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Synthesis and biological evaluation of novel macrocyclic bis-7-azaindolylmaleimides as potent and highly selective glycogen synthase kinase-3β (GSK-3β) inhibitors☆

Lan Shen, Catherine Prouty, Bruce R. Conway, Lori Westover, Jun Z. Xu, Richard A. Look, Xin Chen, Mary Pat Beavers, Jerry Roberts, William V. Murray, Keith T. Demarest and Gee-Hong Kuo*

Drug Discovery Division, Johnson and Johnson Pharmaceutical Research and Development, L.L.C., 1000 Route 202, PO Box 300, Raritan, NJ 08869, USA

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Abstract—Palladium catalyzed cross-coupling reactions were used to synthesize two key intermediates **3** and **5** that resulted in the synthesis of novel series of macrocyclic bis-7-azaindolylmaleimides. Among the three series of macrocycles, the oxygen atom and thiophene containing linkers yielded molecules with higher inhibitory potency at GSK-3β ($K_i = 0.011–0.079$ μM) while the nitrogen atom containing linkers yielded molecules with lower potency ($K_i = 0.150–>1$ μM). Compound **33** and **36** displayed 1–2 orders of magnitude selectivity at GSK-3β against CDK2, PKCβII, Rsk3 and little or no inhibitions to the other 62 protein kinases. Compound **46** was at least 100-fold more selective towards GSK-3β than PKCβII, and it had little or no activity against a panel of 65 protein kinases, almost behaved as a GSK-3β 'specific inhibitor'. All three compounds showed good potency in GS assay. Molecular docking studies were conducted in an attempt to rationalize the GSK-3β selectivity of azaindolylmaleimides. The high selectivity, inhibitory potency and cellular activities of these non-crown-ether typed molecules may provide them as a valuable pharmacological tools in elucidating the complex roles of GSK-3β in cell signaling pathways and the potential usage for the treatment of elevated level of GSK-3β involved diseases.

1. Introduction

Glycogen synthase kinase-3 (GSK-3) is a serine/threonine protein kinase that was first identified over 20 years ago because of its ability to phosphorylate and inhibit glycogen synthase (GS),[1] the rate-limiting enzyme of glycogen biosynthesis.[2] Mammalian GSK-3 exists as two isoforms, GSK-3α and GSK-3β, sharing 98% homology in their catalytic domain.[3]Both isoforms are ubiquitously expressed in cells and tissues, and have similar biochemical properties.[3] Today, it is known that GSK-3 is involved in diverse cellular processes and might have multiple substrates.[4,5] For example, GSK-3 phosphorylates and inhibits the functioning of insulin receptor substrate-1 (IRS-1) and GS, the two key targets in the insulin signaling pathway.[6] Suppression of these targets may limit most insulin-mediated biological responses. In addition, elevated GSK-3 activity was found in diabetic tissues, reinforcing GSK-3 as a promising therapeutic target for insulin resistance and Type 2 diabetes. Tau is a known substrate of GSK-3 in vivo. Tau hyperphosphorylation has been postulated to promote microtubule disassembly, an early event in the progression of Alzheimer's disease.[7] Inhibition of GSK-3 has also been shown to attenuate apoptotic signals.[8] Therefore, GSK-3 inhibitors may be useful for the treatment of Alzheimer's disease and protection against cell death.[9] Lithium ions and valproic acid have been used as mood stabilizers for the chronic treatment of patients with bipolar disorder. Recently, these compounds have been shown to be GSK-3 inhibitors.[10] Finally, studies on fibroblasts from the GSK-3β knockout mice indicate that inhibition of GSK-3 may be useful in treating inflammatory disorders or diseases through the negative regulation of NFκB activity.[11]

Keywords: Specific inhibitor; Kinase; Azaindolemaleimide; Cross–x11dummy;coupling.
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* Corresponding author. Tel.: +1-908-704-4330; fax: +1-908-203-8109; e-mail: gkuo@prdus.jnj.com

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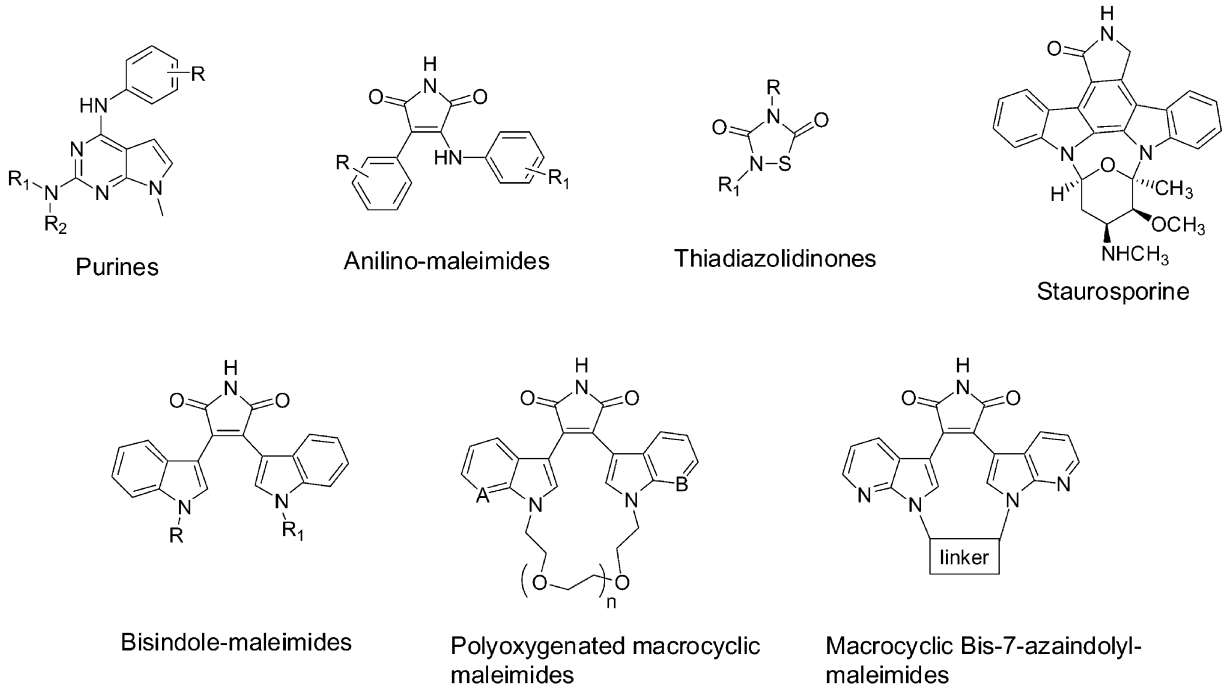


Scheme 1.

Since Chiron disclosed purines[12] (Scheme 1) are capable of exhibiting GSK-3 inhibitory activities, many chemical series[13,14] (pyrazines, pyrimidines, heterocyclic-pyrimidones, amine pyrazoles, bisindole-maleimides, hymenialdisine, paullones, indirubines and anilino-maleimides) have been developed as ATP competitive GSK-3 inhibitors while thiadiazolidinones[15] were reported to be the first ATP non-competitive GSK-3 inhibitors. However, except the anilino-maleimides[16,17] were demonstrated as selective GSK-3 inhibitors over 24 protein kinases, the majority of ATP competitive GSK-3 inhibitors all showed significant activities at other protein kinases. To minimize the potential side effects, it is always desirable to have the clinical agent targeting the enzyme specifically.

