100% (254 nm); MS (ESI): mass calcd. for $C_{32}H_{46}BNO_6$ 551.34, m/z found 552.2 $[M+H]^+$.

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-5-yl)oxy)acetate (37).

Prepared from **7** by Method B. ^{1}H NMR (400MHz, DMSO-d_6) δ 9.05 (s, 1H), 7.54 (d, *J* = 7.2 Hz, 1H), 6.82 (m, 2H), 6.03 (dd, *J* = 17.6, 11.2 Hz, 1H), 5.54 (d, *J* = 8.4 Hz, 1H), 5.00 (m, 2H), 4.81 (s, 2H), 4.70 (m, 2H), 4.51 (d, *J* = 6 Hz, 1H), 3.36 (m, 1H), 2.35 (s, 1H), 2.11-1.97 (m, 4H), 1.68-1.17 (m, 10 H), 0.92 (m, 4 H),0.74 (d, *J* = 7.2 Hz, 3H), 0.56 (d, *J* = 7.2 Hz, 3H). HPLC: 100% (220 nm), 100% (254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((6-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-5-yl)oxy)acetate (38).

Prepared from **7** by Method B. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.28 (s, 1H), 7.39 (d, *J* = 10.4 Hz, 1H), 7.02 (d, *J* = 7.2 Hz, 1H), 6.03 (dd, *J* = 18.0, 11.2 Hz, 1H), 5.53 (d, *J* = 8.0 Hz, 1H), 4.96 (m, 2H), 4.81 (d, 2H), 4.78 (s, 2H), 4.50 (d, *J* = 6 Hz, 1H), 3.36 (m, 1H), 2.35 (s, 1H), 2.08-1.96 (m, 4H), 1.60-1.19 (m, 10 H), 0.98-0.93 (m, 4 H),0.74(d, *J* = 6.8 Hz, 3H), 0.56(d, *J* = 7.2 Hz, 3H). MS (ESI): mass calcd. For $C_{29}H_{38}BO_7F$ 528.42, m/z found 527.2 $[M-H]^-$. HPLC: 100% (220 nm), 100% (254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-5-yl)oxy)acetate (39).

Prepared from **7** by Method B. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.20 (s, 1H), 6.73 (s, 1H), 6.60 (d, *J* = 10 Hz, 1H), 6.03 (dd, *J* = 18.0, 11.2 Hz, 1H), 5.54 (d, *J* = 8.4 Hz, 1H), 4.97 (m, 2H), 4.84 (s, 2H), 4.75 (m, 2H), 4.51 (d, *J* = 6 Hz, 1H), 3.36 (m, 1H), 2.35 (s,

1H), 2.13-1.96 (m, 4H), 1.61-1.17 (m, 10 H), 0.98-0.91 (m, 4 H),0.75(d, *J* = 7.2 Hz, 3H), 0.57 (d, *J* = 6.8 Hz, 3H). MS (ESI): mass calcd. For $C_{29}H_{38}BO_7F$ 528.42, m/z found 586.0 $[M+59-H]^-$. HPLC: 100% (220 nm), 100% (254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((1-hydroxy-2-(methylsulfonyl)-1,2-dihydrobenzo[d][1,2,3]diazaborinin-7-yl)oxy)acetate (40).

Prepared from **7** by Method B. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.18 (s, 1H), 8.80-8.78 (m, 1H), 8.66 (s, 1H), 7.41-7.39 (m, 1H), 6.12-6.08 (m, 1H), 5.12-5.08 (m, 1H), 5.01-4.98 (m, 2H), 4.88-4.86 (m, 2H),3.37 (s, 5H), 2.40 (s, 1H), 2.09-2.03 (m, 4H), 1.70-1.45 (m, 3H), 1.33-1.25 (m, 8H), 1.05-0.95 (m, 4H), 0.82-0.80 (m, 3H), 0.65-0.64 (m, 3H).

(3aR,4R,5R,7S,8S,9aS,12R)-8-hydroxy-4,7,12-trimethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((1-hydroxy-2-methyl-1,2-dihydrobenzo[d][1,2,3]diazaborinin-7-yl)oxy)acetate (41).

Prepared from **7** by Method B. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.31 (s, 1H), 7.90 (s, 1H), 7.76 (br. s., 1H), 7.68 (d, *J* = 8.8 Hz, 1H), 7.28 (d, *J* = 7.3 Hz, 1H), 6.10 (dd, *J* = 10.8, 17.2 Hz, 1H), 5.60 (d, *J* = 7.8 Hz, 1H), 5.14-4.93 (m, 2H), 4.88-4.71 (m, 2H), 4.54 (d, *J* = 5.8 Hz, 1H), 3.47 (s, 2H), 3.41 (br. s., 1H), 3.07 (s, 3H), 2.41 (br. s., 1H), 2.26-1.95 (m, 4H), 1.73-0.92 (m, 10H), 0.90-0.76 (m, 3H), 0.63 (d, *J* = 6.0 Hz, 3H). MS (ESI): mass calcd. for $C_{30}H_{41}BN_2O_6$ 536.3, m/z found 535.3 $(M-H)^-$. HPLC: 90.7% in 220 nm; 93.3% in 254 nm.

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((2-acetyl-1-hydroxy-1,2-dihydrobenzo[d][1,2,3]diazaborinin-7-yl)oxy)acetate (42).

Prepared from **7** by Method B. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.02 (s, 1H), 7.53 (d, *J* = 8.4 Hz, 1H), 7.07-6.94 (m, 2H), 6.16-6.03 (m, 1H), 5.56 (t, *J* = 8.0 Hz, 1H), 5.11-4.96 (m, 2H), 4.77 (t, *J* = 10.0 Hz, 2H), 4.53 (d, J = 5.8 Hz, 1H), 3.39 (d, *J* = 5.2 Hz, 1H), 2.36 (d, *J* = 1.4 Hz, 3H), 2.23-1.94 (m, 4H), 1.63 (br. s., 2H), 1.49-1.09 (m, 7H), 1.00 (d, *J* = 17.8 Hz, 4H), 0.80 (d, *J* = 3.6 Hz, 3H), 0.61 (dd, *J* = 7.0, 12.0 Hz, 3H). MS (ESI): mass

calcd. for $C_{31}H_{41}BN_2O_7$ 564.3, m/z found 581.3 $(M+H_2O-H)^-$. HPLC: 94.9% in 220 nm; 100% in 254 nm.

tert-butyl 1-hydroxy-7-(2-(((3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl)oxy)-2-oxoethoxy)benzo[d][1,2,3]diazaborinine-2(1H)-carboxylate (43).

Prepared from **7** by Method B. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.57 (s, 1H), 8.10 (s, 1H), 7.75 (d, J = 8.2 Hz, 1H), 7.47 (br. s., 1H), 7.38 (d, J = 9.2 Hz, 1H), 6.09 (dd, J = 11.0, 17.8 Hz, 1H), 5.60 (d, J = 8.6 Hz, 1H), 5.11-4.96 (m, 2H), 4.89 (d, J = 6.0 Hz, 1H), 4.53 (d, J = 6.0 Hz, 1H), 2.41 (s, 1H), 2.26-1.96 (m, 4H), 1.65-1.58 (m, 11H), 1.53-1.21 (m, 8H), 1.13-0.97 (m, 5H), 0.81 (d, J = 6.8 Hz, 3H), 0.66 (d, J = 6.8 Hz, 3H). MS (ESI): mass calcd. for $C_{34}H_{47}BN_2O_8$ 622.3, m/z found 639.4 $(M+H_2O-H)^-$. HPLC: 96.4% in 220 nm; 96.5% in 254 nm.

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((1-hydroxy-1,2-dihydrobenzo[d][1,2,3]diazaborinin-7-yl)oxy)acetate (44).

Prepared from **43** by deprotection with HCl/ethyl acetate in dichloromethane. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.90 (s, 1H), 7.96 (s, 1H), 7.75-7.64 (m, 2H), 7.31 (d, J = 8.8 Hz, 1H), 6.09 (dd, J = 11.2, 17.8 Hz, 1H), 5.60 (d, J = 8.2 Hz, 1H), 5.13-4.95 (m, 2H), 4.89-4.74 (m, 2H), 3.41 (d, J = 5.6 Hz, 1H), 2.41 (s, 1H), 2.25-1.97 (m, 4H), 1.71-1.18 (m, 10H), 1.07-0.93 (m, 4H), 0.81 (d, J = 6.8 Hz, 3H), 0.64 (d, J = 6.8 Hz, 3H). MS (ESI): mass calcd. for $C_{29}H_{39}BN_2O_6$ 522.3, m/z found 521.3 $(M-H)^-$. HPLC: 99.7% in 220 nm; 100% in 254 nm.

Methyl 1-hydroxy-7-(2-(((3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl)oxy)-2-oxoethoxy)benzo[d][1,2,3]diazaborinine-2(1H)-carboxylate (45).