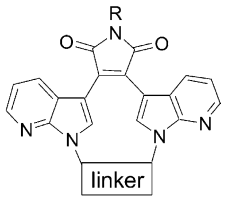
In an attempt to identify a potent and selective protein kinase C gamma (PKCγ) inhibitors for the treatment of chronic pain, we unexpectedly discovered that polyoxygenated-macrocyclic-maleimides (Scheme 1) are potent and highly selective GSK-3β inhibitors if both A and B are nitrogen atoms and $n=2$ or 3 ($n=2$, $IC_{50}=0.034$ μM; $n=3$, $IC_{50}=0.048$ μM).[18] However, these compounds are less attractive due to the cytotoxicity concern of its crown-ether typed structures.[19] This article describes our continued efforts to the identification of several non-crown-ether typed macrocyclic bis-7-azaindolylmaleimides as potent and highly selective GSK-3β inhibitors.

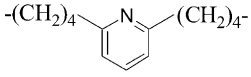
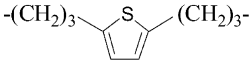
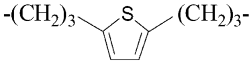
2. Chemistry

The synthesis of macrocyclic bis-7-azaindolylmaleimides described in Table 1 resulted from bis-alkylations of various linkers with the upper maleimides. Two key *N*-protected maleimides were used in these studies (Scheme 2). The first one, *N*-methyl-bis-7-azaindolylmaleimide **3**, was synthesized in two steps from the known 3-iodo-7-azaindole derivative **1**.[20] The tributyltin analogue of **2** was reported to synthesize from **1** in 19% yield.[20] We found out that if trimethyltin chloride was mixed with the iodide **1** at −78 °C before the addition of *n*-butyllithium, the yield of **2** was increased to 55–60%. A palladium-catalyzed cross-coupling reaction[21] of **2** with 2,3-dichloro -*N*-methylmaleimide gave the desired coupling product **3**.[22] Later, it was found out that some of the base-sensitive molecules were decomposed during the base hydrolysis and ammonolysis conditions required for the transformation of *N*-methylmaleimides to *N*-H maleimides. Therefore, we prepared the *N*-2,4-dimethoxybenzyl-bis-7-zazindolylmaleimide **5** that could be deprotected under acidic condition. Treatment of 3,4-dichloro-furan-2,5-dione with 2,4-dimethoxybenzylamine in refluxing acetic acid gave dichloride **4**. A palladium-catalyzed cross-coupling reaction of **2** with **4** gave the desired *N*-2,4-dimethoxybenzyl protected maleimide **5**.

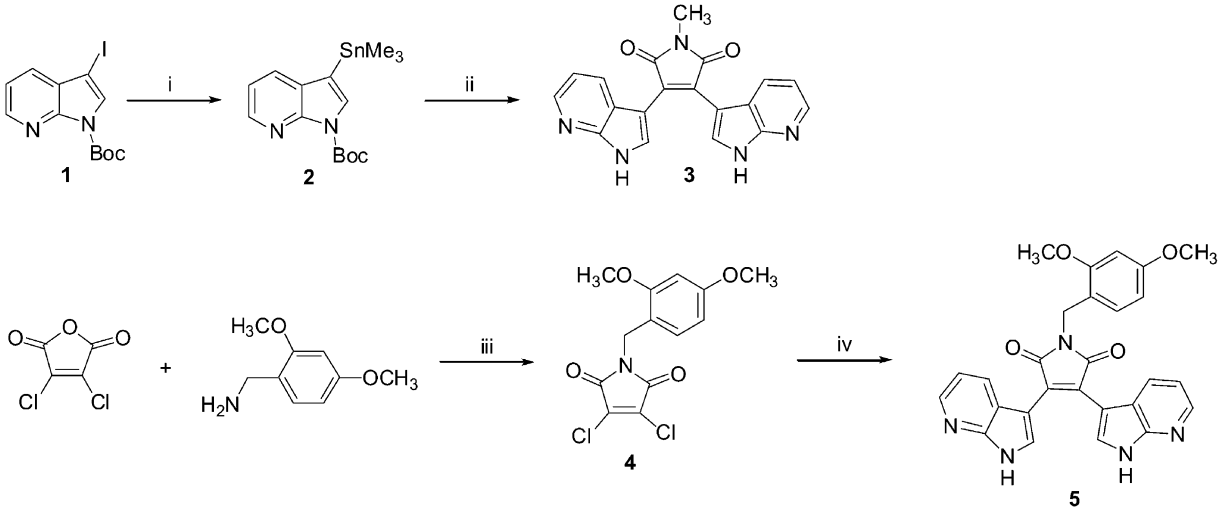
The synthesis of the symmetrical nitrogen containing macrocyclic maleimides **10** and **12** were shown in Scheme 3. Alkylation of *N*-methylmaleimide **3** with the bismesylate **7**, which in turn was prepared from carbamate diol **6**,[23] in the presence of Cs_2CO_3 in DMF[24] gave the cyclic maleimide **8**. Removal of the Boc protecting group, following by hydrolysis and ammonolysis[24] gave the *N*-H cyclic maleimide **10**. Ethylation of **9** gave **11**. Hydrolysis and ammonolysis of **11** provided the *N*-ethylated cyclic maleimide **12**. The synthesis of unsymmetrical nitrogen containing macrocyclic maleimides **19** and **21** were shown in Scheme 4. Reaction of δ-valerolactone and 4-amino-1-butanol gave the diol **13**. Borane reduction followed by *N*-Boc protection, and mesylation gave the required bismesylate **16**. Alkylation

Table 1. Inhibition constants at GSK-3β[a]

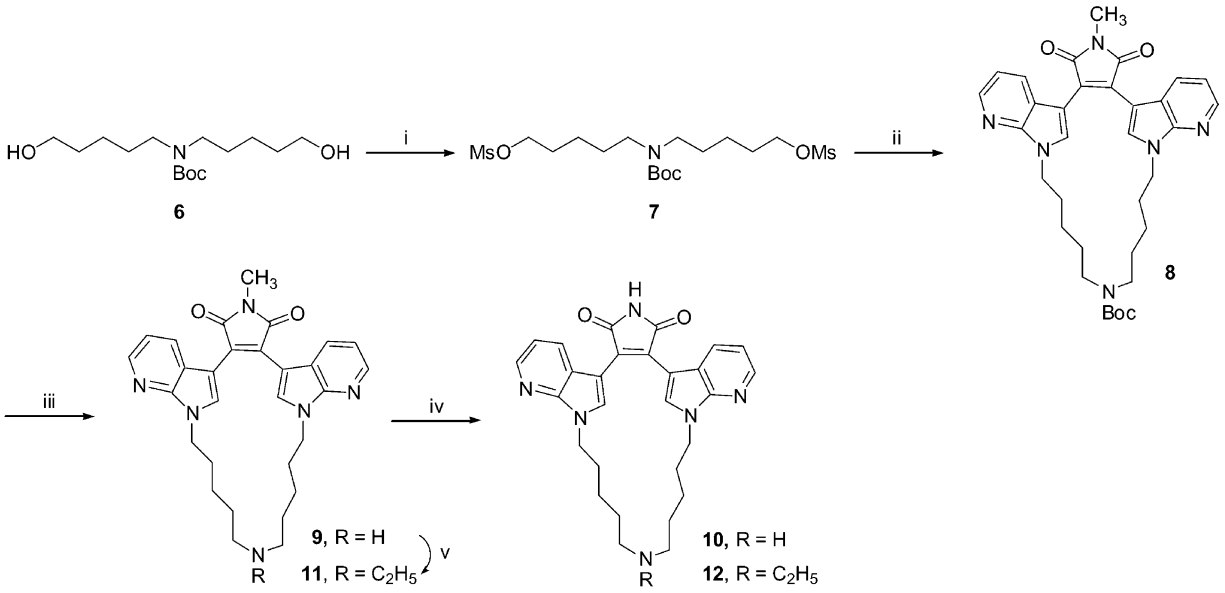


Compd	R	—linker—	GSK-3β $K_i \pm$SEM (μM)
10	H	$-(CH_2)_5NH(CH_2)_5-$	0.318 ± 0.048
12	H	$-(CH_2)_5N(C_2H_5)(CH_2)_5-$	0.541 ± 0.201
19	H	$-(CH_2)_5NH(CH_2)_4-$	31% inhib. @ 1 μM
21	H	$-(CH_2)_5N(C_2H_5)(CH_2)_4-$	44% inhib. @ 1 μM
28	H	$-(CH_2)_4C(=O)(CH_2)_6-$	0.079 ± 0.001
33	H	$-(CH_2)_4C(=O)(CH_2)_4-$	0.058 ± 0.005
36	H	$-(CH_2)_4CH(OH)(CH_2)_4-$	0.011 ± 0.002
41	H		0.150 ± 0.009
46	H		0.036 ± 0.001
45	Protected		0% inhib. @ 1 μM

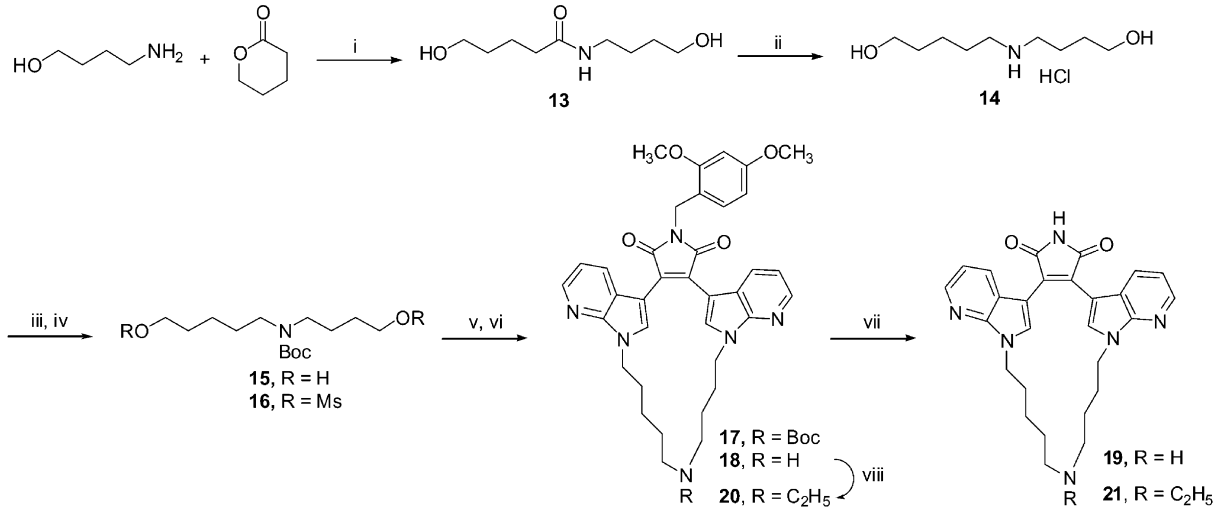
[a] Assay details were described in the Experimental.
[b] SEM, standard error mean.



Scheme 2. (i) Me_3SnCl, −78 °C, *n*-BuLi; (ii) 2,3-dichloro-*N*-methylmaleimide, $PdCl_2(PPh_3)_2$, LiCl, toluene, 100 °C; (iii) HOAc, reflux; (iv) **2**, $PdCl_2(PPh_3)_2$, LiCl, toluene, 100 °C.



Scheme 3. (i) MsCl, pyridine; (ii) **3**, Cs_2CO_3, DMF, 100 °C; (iii) TFA; (iv) (a) KOH, EtOH, 80 °C; 10% citric acid; (b) NH_4OAc, 140 °C; (v) C_2H_5I, THF, 70 °C.

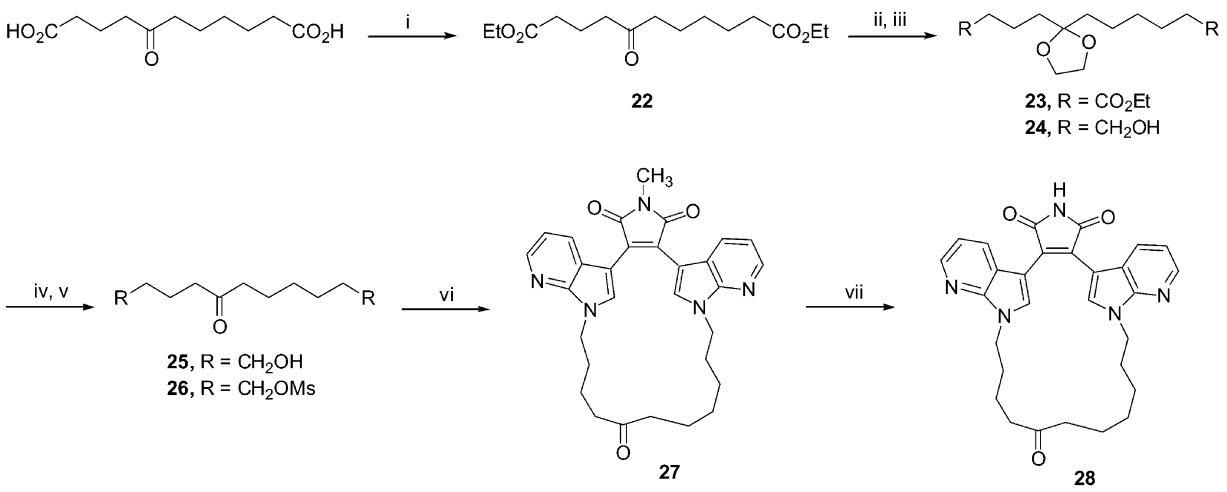


Scheme 4. (i) Xylene; (ii) (a) $BH_3{\cdot}SMe_2$, THF; (b) HCl, Et_2O; (iii) Et_3N, CH_3OH, Boc_2O; (iv) MsCl, pyridine; (v) **5**, Cs_2CO_3, DMF, 70 °C; (vi) TFA; (vii) CH_3SO_3H; (viii) C_2H_5I, THF.