Prepared from **7** by Method B. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.12 (s, 1H), 7.78 (d, J = 8.4 Hz, 1H), 7.48 (s, 1H), 7.39 (dd, J = 2.4, 8.4 Hz, 1H), 6.09 (dd, J = 11.2, 17.6 Hz, 1H), 5.60 (d, J = 7.8 Hz, 1H), 5.13-4.97 (m, 2H), 4.96-4.84 (m, 2H), 3.91 (s, 3H), 3.41 (d, J = 5.8 Hz, 1H), 2.41 (s, 1H), 2.25-1.99 (m, 4H), 1.74-1.20 (m, 10H), 1.10-0.94 (m, 4H),

0.81 (d, *J* = 6.8 Hz, 3H), 0.67 (d, *J* = 6.8 Hz, 3H). MS (ESI): mass calcd. for $C_{31}H_{41}BN_2O_8$ 580.3, m/z found 597.4 $(M+H_2O-H)^-$. HPLC: 97.2% in 220 nm; 96.1% in 254 nm.

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((1-hydroxy-1H-benzo[d][1,2,6]oxazaborinin-7-yl)oxy)acetate (46).

Prepared from **7** by Method B. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.29 (s, 1H), 8.54 (s, 1H), 7.71 (d, *J* = 8.2 Hz, 1H), 7.49 (s, 1H), 7.37 (d, *J* = 6.0 Hz, 1H), 6.13-6.04 (m, 1H), 5.60 (d, *J* = 7.8 Hz, 1H), 5.11-4.96 (m, 2H), 4.87 (d, *J* = 7.2 Hz, 1H), 4.53 (d, *J* = 6.2 Hz, 1H), 3.41 (m, 1H), 2.41 (s, 1H), 2.05 (m, 4H), 1.71-1.44 (m, 4H), 1.40-1.20 (m, 7H), 1.04 (s, 4H), 0.81 (d, *J* = 6.8 Hz, 3H), 0.65 (d, *J* = 7.0 Hz, 3H). MS (ESI): mass calcd. for $C_{29}H_{38}BNO_7$ 523.3, m/z found 522.3 $(M-H)^-$. HPLC: 97.5% in 220 nm; 94.3% in 254 nm.

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((8-fluoro-1-hydroxy-2-(methylsulfonyl)-1,2-dihydrobenzo[d][1,2,3]diazaborinin-7-yl)oxy)acetate (47).

Prepared from **7** by Method B. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.90 (s, 1H), 7.50-7.28 (m, 2H), 6.13 (m, 1H), 5.61 (s, 1H), 5.19-4.84 (m, 3H), 4.54 (s, 1H), 3.24 (s, 3H), 2.41 (s, 1H), 2.07 (m, 4H), 1.73-1.19 (m, 11H), 1.06 (m, 4H), 0.82 (d., *J* = 6.8 Hz, 3H), 0.65 (d, *J* = 6.8 Hz, 3H). MS (ESI): mass calcd. for $C_{30}H_{40}BFN_2O_8S$ 618.3, m/z found 619.2 $(M+H)^+$. HPLC: 95.2% in 220 nm; 98.0% in 254 nm.

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((8-fluoro-1-hydroxy-2-methyl-1,2-dihydrobenzo[d][1,2,3]diazaborinin-7-yl)oxy)acetate (48).

Prepared from **7** by Method B. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.93 (d, *J* = 2.4 Hz, 1H), 7.54-7.44 (m, 2H), 6.10 (dd, *J* = 11.2, 17.6 Hz, 1H), 5.60 (d, *J* = 8.4 Hz, 1H), 5.10-4.98 (m, 2H), 4.94 (d, *J* = 3.6 Hz, 1H), 3.49 (s, 3H), 3.40 (d, *J* = 6.4 Hz, 1H), 2.40 (brs, 1H), 2.24-1.97 (m, 5H), 1.72-1.19 (m, 10H), 1.04 (s, 4H), 0.81 (d, *J* = 7.2 Hz, 3H), 0.63

(d, J = 7.2 Hz, 3H). MS (ESI): mass calcd. for $C_{30}H_{40}BFN_2O_6$ 554.5, m/z found 555.3$[M+H]^+$. HPLC: 100.00% (220 nm), 100.00% (254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((2-acetyl-8-fluoro-1-hydroxy-1,2-dihydrobenzo[d][1,2,3]diazaborinin-7-yl)oxy)acetate (49).

Prepared from **7** by Method B. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.05 (s, 1H), 7.40 (d, J = 8.4 Hz, 1H), 7.17-7.12 (m, 1H), 6.15-6.07 (m, 1H), 5.61 (d, J = 7.6 Hz, 1H), 5.13-5.00 (d, J = 3.6 Hz, 2H), 4.97-4.83 (m, 2H), 4.56-4.50 (m, 1H), 2.44-2.38 (m, 2H), 2.23-2.00 (m, 7H), 1.70-1.19 (m, 11H), 1.05 (d, J = 3.2 Hz, 3H), 0.82 (d, J = 6.8 Hz, 3H), 0.65 (dd, J = 6.8, 17.6 Hz, 2H). MS (ESI): mass calcd. for $C_{31}H_{40}BFN_2O_7$ 582.5, m/z found 583.3$(M+H)^+$. HPLC: 90.0% (220 nm), 90.4% (254 nm).

Methyl 8-fluoro-1-hydroxy-7-(2-(((3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl)oxy)-2-oxoethoxy)benzo[d][1,2,3]diazaborinine-2(1H)-carboxylate (50).

Prepared from **7** by Method B. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.53 (brs, 1H), 7.86 (brs, 1H), 7.48-7.29 (m, 2H), 6.11 (dd, J = 10.8, 17.6 Hz, 1H), 5.60 (d, J = 8.0 Hz, 1H), 5.13-4.99 (m, 2H), 4.98-4.86 (m, 2H), 4.54 (d, J = 6.4 Hz, 1H), 3.84 (s, 2H), 3.44-3.39 (m, 1H), 2.41 (brs, 1H), 2.29-2.00 (m, 3H), 1.73-1.19 (m, 10H), 1.11-0.94 (m, 4H), 0.81 (d, J = 6.4 Hz, 3H), 0.70-0.60 (m, 3H). MS (ESI): mass calcd. for $C_{31}H_{40}BFN_2O_8$ 598.5, m/z found 599.3$(M+H)^+$. HPLC: 89.1% (220 nm), 100.0% (254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((8-fluoro-1-hydroxy-1H-benzo[d][1,2,6]oxazaborinin-7-yl)oxy)acetate (51).

Prepared from **7** by Method B. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.44-9.27 (m, 1H), 8.55 (brs, 1H), 7.54 (brs, 2H), 6.10 (dd, J = 11.2, 17.6 Hz, 1H), 5.59 (d, J = 8.8 Hz, 1H), 5.23-4.90 (m, 4H), 4.54 (brs, 1H), 2.40 (brs, 1H), 2.24-1.97 (m, 4H), 1.72-1.19 (m, 10H), 1.10-0.94 (m, 3H), 0.81 (d, J = 6.4 Hz, 3H), 0.64 (d, J = 7.2 Hz, 3H). MS (ESI): mass calcd. for $C_{29}H_{37}BFNO_7$ 541.4, m/z found 558.3$(M+H_2O-H)^-$. HPLC: 94.9% (220 nm), 95.8% (254 nm).

Methyl 1-hydroxy-7-(2-(((3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl)oxy)-2-oxoethoxy)-8-methylbenzo[d][1,2,3]diazaborinine-2(1H)-carboxylate (52).

Prepared from **7** by Method B. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.15 (brs, 1H), 8.02 (brs, 1H), 7.59 (brs, 1H), 7.33 (brs, 1H), 6.11 (dd, J = 17.6, 11.2 Hz, 1H), 5.61 (d, J = 8.4 Hz, 1H), 5.09-4.98 (m, 2H), 4.90 (s, 1H), 4.52 (d, J = 2.4 Hz, 2H), 3.90 (s, 3H), 3.41 (d, J = 5.6 Hz, 1H), 2.33 (s, 3H), 2.20-2.05 (m, 4H), 1.72-1.55 (m, 2H), 1.54-1.36 (m, 2H), 1.34 (s, 3H), 1.31-1.15 (m, 3H), 1.03 (m, 4H), 0.82 (d, J = 6.8 Hz, 3H), 0.65 (d, J = 6.8 Hz, 3H). MS (ESI): mass calcd. for $C_{32}H_{43}BN_2O_8$, 594.5, m/z found 595.4 $[M+H]^+$. HPLC: 93.0% (220 nm), 88.6% (254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((1-hydroxy-3,4-dihydro-1H-benzo[c][1,2]oxaborinin-7-yl)oxy)acetate (53).

Prepared from **7** by Method B. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.14 (s, 1H), 7.07 (d, J = 8.4 Hz, 1H), 6.90 (d, J = 8.4 Hz, 1H), 6.06 (dd, J = 11.2, 17.6 Hz, 1H), 5.56 (d, J = 8.0 Hz, 1H), 5.05-4.95 (m, 3H), 4.82-4.65 (m, 2H), 3.99 (t, J = 5.6 Hz, 2H), 3.42-3.37 (m, 1H), 2.74 (t, J = 5.6 Hz, 2H), 2.38-2.32 (m, 1H), 2.23-1.98 (m, 4H), 1.72-1.19 (m, 10H), 1.08-0.94 (m, 4H), 0.77 (d, J = 6.4 Hz, 3H), 0.60 (d, J = 6.4 Hz, 3H). MS (ESI): mass calcd. for $C_{30}H_{41}BO_7$ 524.29, m/z found 523.2$[M-H]^-$. HPLC: 99.9% (220 nm), 99.9% (254 nm).

(3aR,4R,5R,7R,8S,9R,9aS,12R)-7-ethyl-8-hydroxy-4,7,9,12-tetramethyl-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (54)

A suspension of **6** (500.0 mg, 938.6 μmol, 1.0 eq) and Pd/C (300.0 mg, 938.6 μmol, 1.0 eq) in THF (30.0 mL) were stirred at 25 °C for 12 hours under 40 psi hydrogen atmosphere. The mixture was filtered and the filtrate was concentrated to give (3aR,4R,5R,7R,8S,9R,9aS,12R)-7-ethyl-8-hydroxy-4,7,9,12-tetramethyl-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-(tosyloxy)acetate (500.0 mg, 902.8 μmol, 96.2% yield, 96.5% purity) as white foam. ^{1}H NMR ($CDCl_3$, 400MHz) δ 7.83 (d, J = 8.4 Hz, 2H), 7.36 (d, J = 8.4 Hz, 2H), 5.66 (d, J = 7.9 Hz, 1H), 4.51 (s, 2H),

3.45-3.37 (m, 1H), 2.46 (s, 3H), 2.41-2.14 (m, 4H), 2.09 (br. s., 1H), 1.85-1.05 (m, 15H), 0.99-0.91 (m, 6H), 0.72 (t, *J* = 7.5 Hz, 3H), 0.61 (d, *J* = 7.1 Hz, 2H)

A solution of (3aR,4R,5R,7R,8S,9R,9aS,12R)-7-ethyl-8-hydroxy-4,7,9,12-tetramethyl-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-(tosyloxy)acetate (300.0 mg, 561.1 μmol, 1.0 eq), **80** (94.22 mg, 561.06 μmol, 1.00 eq) and Na_2CO_3 (178.4 mg, 1.7 mmol, 3.0 eq) in DMSO (15.0 mL) were heated to 30-40°C for 12 hours. Water (20 mL) was added to the mixture, white solid was precipitated. The mixture was filtered to give crude product, which was purified by prep HPLC to give (3aR,4R,5R,7R,8S,9R,9aS,12R)-7-ethyl-8-hydroxy-4,7,9,12-tetramethyl-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl2-((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate **54** (95.00 mg, 179.10 μmol, 31.92% yield, 100% purity) as white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.26 (s, 1H), 7.24-7.21 (m, 1H), 7.12-7.06 (m, 1H), 5.58 (d, *J* = 8.4 Hz, 1H), 4.92-4.83 (m, 2H), 4.84-4.80 (m, 2H), 4.40 (d, *J* = 5.6 Hz, 1H), 3.34-3.32 (m, 1H), 2.37-2.18 (m., 1H), 2.24-2.00 (m, 3H), 1.80-0.93 (m, 20H), 0.88-0.77 (m, 3H), 0.66-0.56 (m, 3H). MS (ESI): mass calcd. for $C_{29}H_{40}BFO_7$ 530.3, m/z found 529.3 $[M-H]^-$. HPLC: 100% in 220 nm; 100% in 254 nm.

(3aR,4R,5R,7R,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-7-((R)-oxiran-2-yl)-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (55).

A mixture of **6** (20.0 g, 37.5 mmol, 1.0 *eq*), m-CPBA (8.9 g, 41.3 mmol, 1.1 *eq*) in dichloromethane (200.0 mL) was degassed and purged with N_2 for 3 times, and then the mixture was stirred at 25 °C for 12 hours under N_2 atmosphere. The mixture was poured into ice-water (w/w = 1/1) (100 mL). The combined organic phase was washed with aq. $NaHCO_3$ (20 mL) and brine (100 mL), dried, filtered and concentrated in vacuum. The residue was purified by silica gel chromatography (petroleum ether/Ethyl acetate=1/1) to give (3aR,4R,5R,7R,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-7-((R)-oxiran-2-yl)-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-(tosyloxy)acetate (11.0 g, 20.0 mmol, 53.4% yield) as a white solid.

A mixture of (3aR,4R,5R,7R,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-7-((R)-oxiran -2-yl)-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-(tosyloxy)acetate (2.0 g, 3.6 mmol, 1.0 *eq.*), 7-fluoro-1-hydroxy-3H-2,1-benzoxaborol-6-ol (612.9 mg, 3.6 mmol, 1.0 *eq.*), Na_2CO_3 (1.2 g, 10.9 mmol, 3.0 *eq.*) in DMSO (30.0 mL) was degassed and purged with N_2 for 3 times, and then the mixture was stirred at 30°C for 12 hours under N_2 atmosphere. The mixture was poured into ice-water (w/w = 1/1) (50 mL) and precipitating solid. The solid was collected to afford (3aR,4R,5R,7R,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-7-((R)-oxiran-2-yl)-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c] [1,2]oxaborol-6-yl)oxy)acetate **55** (1.7 g) as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.27 (s, 1H), 7.25-7.19 (m, 1H), 7.13 (d, J = 8.4 Hz, 1H), 5.6 (d, J = 8.4 Hz, 1H), 4.97 - 4.90 (m, 2H), 4.89-4.75 (m, 2H), 4.35 (d, J = 6.4 Hz, 1H), 3.48 (t, J = 6.4 Hz, 1H), 3.13-3.10 (m, 1H), 2.43-2.37 (m, 1H), 2.30-1.99 (m, 5H), 1.76-1.57 (m, 3H), 1.55-1.23 (m, 6H), 1.18-0.97 (m, 3H), 0.93-0.75 (m, 6H), 0.64 (d, J = 7.2 Hz, 3H) MS (ESI): mass calcd. for $C_{29}H_{38}BFO_8$ 544.26, m/z found 543.3 $[M-H]^-$. HPLC: 96.6% (220 nm), 100.0% (254 nm).

The following compounds are derived from (3aR,4R,5R,7R,8S,9R,9aS,12R)-7-formyl-8-hydroxy-4,7,9,12-tetramethyl-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-(tosyloxy)acetate **57**, which was prepared by reaction of (3aR,4R,5R,7R,8S,9R,9aS,12R)-7-formyl-8-hydroxy-4,7,9,12-tetramethyl-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-hydroxyacetate[28] (**56**) and p-toluenesulfonyl chloride in pyridine.

(3aR,4R,5R,7R,8S,9R,9aS,12R)-7-formyl-8-hydroxy-4,7,9,12-tetramethyl-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (58).

A solution of **57** (300.0 mg, 561.1 μmol, 1.0 eq), **80** (94.2 mg, 561.1 μmol, 1.0 eq) and Na_2CO_3 (178.41 mg, 1.68 mmol, 3.00 eq) in DMSO (10.0 mL) were heated to 30–40 °C for 12 hours. 20 mL water was added to the mixture, white solid precipitated and filtered to give crude product. The crude product was purified by prep HPLC to give (3aR,4R,5R,7R,8S,9R,9aS,12R)-7-formyl-8-hydroxy-4,7,9,12-tetramethyl-3-

oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl2-((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate **58** (81.0 mg, 152.7 μmol, 27.2% yield, 10% purity) as white solid. ^{1}H NMR (DMSO-d_6, 400MHz) δ 9.67 (s, 1H), 9.27 (s, 1H), 7.24 (t, J = 7.9 Hz, 1H), 7.11 (d, J = 8.4 Hz, 1H), 5.42 (d, J = 8.4 Hz, 1H), 4.92 (s, 2H), 4.86-4.75 (m, 3H), 3.57 (t, J = 6.6 Hz, 1H), 2.43-2.03 (m, 4H), 1.76-1.21 (m, 10H), 1.09 (s, 3H), 0.95 (d, J = 7.1 Hz, 3H), 0.89-0.80 (m, 2H), 0.62 (d, J = 7.1 Hz, 3H). MS (ESI): mass calcd. for $C_{28}H_{36}BFO_8$ 530.3, m/z found 529.3 $[M-H]^-$. HPLC: 100% in 220 nm; 100% in 254 nm.