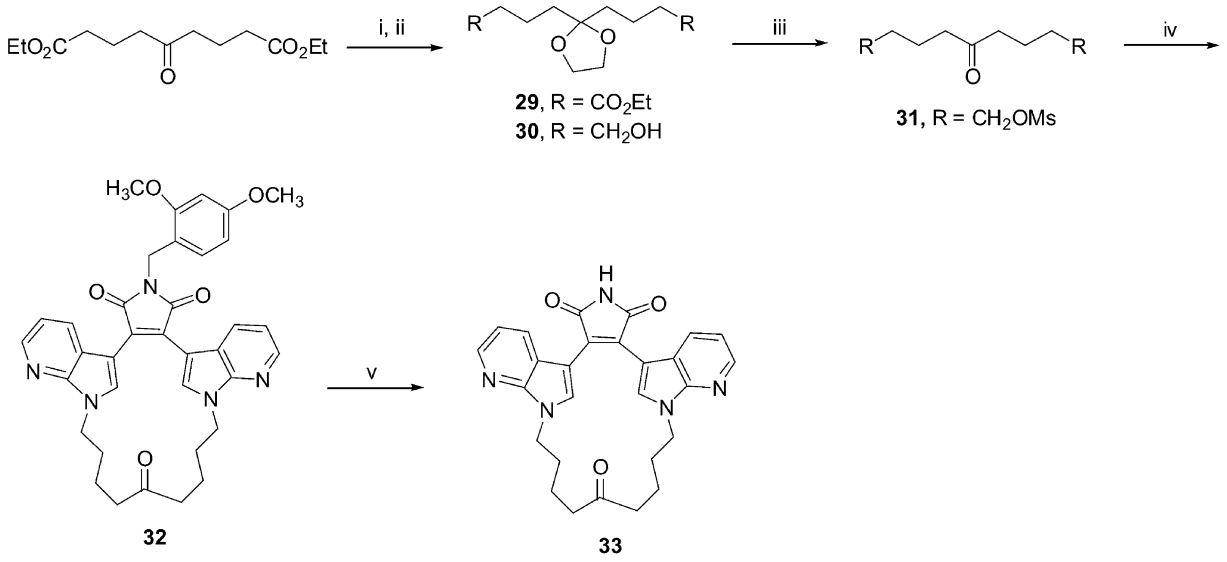
of **5** with **16** gave **17**. Treatment of **17** with trifluoroacetic acid removed the *N*-Boc protecting group selectively to give **18**. As desired, removal of the *N*-2,4-dimethoxybenzyl protecting group was accomplished with the treatment of methanesulfonic acid at 20 °C to provide **19** in quantitative yield.[25] Ethylation of **18** gave **20**. Acid treatment of **20** provided the unsymmetrical *N*-ethylated cyclic maleimide **21**.

The synthesis of unsymmetrical carbonyl group containing macrocyclic maleimide **28** was shown in Scheme 5. The carbonyl group of **22** was protected as cyclic ketyl **23**, followed by reduction to give the diol **24**. Deprotection and mesylation yielded the bismesylate **26**. Alkylation of **3** with **26** gave **27**, followed by hydrolysis and ammonolysis to provide the maleimide **28**. Similarly, reduction of the cyclic ketyl **29** gave the diol **30**. Deprotection and mesylation yielded **31**. Alkylation of **5** with **31** gave **32**, followed by acid promoted deprotection to give the symmetrical maleimide **33** (Scheme 6). Reduction of **31** gave the alcohol **34**. Alkylation of **5** with **34**, following by deprotection of **35** gave the hydroxyl containing macrocyclic maleimide **36** (Scheme 7).

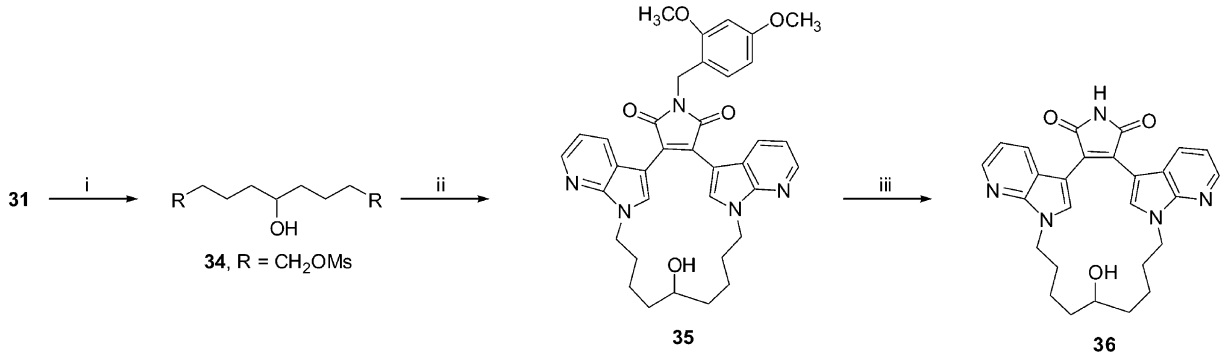
Bis-alkylation of 2,6-lutidine with 3-bromo-propoxy-*tert*-butyldimethylsilanes gave **37**. Deprotection followed by mesylation gave bismesylate **39**. Alkylation of **3** with **39**, following by hydrolysis and ammonolysis yielded the pyridine containing macrocyclic maleimide **41** (Scheme 8). In a similar way, bis-alkylation of thiophene gave **42**. Deprotection, mesylation and alkylation of **44** with **5**, followed by deprotection to give the thiophene containing macrocyclic maleimide **46** (Scheme 9).



Scheme 5. (i) EtOH, HCl (concd.); (ii) $CH(OEt)_3$, TsOH, $HO(CH_2)_2OH$; (iii) $LiAlH_4$; (iv) H_2SO_4, acetone; (v) MsCl, Et_3N; (vi) **3**, Cs_2CO_3, DMF, 100 °C; (vii) (a) KOH, EtOH, 80 °C; 10% citric acid; (b) NH_4OAc, 140 °C.