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-7-(hydroxymethyl)-4,7,9,12-tetramethyl-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (59)

A solution of **57** (300.0 mg, 561.1 μmol, 1.0 eq) and $NaBH(OAc)_3$ (237.8 mg, 1.1 mmol, 2.0 eq) in dichloromethane (15.0 mL) were stirred at 20°C for 2 hours. 20 mL water was added to the mixture, the aqueous layer was treated with dichloromethane (10 mL x 3), the combined organic phase was treated with brine, dried and concentrated in vacuo to give crude (3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-7-(hydroxymethyl)-4,7,9,12-tetramethyl-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-(tosyloxy)acetate (300.0 mg, crude) as colorless oil.

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-7-(hydroxymethyl)-4,7,9,12-tetramethyl-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-(tosyloxy)acetate (300.0 mg, 559.0 μmol, 1.0 eq), **80** (93.9 mg, 559.0 μmol, 1.0 eq) and Na_2CO_3 (177.7 mg, 1.7 mmol, 3.0 eq) in DMSO (5.0 mL) were heated to 30-40°C for 12 hours. 20 mL water was added to the mixture; white solid was precipitated and filtered to give crude product. The crude product was purified by prep HPLC to give (3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-7-(hydroxymethyl)-4,7,9,12-tetramethyl-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl2-((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate **59** (47.0 mg, 88.3 μmol, 15.8% yield) as white solid. ^{1}H NMR (DMSO-d_6, 400MHz) δ 9.26 (s, 1H), 7.22-7.20 (m, 1H), 7.10 7.00 (m, 1H), 5.55-5.53 (m, 1H), 5.17-5.15 (m, 1H), 4.92 (s, 1H), 4.83-4.72 (m, 3H), 4.56-4.48 (m, 2H), 3.90 (d, J = 11.0 Hz, 1H), 3.41 (d, J = 6.2 Hz, 1H), 3.32 (d, J = 11.0 Hz,

1H), 2.32-2.30 (m., 2H), 2.26-1.98 (m, 3H), 1.88-1.21 (m, 10H), 1.02 (s, 3H), 0.83 (d, J = 7.1 Hz, 3H), 0.61 (d, J = 6.6 Hz, 3H). MS (ESI): mass calcd. for $C_{28}H_{38}BFO_8$ 532.3, m/z found 531.3 $[M-H]^-$. HPLC: 100% in 220 nm; 100% in 254 nm.

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-7-((hydroxyimino)methyl)-4,7,9,12-tetramethyl-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (60).

A solution of **58** (400.0 mg, 754.2 μmol, 1.0 eq) and hydroxylamine hydrochloride (62.9 mg, 905.0 μmol, 1.2 eq) in H_2O (5.0 mL) and ethanol (10.0 mL) were stirred at 15 °C for 12 hours. HPLC and LCMS showed the desired product as major component and starting material consumed. Water (30 mL) was added to the mixture, white solid precipitated, the mixture was filtered to give crude product. The crude product was purified by prep HPLC. The solvent was concentrated to about 15 mL, then lyophilized to give (3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-7-((hydroxyimino)methyl)-4,7,9,12-tetramethyl-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl2-((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate **60** (99.0 mg, 174.3 μmol, 23.1% yield, 96.0% purity) as white solid. ^{1}H NMR (DMSO-d_6, 400MHz) δ 10.41 (br. s., 1H), 9.25 (br. s., 1H), 7.51 (s, 1H), 7.23 (t, J = 7.9 Hz, 1H), 7.10 (d, J = 7.9 Hz, 1H), 5.55 (d, J = 7.9 Hz, 1H), 4.92 (s, 2H), 4.78 (s, 2H), 3.46 (d, J = 6.6 Hz, 1H), 2.41 (br. s., 1H), 2.25-2.01 (m, 3H), 1.75-0.94 (m, 16H), 0.85 (d, J = 7.1 Hz, 3H), 0.62 (d, J = 6.6 Hz, 3H). MS (ESI): mass calcd. for $C_{28}H_{37}BFNO_8$ 545.26 m/z found 546.3 $[M+H]^+$. HPLC: 96.2% in 220 nm; 100% in 254 nm.

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-7-((methoxyimino)methyl)-4,7,9,12-tetramethyl-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (61).

This compound was prepared in a manner similar to that described for **60**, using O-methyl hydroxylamine in place of hydroxylamine. ^{1}H NMR (DMSO-d_6, 400MHz,) δ 9.25 (br. s., 1H), 7.50 (s, 1H), 7.25 (t, J = 8.0 Hz, 1H), 7.10 (d, J = 8.4 Hz, 1H), 5.60 (d, J = 8.0 Hz, 1H), 4.92 (s, 2H), 4.78 (s, 2H), 3.62 (s, 3H), 3.48 (d, J = 5.6 Hz, 1H), 2.41 (br. s., 1H), 2.24-1.97 (m, 4H), 1.72-1.40 (m, 5H), 1.35-1.19 (m, 5H), 1.15-0.96 (m, 5H), 0.85

(d, J = 6.4 Hz, 3H), 0.63 (d, J = 5.6 Hz, 3H) MS (ESI): mass calcd. for $C_{29}H_{39}BFNO_8$ 559.28, m/z found 560.3 $[M+H]^+$. HPLC: 97.5% (220 nm), 100.0% (254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-7-((isopropoxyimino)methyl)-4,7,9,12-tetramethyl-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (62).

This compound was prepared in a manner similar to that described for **60**, using O-isopropyl hydroxylamine in place of hydroxylamine. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.26 (br. s., 1H), 7.47 (s, 1H), 7.23 (t, J = 8.0 Hz, 1H), 7.10 (d, J = 8.0 Hz, 1H), 5.57 (d, J = 8.0 Hz, 1H), 4.92 (s, 1H), 4.76 (s, 1H), 4.16 (dt, J = 5.6, 12.1 Hz, 1H), 3.48 (d, J = 5.2 Hz, 2H), 2.41 (br. s., 1H), 2.09 (d, J = 15.6 Hz, 4H), 1.73-1.21 (m, 12H), 1.19-0.97 (m, 10H), 0.86 (d, J = 6.4 Hz, 3H), 0.63 (d, J = 6.4 Hz, 3H). MS (ESI): mass calcd. for $C_{31}H_{43}BFNO_8$ 587.48, m/z found 586.3 $[M-H]^-$. HPLC: 98.8% (220 nm), 85.1% (254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-7-(aminomethyl)-8-hydroxy-4,7,9,12-tetramethyl-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (63).

To a solution of **60** (570.0 mg, 1.0 mmol, 1.0 *eq*) in methanol (10.0 mL) was added Ra-Ni (0.5 g) under N_2 atmosphere. The suspension was degassed and purged with H_2 for 3 times. The mixture was stirred under H_2 (50 psi) at 25 °C for 2 hours. The reaction mixture was filtered and the filter was concentrated to give a residue, which was purified by prep HPLC to give (3aR,4R,5R,7S,8S,9R,9aS,12R)-7-(aminomethyl)-8-hydroxy-4,7,9,12-tetramethyl -3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((7-fluoro-1-hydroxy-1,3-dihydro benzo[c][1,2]oxaborol-6-yl)oxy)acetate **63** (300.0 mg, 464.8 μmol, 44.2% yield, TFA) as a light yellow solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.27 (br. s., 1H), 7.55 (br. s., 2H), 7.25 (t, J = 7.6 Hz, 1H), 7.15-7.06 (m, 1H), 5.49-5.28 (m, 2H), 4.91 (br. s., 1H), 4.78 (br. s., 1H), 3.51 (br. s., 1H), 3.18 (br. s., 1H), 2.92 (br. s., 1H), 2.42 (d, J = 8.4 Hz, 2H), 2.25-1.90 (m, 4H), 1.69-1.21 (m, 10H), 1.04 (br. s., 3H), 0.84 (d, J = 5.6 Hz, 2H), 0.64 (d, J = 6.4 Hz, 3H) MS (ESI): mass calcd. for $C_{30}H_{40}BF_4NO_9$ 531.28, m/z found 532.3 $[M+H]^+$. HPLC: 97.1% (220 nm), 93.6% (254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-7-((methylamino)methyl)-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (64).

Sodium cyanoborohydride (142.2 mg, 2.3 mmol, 4.0 eq) was added to a solution of **58** (300.0 mg, 565.6 μmol, 1.0 eq) and methylamine (4.0 eq) in methanol (10.0 mL) and acetic acid (1.0 mL). The mixture was stirred at 15 °C for 12 hours. 30 mL water was added to the mixture, and the mixture was treated with dichloromethane (3 ×30 mL). The combined organic phase was concentrated to give crude product. The crude product was purified by prep HPLC. The solvent was concentrated to about 15-20 mL solution left then was lyophilized to give (3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-7-((methylamino)methyl)-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl2-((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate **64** (52.0 mg, 95.3 μmol, 16.8% yield) as white TFA salt solid. ^{1}H NMR DMSO-d_6, (400 MHz) δ 9.30 (br. s., 1H), 8.01 (br. s., 2H), 7.31 (t, J = 8.0 Hz, 1H), 7.13 (d, J = 7.8 Hz, 1H), 5.36 (d, J = 8.0 Hz, 1H), 4.93 (s, 2H), 4.87-4.71 (m, 2H), 3.47 (d, J = 5.3 Hz, 1H), 3.27 (d, J = 8.8 Hz, 1H), 3.12 (d, J = 10.5 Hz, 1H), 2.62-2.57 (m, 3H), 2.26-1.93 (m, 6H), 1.71-1.21 (m, 10H), 1.14-0.94 (m, 4H), 0.84 (d, J = 6.3 Hz, 3H), 0.64 (d, J = 7.0 Hz, 3H). MS (ESI): mass calcd. for $C_{31}H_{42}BF_4NO_9$ 545.3 m/z found 546.3 $[M+H]^+$. HPLC: 96.4% in 220 nm; 100% in 254 nm.

The following compounds were prepared in a similar manner to that described for **64** from **58** and the appropriate amine.

(3aR,4R,5R,7S,8S,9R,9aS,12R)-7-((ethylamino)methyl)-8-hydroxy-4,7,9,12-tetramethyl-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (65).

^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.28 (br. s., 1H), 7.88 (br. s., 1H), 7.73 (br. s., 1H), 7.31 (t, J = 8.2 Hz, 1H), 7.12 (d, J = 8.4 Hz, 1H), 5.42 (br. s., 1H), 5.36 (d, J = 8.4 Hz, 1H), 4.93 (s, 2H), 4.86-4.74 (m, 2H), 3.49 (d, J = 5.7 Hz, 1H), 3.33-3.22 (m, 1H), 3.14-2.94 (m, 3H), 2.42 (br. s., 1H), 2.25-1.94 (m, 4H), 1.72-0.94 (m, 18H), 0.84 (d, J = 7.1

Hz, 2H), 0.64 (d, *J* = 7.1 Hz, 3H). MS (ESI): mass calcd. for $C_{32}H_{44}BF_4NO_9$ 559.3 m/z found 560.3 $[M+H]^+$. HPLC: 98.2% in 220 nm; 100% in 254 nm.

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-((propylamino)methyl)decahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (66).

^{1}H NMR (DMSO-d_6, 400MHz,) δ 9.29 (br. s., 1H), 7.96 (br. s., 2H), 7.77 (br. s., 1H), 7.31 (t, *J* = 7.9 Hz, 1H), 7.11 (d, *J* = 7.9 Hz, 1H), 5.48 (br. s., 1H), 5.35 (d, *J* = 7.9 Hz, 1H), 4.93 (s, 2H), 4.87-4.75 (m, 2H), 3.49 (d, *J* = 5.7 Hz, 1H), 3.29 (t, *J* = 10.1 Hz, 1H), 3.17-3.04 (m, 1H), 2.97-2.82 (m, 2H), 2.41 (br. s., 1H), 2.24-1.91 (m, 4H), 1.75-0.95 (m, 17H), 0.90-0.79 (m, 4H), 0.65 (d, *J* = 7.1 Hz, 3H). MS (ESI): mass calcd. for $C_{33}H_{46}BF_4NO_9$ 573.3 m/z found 574.3 $[M+H]^+$. HPLC: 98.9% in 220 nm; 100% in 254 nm.

(3aR,4R,5R,7S,8S,9R,9aS,12R)-7-((butylamino)methyl)-8-hydroxy-4,7,9,12-tetramethyl-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (67).

^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.28 (br. s., 1H), 7.97 (br. s., 1H), 7.76 (br. s., 1H), 7.31 (t, *J* = 8.2 Hz, 1H), 7.11 (d, *J* = 7.9 Hz, 1H), 5.35 (d, *J* = 8.4 Hz, 1H), 4.92 (s, 2H), 4.86 - 4.75 (m, 2H), 3.49 (d, *J* = 6.2 Hz, 1H), 3.28 (t, *J* = 9.9 Hz, 1H), 3.18-3.06 (m, 1H), 2.93 (br. s., 2H), 2.42 (br. s., 1H), 2.25-1.91 (m, 5H), 1.73-1.20 (m, 15H), 1.17-0.96 (m, 4H), 0.90-0.79 (m, 5H), 0.65 (d, *J* = 6.6 Hz, 3H). MS (ESI): mass calcd. for $C_{34}H_{48}BF_4NO_9$ 587.3 m/z found 588.5 $[M+H]^+$. HPLC: 98.6% in 220 nm; 91.6% in 254 nm.

(3aR,4R,5R,7S,8S,9R,9aS,12R)-7-((cyclopropylamino)methyl)-8-hydroxy-4,7,9,12-tetramethyl-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (68).

^{1}H NMR (DMSO-d_6, 400MHz,) δ 9.29 (br. s., 1H), 8.29 (br. s., 1H), 7.99 (br. s., 1H), 7.32 (t, *J* = 8.2 Hz, 1H), 7.12 (d, *J* = 7.9 Hz, 1H), 5.42 (d, *J* = 8.4 Hz, 2H), 4.93 (s, 2H), 4.87-4.75 (m, 2H), 3.48 (d, *J* = 6.2 Hz, 1H), 3.39-3.22 (m, 2H), 2.73 (br. s., 1H), 2.42 (br. s., 1H), 2.25-1.91 (m, 5H), 1.74-1.22 (m, 10H), 1.13-0.92 (m, 6H), 0.89-0.68 (m, 6H),

0.65 (d, *J* = 7.1 Hz, 3H). MS (ESI): mass calcd. for $C_{33}H_{44}BF_4NO_9$ 571.3 m/z found 572.3 $[M+H]^+$. HPLC: 97.7% in 220 nm; 100% in 254 nm.

(3aR,4R,5R,7S,8S,9R,9aS,12R)-7-((dimethylamino)methyl)-8-hydroxy-4,7,9,12-tetramethyl-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (69).

^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.29 (br. s., 1H), 8.81 (br. s., 1H), 7.27 (t, *J* = 7.7 Hz, 1H), 7.12 (d, *J* = 8.4 Hz, 1H), 5.94 (br. s., 1H), 5.35 (d, *J* = 7.9 Hz, 1H), 4.93 (s, 2H), 4.83 (br. s., 2H), 3.72-3.40 (m, 4H), 3.10 (d, *J* = 13.2 Hz, 1H), 2.86-2.84 (m, 4H), 2.41 (br. s., 1H), 2.27-1.99 (m, 4H), 1.77-1.18 (m, 12H), 1.12-0.96 (m, 2H), 0.88 (d, J = 6.2 Hz, 3H), 0.65 (d, *J* = 5.7 Hz, 3H). MS (ESI): mass calcd. for $C_{32}H_{44}BF_4NO_9$ 559.3 m/z found 560.3 $[M+H]^+$. HPLC: 96.8% in 220 nm; 100% in 254 nm.

(3aR,4R,5R,7S,8S,9R,9aS,12R)-7-(acetamidomethyl)-8-hydroxy-4,7,9,12-tetramethyl-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (70).

A mixture of **63** (300.0 mg, 564.5 μmol, 1.0 *eq.*), acetic anhydride (63.4 mg, 620.9 μmol, 1.1 *eq.*), acetic acid (67.8 mg, 1.1 mmol, 2.0 *eq.*) in dichloromethane (20.0 mL) was degassed and purged with N_2 for 3 times, and then the mixture was stirred at 60 °C for 12 hours under N_2 atmosphere. LC-MS showed **63** was consumed completely and one main peak with desired MS was detected. The solvent was removed. The residue was purified by prep HPLC to give (3aR,4R,5R,7S, 8S,9R,9aS,12R)-7-(acetamidomethyl)-8-hydroxy-4,7,9,12-tetramethyl-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol- 6-yl)oxy)acetate **70** (97.0 mg, 169.1 μmol, 29.9% yield) was obtained as a white solid. ^{1}H NMR (DMSO-d_6, 400MHz) δ 7.28 (t, *J* = 8.4 Hz, 1H), 7.09 (d, *J* = 8.4 Hz, 1H), 7.00-6.94 (m, 1H), 5.51 (d, *J* = 8.4 Hz, 1H), 4.90 (s, 2H), 4.85-4.74 (m, 2H), 3.60 (dd, *J* = 6.4, 13.8 Hz, 1H), 3.43 (d, *J* = 5.6 Hz, 1H), 3.00 (dd, *J* = 4.4, 13.8 Hz, 1H), 2.36 (br. s., 1H), 2.31 (br. s., 1H), 2.23-1.99 (m, 3H), 1.94-1.82 (m, 1H), 1.81-1.73 (m, 2H), 1.69-1.55 (m, 2H), 1.48 (br. s., 1H), 1.40-1.20 (m, 6H), 1.05-0.87 (m, 4H), 0.83 (d, *J* = 6.4 Hz, 2H), 0.62 (d, *J* = 6.4 Hz, 3H) MS (ESI): mass calcd. for $C_{30}H_{41}BFNO_8$ 573.29, m/z found 574.3 $[M+H]^+$. HPLC: 100.0% (220 nm), 100.0% (254 nm).