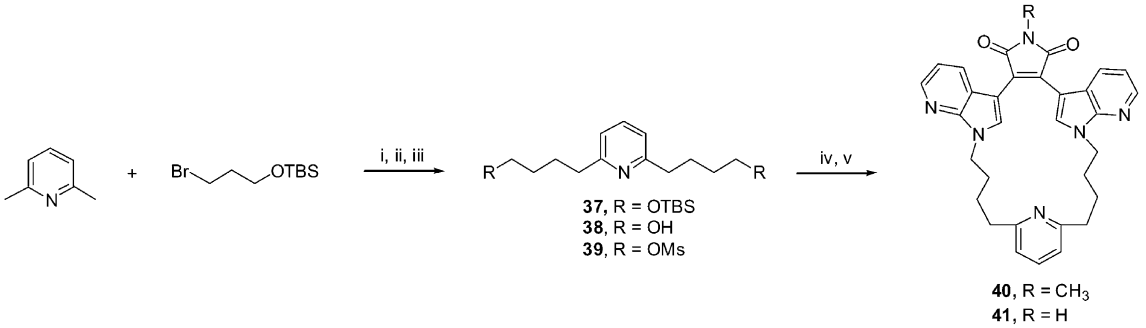
Scheme 6. (i) $CH(OEt)_3$, TsOH, $HO(CH_2)_2OH$; (ii) $LiAlH_4$; (iii) MsCl, Et_3N; (iv) **5**, Cs_2CO_3, DMF, 70 °C; (v) CH_3SO_3H.



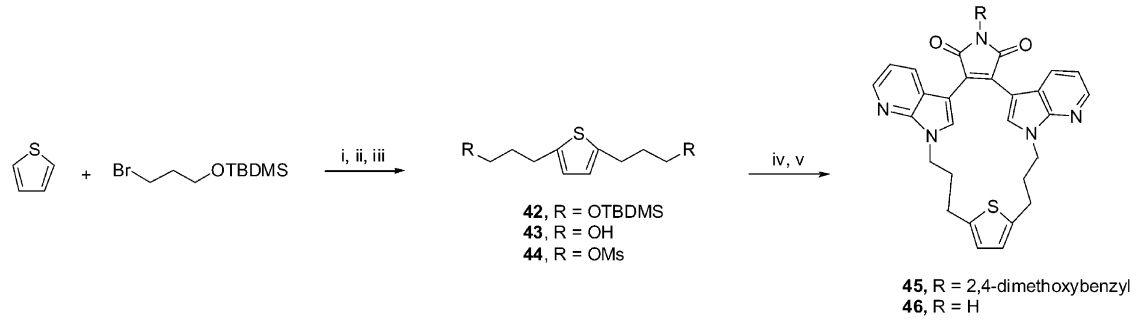
Scheme 7. (i) $NaBH_4$, EtOH, THF; (ii) **5**, Cs_2CO_3, DMF, 80 °C; (iii) CH_3SO_3H.

3. Results and discussion

In the polyoxygenated macrocyclic maleimide series, the 19-membered macrocycle ($n=2$) gave the best potency at GSK-3β. Thus, we first prepared compound **10** (the nitrogen containing linker, Table 1) with the same ring size; however, this replacement resulted in ~10-fold lower potency ($K_i=0.318$ μM). It was thought that the reduced potency might be due to the unfavorable extra hydrogen bond donor –NH moiety presented in the linker. Compound **12** was then synthesized with the –NH moiety replaced with $-NC_2H_5$ moiety. However, no improvement was observed ($K_i=0.541$ μM for **12** vs 0.318 μM for **10**). Since it was evident that conformationally



Scheme 8. (i) *n*-BuLi, THF; (ii) TBAF; (iii) MsCl, Et_3N; (iv) **3**, Cs_2CO_3, DMF, 100 °C; (v) (a) KOH, EtOH, 80 °C; 10% citric acid; (b) NH_4OAc, 140 °C.



Scheme 9. (i) *n*-BuLi, THF; (ii) TBAF; (iii) MsCl, Et_3N; (iv) **5**, Cs_2CO_3, DMF, 90 °C; (v) CH_3SO_3H.

constrained analogues may exhibit better binding affinities,[26] we next examined the potency of the smaller macrocycles **19** and **21**. With disappointment, both 18-membered macrocycles containing either –NH or $–NC_2H_5$ in the linker displayed even poorer potency (for **19**, 31% inhibition at 1 μM; for **21**, 44% inhibition at 1 μM). It was hypothesized that the incorporation of a nitrogen atom in the bottom linker may not be desirable to achieve high inhibitory potency at GSK-3β.

We then went back to re-visit the oxygen atom containing linkers but to place the oxygen atom out of the chain instead. Interestingly, the carbonyl group containing 19-membered macrocycle **28** ($K_i = 0.079$ μM) did display approximately 4-fold greater inhibitory potency when compared to the nitrogen containing 19-membered macrocycle **10** ($K_i = 0.318$ μM). We continued to prepare a smaller analogue, the 17-membered macrocycle **33**. The conformationally more constrained **33** displayed modest improvement in potency ($K_i = 0.058$ μM) over **28**. Replacement of the carbonyl group of **33** with a hydroxyl group gave **36** ($K_i = 0.011$ μM), which exhibited another 5-fold increase in potency relative to **33**. The high potency of **36** may partially contributed from the hydrogen bond donor capability of –OH group. Replacing the hydroxyl group in **36** with a pyridine ring gave **41**. When comparing macrocycle **41** to macrocycle **12** (which has a comparable ring size), the aromatic nitrogen ring was more favorable than the *tert*-alkyl amine moiety for the inhibitory activity (for **41**, $K_i = 0.150$ μM). Replacement of the hydroxyl group in **36** with a thiophene ring and shortening two carbon atoms gave macrocycle **46**. Compound **46** displayed good potency ($K_i = 0.036$ μM) although this compound was not as potent as the hydroxyl analogue **36**. Overall, it appears that altering the functional groups in the linker has a greater impact on the GSK-3β inhibitory potency than altering the ring sizes does, at least between the 17- to 19-membered macrocycles range. The total abolished potency of 2,4-dimethoxybenzyl-protected maleimide **45** (0% inhibition at 1 μM) suggested that the maleimide NH seems to be critical for the binding.

4. Kinase selectivity

Recently, literature reports have described potent cyclin-dependent kinase (CDK) inhibitors that could also inhibit GSK-3β.[27] The bisindole-maleimide derivatives (Scheme 1) were identified as dual PKC/GSK-3 inhibitors,[28] and have been reported to be competitive inhibitors of ATP binding presumably by interacting at the ATP binding site.[29] Our compounds, in common with most of the other protein kinase inhibitors, also inhibited GSK-3β in an ATP competitive manner.[30] Therefore, it is critical to evaluate our compounds against a panel of kinases including CDK and PKC isoenzymes in order to determine the selectivity. In addition, many reported specific inhibitors of protein kinases were found to be non-specific when re-examined against a large panel of protein kinases by Cohen.[31] Therefore, we submitted compounds **33**, **36** and **46** to UBI (Upstate Biotech Inc.) for broad screening against a panel of 66 protein kinases. In the presence of 100 μM ATP, compound **33** inhibited GSK-3β kinase activity by 96% at a concentration of 10 μM. Compounds **36** or **46** inhibited GSK-3β kinase activity by 99% at 10 μM (Table 2). Interestingly, compounds **33** and **36** only showed very weak inhibitory activities at the other 63

Table 2. Activities at various protein kinases[a]