2-tetramethyl-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)oxy)acetate (71).

To a solution of **70** (1.0 g, 1.8 mmol, 1.0 *eq.*) in ethanol (15.0 mL) was added pyridin-1-ium-1-ylboranuide (1.7 g, 17.9 mmol, 10.0 *eq.*). Subsequently, methanol/HCl (4 M, 8.0 mL, 17.9 *eq.*) was added under N_2 atmosphere at 0°C. The mixture was stirred at 25 °C for 12 hours. The mixture was adjusted to pH~6 with saturated $NaHCO_3$ and extracted with ethyl acetate (20mL x 2). After concentrated in vacuo, the residue was purified by prep HPLC to afford (3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-7-((methoxyamino)methyl)-4,7,9,12-tetramethyl-3-oxodecahydro-4,9a-propanocyclopenta[8]annulen-5-yl 2-((7-fluoro-1-hydroxy-1,3-dihydro -benzo[c][1,2]oxaborol-6-yl)oxy)acetate **71** (432.0 mg, 769.4 μmol, 43.0% yield) as a white solid. ^{1}H NMR (DMSO-d_6, 400MHz) δ 7.28 (t, J = 8.0 Hz, 1H), 7.12 (d, J = 8.0 Hz, 1H), 5.45-5.36 (m, 1H), 4.93 (s, 2H), 4.81 (d, J = 2.4 Hz, 2H), 4.31 (d, J = 5.6 Hz, 1H), 3.77-3.71 (m, 4H), 3.50-3.44 (m, 1H), 3.26 (t, J = 13.2 Hz, 1H), 2.40 (br. s., 1H), 2.23-1.93 (m, 4H), 1.77-1.23 (m, 10H), 1.19-0.97 (m, 4H), 0.87 (d, J = 6.4 Hz, 3H), 0.65 (dd, J = 3.2, 6.8 Hz, 3H). MS (ESI): mass calcd. for $C_{31}H_{42}BF_4NO_{10}$ 561.29, m/z found 562.4 $[M+H]^+$. HPLC: 95.4% (220 nm), 95.1% (254 nm).

(3aR,4R,5R,7S,9R,9aS,12R)-4,7,9,12-tetramethyl-3,8-dioxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl (1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)carbamate (74)

Trichloromethylchloroformate (215.0 mL, 1.5 mmol) and triethylamine (495.0 mL, 3.6 mmol) were added to an ice cooled solution of (3R,3aR,4R,5R,7S,9R,9aR,12R)-5-hydroxy-3-methoxy -4,7,9,12-tetramethyl-7-vinyloctahydro-4,9a-propanocyclopenta[8]annulen-8(9H)-one **72** (1.0 g, 3.0 mmol) in THF (10 mL). The mixture was stirred at room temperature for 2 h and then treated with further quantities of trichloromethylchloroformate (215.0 mL, 1.5 mmol) and triethylamine (495.0 mL, 3.6 mmol). After a further 2h, the mixture was diluted with ethyl acetate and washed with brine. The organic phase was dried, concentrated to give (3R,3aR,4R,5R,7S,9R,9aR,12R)-3-methoxy-4,7,9,12-tetramethyl-8-oxo-7-

vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl carbonochloridate **73** without further purification.(1.2 g, 100.0%).

To a solution of 6-aminobenzo[c][1,2]oxaborol-1(3H)-ol (200.0 mg, 1.4 mmol) in DCM (15 mL) were added triethylamine (137.0 mg, 1.4 mmol) and **73** (1.2 g, 3.0 mmol). The reaction mixture was stirred at room temperature overnight, at which time TLC showed the starting materials were consumed completely. The mixture was extracted with ethyl acetate, washed with brine, dried and concentrated *in vacuo* give the crude intermediate, to which was added a solution of zinc chloride in concentrated HCl. The mixture was stirred at room temperature for 0.5 h. Then the mixture was added water and extracted with ethyl acetate. The crude product was purified by prep HPLC to give (3aR,4R,5R,7S,8S,9R, 9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl (1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)carbamate **74** (61.0 mg). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.38 (s, 1H), 7.89(s, 1H), 7.43 (d, J = 7.6 Hz, 1H), 7.27 (d, J=8.0 Hz, 1H), 6.26(m, 1H), 5.58 (s, 1H), 5.13 (m, 2H), 4.89 (s, 2H), 2.38 (m,1H), 2.05 (m, 4H), 1.06-1.50 (m, 13H), 1.07 (m, 4H), 0.82 (d, 3H), 0.69 (d, 3H).

The following compounds were prepared from **73** in a similar manner:

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl (5-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)carbamate (75)

^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.25 (s, 2H), 7.99 (s, 1H), 7.32 (d, J = 10.4 Hz, 1H), 6.74 (dd, J = 10.8, 17.6 Hz, 1H), 5.64(d, J = 9.6 Hz, 1H), 5.29 (d, J = 10.8 Hz, 1H), 4.98 (m,3H), 3.40 (s, 1H), 3.12 (s, 3H), 2.88 (m, 1H), 2.34 (m, 1H), 2.04 (s, 3H), 1.90 (m, 1H), 1.72 (m, 1H), 1.67 (d, 2H), 1.43 (m, 2H), 1.25-1.07 (m, 9H), 0.87-0.75 (m, 5H). MS (ESI): mass calcd. for $C_{29}H_{39}BNO_6$ 527.2, m/z found 526.2 $[M-H]^-$. HPLC: 99.6% (220 nm), 100% (254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl ((1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)methyl)carbamate (76)

^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.70 (t, J = 5.8 Hz, 1H), 7.61 (s, 1H), 7.38-7.33 (m, 2H), 6.74 (dd, J = 10.8, 17.6 Hz, 1H), 5.53 (d, J = 9.6 Hz, 1H), 5.24 (d, J = 10.4 Hz, 1H), 4.96-4.90 (m, 3H), 4.32-4.16 (m, 2H), 3.13-3.10 (m, 3H), 2.85 (d, J = 6.4 Hz, 1H), 2.36-2.33 (m, 1H), 2.07-1.61 (m, 5H), 1.41-1.38 (m, 2H), 1.18-1.01 (m, 11H), 0.90 (d, J = 6.4 Hz, 3H), 0.78 (d, J = 6.8 Hz, 3H). MS (ESI): mass calcd. for $C_{30}H_{42}BNO_6$ 523.3, m/z found 522.2 $[M-H]^-$. HPLC: 99.0% (220 nm), 100% (254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-4,7,9,12-tetramethyl-3-oxo-7-vinyldecahydro-4,9a-propanocyclopenta[8]annulen-5-yl ((7-fluoro-1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)methyl)carbamate (77)

^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.23 (s, 1H), 7.50 (t, J = 6.0 Hz, 1H), 7.38 (t, J = 7.2 Hz, 1H), 7.14 (d, J = 8.0 Hz, 1H), 6.20 (dd, J = 11.2, 18.0 Hz, 1H), 5.41 (d, J = 8.8 Hz, 1H), 5.09-4.86 (m, 4H), 4.25-4.10 (m, 2H), 3.38 (d, J = 5.6 Hz, 1H), 2.29 (s., 1H), 2.22-1.95 (m, 4H), 1.69-1.14 (m, 10H), 1.06-0.88 (m, 4H), 0.78 (d, J = 6.6 Hz, 3H), 0.62 (d, J = 5.2 Hz, 3H). MS (ESI): mass calcd. for $C_{29}H_{39}BFNO_6$ 527.29, m/z found 526.3 $[M-H]^-$. HPLC: 100% (220 nm), 100% (254 nm).

(3aR,4R,5R,7S,8S,9R,9aS,12R)-8-hydroxy-7-((methoxyamino)methyl)-4,7,9,11,2-difluoro-3-(methoxymethoxy)benzene 80.

To a solution of 2,3-difluorophenol (300.0 g, 2.3 mol, 1.0 eq) in dichloromethane (1.5 L) was added methoxymethyl chloride (278.9 g, 3.5 mol, 1.5 eq) and DIEA (597.1 g, 4.6 mol, 2.0 eq). The mixture was stirred at 0°C for 2 hours. TLC indicated that starting material was consumed. The reaction mixture was quenched by addition of H_2O (1500 mL) at 0 °C, and then was adjusted to pH = 6. The combined organic layers were washed with saturation NH_4Cl (3 × 500 mL), dried, filtered and concentrated under reduced pressure to give a residue. 1,2-difluoro-3-(methoxymethoxy)benzene (400.0 g, 2.3 mol, 99.4% yield) was obtained as yellow oil which was used into the next step without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.03-6.93 (m, 1H), 6.89-6.78 (m, 1H), 5.28-5.20 (m, 2H), 3.58-3.50 (m, 3H).

3,4-Difluoro-2-(methoxymethoxy)benzaldehyde 81

To a solution of **80** (100.0 g, 574.3 mmol, 1.0 eq.) in THF (1.5 L) was added n-butyllithium (2.5 M, 298.6 mL, 1.3 eq.). The mixture was stirred at -78 °C for 7 hours. Then ethyl formate (85.1 g, 1150 mmol, 2.0 eq.) was added and the mixture was stirred for another 1 hour. TLC indicated that starting material was consumed completely. The reaction mixture was quenched by addition of H2O (1000 mL) at 0°C, and then was extracted with ethyl acetate (3 × 800 mL). The combined organic layers were washed with brine 1000 mL, dried, filtered and concentrated under reduced pressure to give a residue. The 3,4-difluoro-2-(methoxymethoxy)benzaldehyde **81** (492.0 g, crude) as a yellow oil was used into the next step without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.40-10.29 (m, 1H), 7.73-7.57 (m, 1H), 7.11-6.98 (m, 1H), 5.33 (s, 2H), 3.63-3.58 (m, 3H).

4-(Benzyloxy)-3-fluoro-2-(methoxymethoxy)benzaldehyde 82

To a solution of **81** (256.0 g, 1.27 mol, 1.0 eq.) in DMF (1.5 L) was added Cs_2CO_3 (618.9 g, 1.90 mol, 1.5 eq.) and benzyl alcohol (136.9 g, 1.3 mol, 1.0 eq.). The mixture was stirred at 70°C for 10 hours. TLC indicated that starting material was consumed completely. The reaction mixture was quenched by addition of H_2O (1000 mL) at 0 °C, and then diluted with ethyl acetate (1200 mL) and extracted with ethyl acetate (1200 mL x 2). The combined organic layers were washed with H_2O (2 × 1000 mL), dried, filtered and concentrated under reduced pressure to give a residue. 4-(benzyloxy)-3-fluoro-2-(methoxymethoxy)benzaldehyde **82** (400.0 g, crude) as a yellow oil, which was used into the next step without further purification.

4-(Benzyloxy)-3-fluoro-2-hydroxybenzaldehyde 83

To a solution of **82** (760.0 g, 2.6 mol, 1.0 eq.) in CH_3OH (600.0 mL) was added aq. HCl (2 M, 200.0 mL). The mixture was stirred at 40 °C for 4 hours. TLC indicated that starting material was consumed completely. The reaction was quenched by addition of H_2O (300 mL) and the mixture was concentrated under reduced pressure to remove CH_3OH. The residue was diluted with dichloromethane 500 mL and extracted with dichloromethane 1000mL (2 × 500 mL). The combined organic layers were washed with brine (3 × 500 mL), dried, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, petroleum

ether/ethyl acetate = 20:1 to 5:1). 4-(benzyloxy)-3-fluoro-2-hydroxybenzaldehyde **83** (225.0 g, 913.8 mmol, 34.9% yield) was obtained as a black-brown solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.97 (s, 1H), 10.06 (s, 1H), 7.34-7.52 (m, 6H), 6.90-6.98 (m, 1H), 5.28 (s, 2H).

3-(Benzyloxy)-2-fluoro-6-formylphenyl trifluoromethanesulfonate 84

To a solution of **83** (117.0 g, 475.2 mmol, 1.0 eq.), pyridine (75.2 g, 950.3 mmol, 2.0 eq.) and DMAP (5.8 g, 47.5 mmol, 0.1 eq.) in dichloromethane (1.5 L) was added Tf_2O (201.1 g, 712.8 mmol, 1.5 eq.) dropwise. The mixture was stirred at 0°C for 2 hours. HPLC indicated that starting material was consumed completely. The reaction mixture was quenched by addition of H_2O (1000 mL), and then extracted with dichloromethane (2 × 1000 mL). The combined organic layers were washed with brine (500 mL), dried, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 20:1 to 2:1). 3-(benzyloxy)-2-fluoro-6-formylphenyl trifluoromethanesulfonate **84** (150.0 g, 396.5 mmol, 83.5% yield) was obtained as a yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.08 (s, 1H), 7.71 (d, J = 7.8 Hz, 1H), 7.49-7.36 (m, 5H), 7.19-7.13 (m, 1H), 5.28 (s, 2H).

4-(Benzyloxy)-3-fluoro-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde 85

To a solution of **84** (52.0 g, 137.5 mmol, 1.00 eq.), potassium acetate (40.5 g, 412.4 mmol, 3.0 eq.) and dipinacol diborane (104.7 g, 412.4 mmol, 3.0 eq.) in dioxane (1.0 L) was added $Pd(dppf)Cl_2$ (2.0 g, 2.75 mmol, 0.02 eq.). The mixture was stirred at 70°C for 16 hours. HPLC indicated that starting material was consumed completely. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 50:1 to 5:1). 4-(benzyloxy)-3-fluoro-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde **85** (37.0 g, 103.9 mmol, 75.6% yield) was obtained as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.81 (d, J = 3.0 Hz, 1H), 7.81 (d, J = 8.3 Hz, 1H), 7.54-7.33 (m, 7H), 5.31 (s, 2H), 1.39-1.28 (m, 12H).

6-(Benzyloxy)-7-fluorobenzo[c][1,2]oxaborol-1(3H)-ol 86

To a solution of **85** (20.0 g, 56.2 mmol, 1.00 eq) in THF (50.0 mL) was added $NaBH_4$ (3.2 g, 84.2 mmol, 1.5 eq). The mixture was stirred at 0 °C for 1 hour. TLC indicated that starting material was consumed completely. The reaction mixture was quenched by addition H_2O (100 mL) at 0 °C, and then adjusted pH = 5, removed the THF, then filtered and concentrated under reduced pressure to give a residue. 6-(benzyloxy)-7-fluorobenzo[c][1,2]oxaborol-1(3H)-ol **86** (43.0 g, 166.6 mmol, 98.9% yield) was obtained as a white solid. 1H NMR (DMSO-d_6, 400 MHz) δ 7.31-7.48 (m, 5H), 7.17 (t, *J* = 7.78 Hz, 1H), 7.01 (d, J = 8.03 Hz, 1H), 5.18 (s, 2H), 5.04 (s, 2H), 4.89 (s, 1H).

7-fluorobenzo[c][1,2]oxaborole-1,6(3H)-diol 87

To a solution of **86** (15.0 g, 58.1 mmol, 1.0 eq) in ethyl acetate (400.0 mL) was added Pd/C (2.0 g). The mixture was stirred at 25 °C for 2 hours under H_2 atmosphere (50 psi). TLC indicated that starting material was consumed completely. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. 7-fluorobenzo[c][1,2]oxaborole-1,6(3H)-diol **87** (7.0 g, 41.7 mmol, 71.7% yield) was obtained as a white solid. ^{1}H NMR (DMSO-d_6, 400MHz) δ 9.59 (s, 1H), 9.14 (s, 1H), 7.05-7.12 (m, 1H), 6.97-7.03 (m, 1H), 4.88 (s, 2H). MS (ESI): mass calcd. for $C_7H_6BFO_3$ 168.04, m/z found 167.1[M-H]-. HPLC: 92.5% (220 nm), 94.4% (254 nm).

Anti-*w*Alb High-Content *in vitro* Assay

C6/36 cells (ECACC #89051705, derived from *Aedes albopictus* larvae) were infected with *Wolbachia pipientis* derived from the supernatant of cultured *A. albopictus* Aa23 cells to create a stably *Wolbachia*-infected cell line C6/36 (*w*AlbB). This cell line was sub-passaged 6–8 days prior to plating out at a density of 2000 viable cells per well in a 384-well CellCarrier plate suspended in Liebovitz media supplemented with 20% fetal bovine serum, 2% tryptose phosphate broth and 1% non-essential amino acids. Compounds were dissolved and diluted in DMSO, and compound solution was added to each well to provide a final DMSO concentration < 1% and a total volume of 100 µL per well.