Protein Kinase	Activity (% of control)		
	33	**36**	**46**
GSK3β (h)	4	1	1
AMPK (r)	98	100	108
Blk (m)	77	85	93
CAMKII (r)	89	91	92
CAMKIV (h)	78	80	70
CDK1/cyclinB (h)	61	62	93
CDK2/cyclinA	50	43	80
CDK5/p35 (h)	98	93	100
CDK6/cyclin D3	85	86	109
CDK7/cyclinH	86	91	103
CHK1 (h)	104	103	123
CHK2 (h)	101	101	81
CK1 (y)	78	62	84
CK2 (h)	116	112	102
CSK	93	80	89
Fes (h)	101	97	96
Fyn (h)	107	103	68
FGFR3	78	64	72
IGF-1R	104	99	93
IKK α	111	116	100
IKK β	106	122	111
IR	92	97	103
JNK1α1 (h)	97	92	106
JNK2α2 (h)	106	99	113
JNK3 (r)	72	56	77
Lck (h)	86	77	77
Lyn	73	77	87
MAPK1 (h)	85	92	95
MAPK2 (h)	101	98	105
MAPK2 (m)	114	112	105
MAPKAP-K2 (h)	100	123	101
MEK1 (h)	100	101	92
MKK4 (m)	91	92	90
MKK6 (h)	103	106	101
MKK7β (h)	147	133	113
MSK1 (h)	64	63	70
PAK2 (h)	99	103	112
PDGFR α	99	106	111
PDGFR β	98	95	89
PDK1 (h)	88	94	94
PKA (b)	99	96	91
PKA (h)	97	113	112
PKBα (h)	106	106	104
PKBβ (h)	71	83	68
PKCα (h)	77	70	84
PKCβII (h)	37	28	56
PKCγ (h)	99	92	102
PKCε (h)	77	71	77
PKCθ	100	85	97
PRAK (h)	98	86	87
c-RAF (h)	99	96	91
ROCK-II (h)	97	98	104
ROCK-II (r)	93	92	98
Rsk1 (r)	81	79	72
Rsk2 (h)	84	99	85
Rsk3 (h)	56	38	74
SAPK2a (h)	119	121	114
SAPK2b (h)	105	105	95
SAPK3 (h)	96	96	113
SAPK4 (h)	94	92	99
p70S6K (h)	108	91	100
SGK (h)	98	103	97
cSRC (h)	94	85	96
Syk	86	97	124
Yes (h)	87	95	82
ZAP-70 (h)	114	125	123

(a) Protein kinase were assayed with 10 μM of **33**, **36** and **46** in the presence of 100 μM ATP. Activities were given as the mean percentage of that in control incubations (averages of duplicate determinations). Assay details were described in the Experimental.

(or 62 for **36**) kinases, and moderate inhibitions at CDK2/cyclin A and PKCβII (and Rsk3 for **36**). The K_i value of these compounds at CDK2, PKCβII and Rsk3 assays were shown in Table 3. Compound **33** exhibited ~20-fold selectivity at GSK-3β versus CDK2/cyclin A and PKCβII whereas compound **36** exhibited ~60-fold selectivity against the three low selective kinases. Remarkably, compound **46** exhibited very low activity against all of the other 65 kinases, and had 97-fold selectivity against the poorly selective one, therefore representing a 'specific inhibitor' of GSK-3β.

5. Cellular activity

Among the multiple cellular processes in which GSK-3 has been implicated, the ability to phosphorylate and inhibit GS was the first regulation process being discovered and is perhaps the most thoroughly studied.[1] Therefore, the cell-based assay examining GS activation represents a direct functional assay to measure the cellular activity of GSK-3 inhibitors. Compounds **33**, **36**, **46** and LiCl were tested for the ability to increase GS activity in human embryonic kidney (HEK293) cells. As described above, LiCl is a known inhibitor of GSK-3β. LiCl increases GS activity in isolated rat adipocytes and hepatocytes,[32] and produces intracellular effects similar to that achieved by GSK-3 inhibition.[33,34] Compound **33** (EC_{50} = 1.26 μM), compound **36** (EC_{50} = 0.33 μM) and compound **46** (EC_{50} = 1.25 μM) all exhibited much greater potency than LiCl (EC_{50} > 3000 μM) in the HEK293 cells (Table 4).

6. Molecular docking studies

Compounds **33**, **36** and **46** are very selective at GSK-3β, demonstrating only moderate or poor activity for other Ser/Thr kinases (Tables 2 and 3). In an attempt to rationalize this high selectivity, the molecular docking studies of **46** were conducted, based on the X-ray structure of GSK-3β.[35] Compound **46** was docked into the ATP binding site of GSK-3β. The binding mode derived from the best docking pose is illustrated in Fig. 1. According to this model, compound **46** may form three H-bonds with GSK-3β when binding into the ATP-binding site. The first H-bond may form between the backbone carbonyl oxygen of ASP133 to the imide NH. The second H-bond is generated between the backbone nitrogen of VAL135 to the imide carbonyl oxygen. The third H-bond may form between the side-chain guanidine group of ARG141 to the azaindole nitrogen atom. The first two H-bonds that are formed with the backbone atoms are identical to those observed when staurosporine (Scheme 1) binds to various kinases, such as CDK2,[36] LcK[37] and so on. Meanwhile, the H-bond with ARG141 turns out to be unique to GSK-3β and provides an interesting model to explain the high GSK-3β selectivity observed for our 7-azaindolemaleimides.

The aligned sequences of several kinases selected from the UBI panel is shown in Fig. 2. This alignment was adopted from the Protein Kinase Resource (PKR)

Table 3. $K_i \pm$SEM (μM) and K_i ratio at selected kinase[a]

Compd	$K_i \pm$SEM (μM)				K_i ratio		
	GSK-3β	CDK2/cyclin A	PKCβII	RsK3	CDK2/GSK-3β	PKCβII/GSK-3β	RsK3/GSK-3β
33	0.058±0.005	1.233±0.057	1.050±0.024	NT[b]	21	18	—
36	0.011±0.002	0.682±0.022	0.710±0.025	0.789±0.097	62	65	72
46	0.036±0.001	NT	3.488±0.067	NT	—	97	—

[a] Assay details were described in the Experimental. [b] Not tested due to less than 50% inhibition.

Table 4. Glycogen synthase activity in HEK293 cells[a]

Compd	$EC_{50} \pm$SEM (μM)
33	1.26±0.16
36	0.33±0.05
46	1.25±0.44
LiCl	>3000

(a) Assay details were described in the Experimental.

database,[38] which includes hundreds of kinases whose sequences have been aligned by Hanks and Quinn.[39] It clearly demonstrates that the positive-charged ARG141 is quite unique to GSK-3β, with many of the other kinases having a negative-charged residue at the same alignment position. Conceivably, the replacement of ARG141 by a negative-charged residue will not only disrupt the H-bonding with the azaindole nitrogen, but also introduce an unfavorable repellent interaction with the lone-pair electrons on that nitrogen. This unique binding site may explain why the bis-7-azaindole **46** exhibit high selectivity at GSK-3β versus other kinases.