Following 7 days of sterile incubation at 26 ˚C, staining media containing SYTO 11 DNA dye was added to each well. After 15 minutes, all media was removed from each well and fresh media (no stain) was added. Imaging of each well was accomplished using a

Perkin Elmer Operetta high-content imaging system. Five fields per well were imaged using a confocal 60× objective with the Fluorescein filter (excitation filter: 460–490 nm; emission filter: 500–550 nm). Images were analyzed using the Perkin Elmer Harmony software to score each intact cell on the basis of texture complexity of the cytoplasm. Full details of the image analysis have been published.[25]

Compound sample wells were analyzed and normalized (along with the positive controls) against the vehicle (untreated) control to give a percentage reduction of Wolbachia-infected cells. Using the cell number analysis, compounds with a host cell number amounting to less than 50% of the vehicle control were classified as toxic and retested at a reduced compound concentration. Dose-response curves were generated with percentage reduction of Wolbachia-infected cells versus compound concentration, using 5–10 compound serial dilutions. Data were analyzed and compound EC_{50}s determined using a 4-parameter logistic nonlinear regression model. EC_{50} is defined as the compound concentration producing a 50% reduction of Wolbachia in the C6/36 cell line.

Anti-*w*Mel *Wolbachia* High-Content *in vitro* Assay

The assay was developed based on previous work[25] and carried out as previously described.[50] Briefly, *D. melanogaster* LDW1 cells naturally infected with *w*Mel strain of *Wolbachia* were maintained in Shields and Sang M3 (SSM3) Insect Medium (Sigma, S3652) supplemented with 10% heat-inactivated Fetal Bovine Serum (FBS) (qualified, One Shot™ Format, Gibco, 26140-111) at 25 °C, in flasks with unvented caps. Greiner 1536-well assay plates (Part. No. 789091) were coated with 1 mg/mL solution of Concanavalin-A lectin (MP BIOMEDICALS, 02195283) to facilitate cell adhesion. Compounds were acoustically transferred into plates using the Echo 555 Liquid Handler (Labcyte Inc.). Cells were seeded at 4,000 cells/well in SSM3 medium supplemented with 2% FBS using the MultiFlo FX Multi-Mode Dispenser (Biotek). After incubating at 25 °C for six days, the cells were fixed with 4% paraformaldehyde (PFA) for at least 10 min and washed with phosphate buffered saline, pH 7, 0.1% Tween 20. Fluorescence in situ hybridization (FISH) was used to stain *Wolbachia* and 3 μM DAPI was used to stain DNA. The "bottle valve" dispenser with an angled-head (Kalypsys Inc, San Diego, CA) was used for fixation and staining. Stained cells were imaged using the CX5 Insight

Cellomics high content imaging instrument with a 10× objective (Thermo). Each well was analyzed using Compartmental Analysis in HCS Studio (Thermo) for cell number and *Wolbachia* content. Analysis outputs included 1) the total object number per well, and 2) the total fluorescence intensity of all spots within each cell (averaged per well). Data were analyzed in Genedata Screener, Version 13.0.1-Standard by normalizing to DMSO (neutral) and inhibitor control-treated wells (12.5 uM doxycycline and 125 nM rifampicin). To determine the EC_{50}s, dose response curves (11`point, 1:3 dilution) were fitted using the four parameter Hill Equation. Each compound was tested in triplicate.

Ethics Statement on Animal Studies

All *in vivo* experiments involving *B. malayi* were conducted at the Liverpool School of Tropical Medicine (LSTM) and were approved by the ethical commitees of the University of Liverpool and LSTM, and were conducted according to Home Office (UK) requirements. All animal experiments with *L. sigmodontis* were conducted at the Institute for Medical Microbiology, Immunology and Parasitology (IMMIP), University Hospital of Bonn, in accordance to the European Union animal welfare guidelines. All protocols were approved by the local authorities (Landesamt für Natur, Umwelt und Verbraucherschutz, Cologne, Germany (AZ 84-02.04.2015.A507; 84-02.04.2012.A140). *In vivo* pharmacokinetic studies in mice were conducted either at Anacor Pharmaceuticals or Shanghai ChemPartner under appropriate review by Institutional Animal Care and Use Committees (IACUC) at each site.

***B. malayi* SCID mouse infection models**

A *B. malayi* SCID mouse infection model for anti-*Wolbachia* drug screening was used as previously described[34, 51-53] In brief, *B. malayi* L3 larvae were generated by membrane blood feeding Liverpool strain filarial susceptible *Aedes aegypti* mosquitoes with microfilariae isolated from patently infected *Meriones unguiculatus* gerbils. CB.17 SCID mice were purchased from Charles River UK. Groups of 3 or 5 SCID mice were infected with either 50 or 100 L3, ip, for larval or adult *B. malayi* efficacy testing, respectively. Drugs were dosed from point of infection for larval testing or after an incubation period of six weeks for adult-stage testing with variable length washout. Test drugs were administered orally at indicated doses and as detailed below. Doxycycline or minocycline

positive controls were administered in water at indicated doses. At two-weeks (larval testing) or 12-weeks post-infection (adult testing) motile *B. malayi* L4 or female parasites were isolated by peritoneal lavage, enumerated by microscopy and individually snap frozen. Total genomic DNA was isolated per worm and QPCR quantifications of single copy *B. malayi Wolbachia* were undertaken per group of 10 worms per treatment / control, as previously described.[34, 54] Efficacy of *Wolbachia* depletion is reported as median *Wolbachia* load per drug group expressed as a percentage of median vehicle control levels from animals treated only with vehicle.

***L. sigmodontis* in vivo mouse model**

Female BALB/c wild type mice were obtained from Janvier (Saint-Berthevin, France) and were housed at the animal facility of the IMMIP in individually ventilated cages on a 12h light/dark cycle with food and water ad libidum. Mice were infected at 6-8 weeks of age via exposure to the natural mite vector *Ornithonyssus bacoti* containing infectious *L. sigmodontis* L3 larvae .[36] To compare the infection, the same batch of mite-containing bedding was used to infect all animals of one experiment. 35 days post infection, a time point adult worms have developed within the thoracic cavity, mice were treated per oral gavage with the test compounds at concentrations indicated in the result section. As a vehicle, 1% CMC + 0.1% Tween80 was used. Negative controls were treated with vehicle only and as positive control, mice were treated orally with doxycycline (Sigma) in 10% DMSO/1xPBS. Necropsies were performed at 64-77 days post infection using an overdose of isoflurane (Baxter, Germany) and adult worms were isolated from the thoracic cavity and peritoneum and enumerated. Realtime PCR was performed from remaining female adult worms to quantify *Wolbachia* FtsZ and L. sigmodontis actin values by qRT-PCR as previously described.[35]

General PK Study Protocol

Animals were allowed to acclimate a minimum of 72 hours prior to selection for study. On the morning of dosing, animals were weighed and grouped randomly. Animals received the test material by tail-vein injection (IV), or oral gavage (PO). After dosing, all clinical signs and observations were recorded, and if adverse effects were noted, the exact time that the animals returned to normal was also recorded. At prescribed

timepoints, animals were anesthetized by isoflurane inhalation and blood samples were collected via retro-orbital bleed or cardiac puncture. After the blood sample collection, animals were euthanized by CO_2 inhalation followed by cervical dislocation.

Blood samples were collected into microtainer tubes (K_2EDTA) on ice, and processed to provide plasma by centrifugation for 6 minutes at approximately 2000 × g or 6,000 rpm. Processed samples were transferrd to analysis tube and placed on dry ice. Samples were analyzed by LC/MS/MS.

Associated Content

Supporting Information.

Molecular formula strings (SMILES) and biochemical and in vitro ADME data.

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Abbreviations Used.

NTDs, Neglected Tropical Diseases; DALYs, disability-adjusted life years; MDA, mass drug administration; PTC, peptidyl-transfer center; MDR1-MDCK, multidrug resistance-1 transfected Madin Darby canine kidney cell line; $f_{unbound}$, fraction unbound to plasma protein; mouse S9, supernatant from 9000g x 20 min fractionation of mouse liver preparation; C_{max}, maximal plasma concentration; SCID, severe combined immune deficiency; L3-stage, third stage larvae; qPCR, quantitative polymerase chain reaction; BID, twice daily; QD, once daily; P_{app}, apparent permeability; Cl_{int}, intrinsic clearance; wsp, Wolbachia surface protein; FtsZ, prokaryotic homolog of mammalian tubulin; BALB/c, Bagg albino strain mice; br, broad; s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, multiplet.

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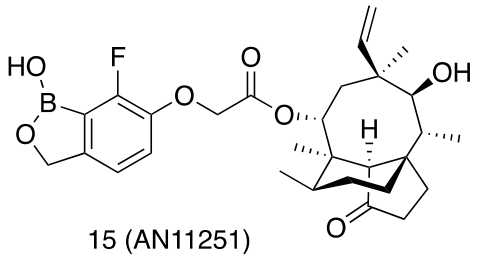
Table of Contents Graphic



15 (AN11251)

Wolbachia EC_{50} = 15 nM (C6/36 cell line)
Wolbachia EC_{50} = 1.5 nM (LDW1 cell line)

>99% reduction of *Wolbachia* in *Litomosoides sigmodontis* mouse model, PO @ 50 mg/kg, BID x 14 days