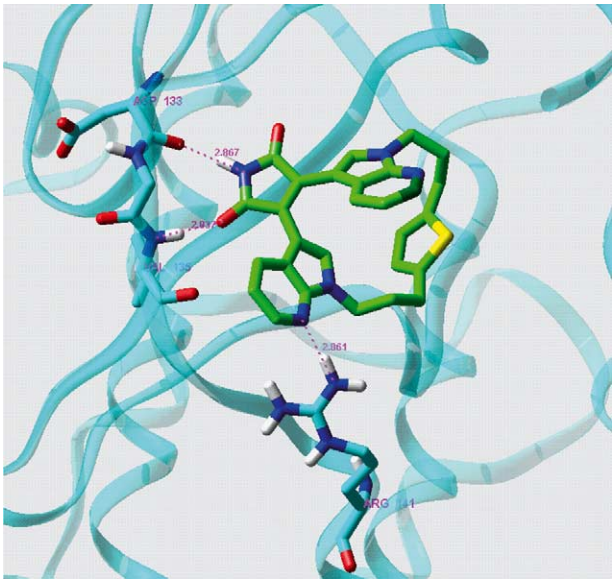


Figure 1. Illustration of the binding mode of compound **46** in the ATP-binding site of GSK-3β, suggested by the molecular docking studies. Compound **46** and its H-bonding partners (ASP133, VAL135, and ARG141) are represented in the stick model. The protein structure is described in the ribbon model. The key H-bonds between compound **46** and GSK-3β are indicated by the pink dotted lines, together with the bond lengths in the unit of angstrom. (Atom color scheme: hydrogen in white; oxygen in red; nitrogen in blue; carbon on compound **46** in green; and carbon on GSK-3β in light blue.).

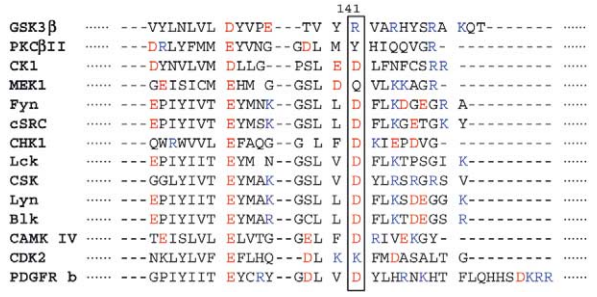


Figure 2. Sequence alignment of some kinases selected from PKR database, to demonstrate the uniqueness of ARG141 at GSK-3β. The positive-charged residues are colored in blue, while the negative-charged residues are colored in red.

There are also a few kinases with a neutral or even positive-charged residue at the alignment position corresponding to ARG141 at GSK-3β, as shown in Fig. 2. For example, CDK2 bears a positive-charged LYS86 at that position. However, a close inspection of the X-ray structure of CDK2 and staurosporine complex (1aq1.pdb)[36] reveals that the positive-charged side chain of LYS86 is actually oriented into a different space, and instead, the negative-charged side chain of ASP83 is the closest residue to the 7-position of the indole ring of staurosporine. Therefore, we speculate that the selectivity difference of the 7-azaindolemaleimides observed may contribute significantly from the different interaction strengths with ARG141 at GSK-3β or the spatially equivalent residues at other kinases.

The lower GSK-3β potency observed for molecules that contain an aliphatic nitrogen-atom linker (compounds **10**, **12**, **19** and **21**) may partially explained as the penalty paid from an unfavorable desolvation with water during the binding processes. A full discussion of the structure activity relationships between these compounds requires a careful consideration of several factors including H-bonding, solvation/desolvation effects, van der Waals forces and electrostatic interactions, the subject of future computational studies.

7. Conclusion

Palladium-catalyzed cross-coupling reactions were used to synthesize two key intermediates **3** and **5** that resulted in the synthesis of novel series of macrocyclic bis-7-azaindolylmaleimides. *N*-2,4-Dimethoxybenzylmaleimide **5** could be deprotected under acidic condition is particularly useful for the synthesis of molecules that may not survived the base-hydrolysis/ammonolysis reaction conditions. Among the three series of macrocycles,

the oxygen atom and thiophene containing linkers yielded molecules with higher inhibitory potency at GSK-3β ($K_i = 0.011$–0.079 μM) while the nitrogen atom containing linkers yielded molecules with lower potency ($K_i = 0.150$–>1 μM). The ring-size of the macrocycles seems to have less impact on the potency. Compound **33** and **36** displayed 1–2 orders of magnitude selectivity at GSK-3β against CDK2, PKCβII, Rsk3 and little or no inhibitions versus 62 other protein kinases. Compound **46** displayed a 100-fold greater selectivity at GSK-3β versus a panel of 65 protein kinases, and behaved as a GSK-3β 'specific inhibitor'. All three compounds showed good potency in GS assay. Molecular docking studies were conducted in an attempt to rationalize the GSK-3β selectivity of bis-7-azaindolylmaleimides. The high selectivity, inhibitory potency and cellular activities of these non-crown-ether typed molecules suggested that these compounds may prove to be valuable pharmacological tools in elucidating the complex roles of GSK-3β in cell signaling pathways and the potential usage for the treatment of elevated level of GSK-3β involved diseases.

8. Experimental

8.1. Chemistry

8.1.1. General information. ^{1}H NMR spectra were measured on a Bruker AC-300 (300 MHz) spectrometer using tetramethylsilane as an internal standard. Elemental analyses were obtained by Quantitative Technologies Inc. (Whitehouse, NJ, USA), and the results were within 0.4% of the calculated values unless otherwise mentioned. Melting points were determined in open capillary tubes with a Thomas-Hoover apparatus and were uncorrected. The optical rotations were measured at 25 °C with an Autopol III polarimeter. Electrospray mass spectra (MS-ES) were recorded on a Hewlett Packard 59987A spectrometer. High resolution mass spectra (HRMS) were obtained on a Micromass Autospec. E. spectrometer. The term 'DMAP' refers to dimethylaminopyridine, 'TFA' refers to trifluoroacetic acid, 'NMP' to 1-methyl-2-pyrrolidinone, 'DPPF' to 1, 1′-bis(diphenylphosphino)ferrocene, '$Pd_2(dba)_3$' to tris (dibenzylideneacetone)dipalladium(0)-chloroform adduct and 'DPPA' refers to diphenylphosphoryl azide.

8.1.2. 3-Trimethylstannanyl-pyrrolo[2,3-*b*]pyridine-1-carboxylic acid *tert*-butyl Ester (2). To a THF solution (15 mL) of 7-aza-1-(*tert*-butyloxycarbonyl)-3-iodoindole **1** (1.82 g, 5.3 mmol) at −78 °C was added trimethyltin chloride (26.5 mL, 1 M in THF, 26.5 mmol) under nitrogen. After 10 min, *n*-BuLi (10 mL, 1.6 M in hexane, 16 mmol) was added dropwise at −78 °C and the reaction mixture was allowed to warm up to 20 °C overnight. Water (4 mL) was added and the solvent was removed under reduced pressure. The residue was diluted with hexane (250 mL) and the organic layer was washed with water, dried (Na_2SO_4) and concentrated. The product was purified by column chromatography (SiO_2) to give 1.198 g (60%) of organostannane **2** as an oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.45 (d, $J = 4.9$ Hz, 1H), 7.77 (d, $J = 7.6$ Hz, 1H), 7.48 (s, 1H), 7.13 (dd, $J = 7.7$, 4.8 Hz, 1H), 1.65 (s, 9H), 0.36 (m, 9H); MS (ES) m/z: 405 (M + Na).

8.1.3. 1-Methyl-3,4-bis-(1*H*-pyrrolo[2,3-*b*]pyridine-3-yl)-pyrrole-2,5-dione (3). A mixture of **2** (185 mg, 0.486 mmol), 2,3-dichloro-*N*-methylmaleimide (29 mg, 0.162 mmol), $PdCl_2(PPh_3)_2$ (5.4 mg, 0.0077 mmol) and LiCl (32 mg, 0.77 mmol) in anhydrous toluene (2 mL) was stirred at 95 °C overnight. The solvent was removed under reduced pressure. The product was purified by column chromatography (SiO_2) to give 23 mg (41%) of **3** as an orange-red solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.35 (s, 2H), 8.12 (brd, $J = 3.9$ Hz, 2H), 7.92 (s, 2H), 7.08 (d, $J = 7.7$ Hz, 2H), 6.73 (m, 2H), 3.06 (s, 3H); MS (ES) m/z: 344 ($M + H^+$). Anal. calcd for $C_{19}H_{13}N_5O_2 \cdot 2.2TFA \cdot 0.5H_2O$: C, 46.59; H, 2.71; N, 11.61. Found: C, 46.25; H, 2.41; N, 11.34.

8.1.4. 3,4-Dichloro-1-(2,4-dimethoxy-benzyl)-pyrrole-2,5-dione (4). A mixture of 2,3-dichloromaleic anhydride (1.02 g, 6.10 mmol), 2,4-dimethoxybenzylic amine (1.02 g, 6.10 mmol) in glacial acetic acid (18 mL) was heated to 80 C for 5 h. After it was cooled to 20 °C, the mixture was concentrated under reduced pressure, and diluted with CH_2Cl_2 (50 mL). The mixture was sequentially washed with water (15 mL), 2 M aqueous Na_2CO_3 (15 mL), water (15 mL) and brine (15 mL). After the combined organic phases were concentrated, the residue was filtered through a short pad of SiO_2, eluting with CH_2Cl_2 to give **4** (1.42 g, 74%) as a light brown solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20 (d, $J = 8.7$ Hz, 1H), 6.44 (d, $J = 2.3$ Hz, 1H), 6.42 (s, 1H), 4.72 (s, 2H), 3.79 (s, 3H), 3.78 (s, 3H).

8.1.5. 1-(2,4-Dimethoxy-benzyl)-3,4-bis-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-pyrrole-2,5-dione (5). A mixture of **2** (500 mg, 1.31 mmol), **4** (180 mg, 0.57 mmol), $PdCl_2(PPh_3)_2$ (80 mg, 0.11 mmol) and LiCl (240 mg, 8.6 mmol) in toluene (9.0 mL) was heated at 100 °C for 20 h. After the solvent was removed under reduced pressure, the residue was dry-loaded on silica gel, eluting with EtOAc/hexane to give **5** (160 mg, 58%) as an orange red solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.30 (s, 2H), 8.12 (d, $J = 4.6$ Hz, 2H), 7.93 (d, $J = 2.8$ Hz, 2H), 7.08 (m, 3H), 6.73 (dd, $J = 8.0$, 4.7 Hz, 2H), 6.58 (d, $J = 2.1$ Hz, 1H), 6.48 (d, $J = 8.4$ Hz, 1H), 4.68 (s, 2H), 3.82 (s, 3H), 3.74 (s, 3H); MS (ES) m/z: 480 ($M + H^+$). Anal. calcd for $C_{27}H_{21}N_5O_4 \cdot 0.3H_2O$: C, 66.88; H, 4.49; N, 14.44. Found: C, 66.51; H, 4.23; N, 14.61.

8.1.6. Methanesulfonic acid 5-[*tert*-butoxycarbonyl-(5-methanesulfonyloxy-pentyl)-amino]-pentyl ester (7). To a solution of carbamate diol **6** (1.06 g, 3.66 mmol,[23]) in CH_2Cl_2 (13 mL) was added pyridine (1.2 mL, 14.6 mmol) and MsCl (1.1 mL, 14.6 mmol) at 0 °C. The mixture was stirred at 20 °C for 1.5 h. It was diluted with diethyl ether (10 mL), and washed sequentially with cold aqueous HCl (5%), NaOH (5%), water and brine. The organic solution was dried ($MgSO_4$), filtered and concentrated. The product was purified by column chromatograph on silica gel (eluting with hexane/

EtOAc) to give **7** as a colorless oil (1.20 g, 74%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.23 (t, J = 6.4 Hz, 4H), 3.16 (s, br, 4H), 3.01 (s, 6H), 1.78 (m, 4H), 1.55 (m, 4H), 1.45 (s, 9H), 1.40 (m, 4H); MS (ES) m/z: 468 (M + Na).

8.1.7. 7,8,9,10,13,14,15,16,24,25-Decahydro-24-methyl-23,25-dioxo-6*H*,23*H*-5,26:17,22-dimethenodipyrido[2,3-*n*:3′,2′-*t*]pyrrolo[3,4-*q*][1,7,13]triazacycloheneicosine-11(12*H*)-carboxylic acid, 1,1-dimethylethyl ester (8). A mixture of **3** (41 mg, 0.12 mmol) and Cs_2CO_3 (190 mg, 0.58 mmol) in DMF (20 mL) was heated to 100 °C. A DMF solution (5 mL) of the bismesylate **7** (77 mg, 0.17 mmol) was added via syringe pump over 1.5 h. After the addition was complete, the reaction mixture was stirred at 20 °C for 21 h. The mixture was quenched with aqueous ammonium chloride (30 mL), and extracted with CH_2Cl_2 (2 × 30 mL). The combined organic layers was washed with water (3 × 20 mL) and brine (15 mL). It was then dried (Na_2SO_4) and concentrated. The product was purified by column chromatography on silica gel eluting with hexane/EtOAc to give **8** (36 mg, 50%) as an orange solid: ^{1}H NMR (300 MHz, CD_3OD) δ 8.29 (dd, J = 4.7, 1.5 Hz, 2H), 7.66 (s, br, 2H), 7.58 (s, 2H), 7.05 (dd, J = 8.0, 4.7 Hz, 2H), 4.30 (t, J = 6.5 Hz, 4H), 3.15 (s, 3H), 2.73 (s, br, 4H), 1.75 (t, J = 6.6 Hz, 4H), 1.42 (s, 9H), 1.34 (m, 4H), 1.03 (m, 4H); MS (ES) m/z: 597 (M + H$^+$).

8.1.8. 7,8,9,10,11,12,13,14,15,16-Decahydro-24-methyl-6*H*,23*H*-5,26:17,22-dimethenodipyrido[2,3-*n*:3′,2′-*t*][pyrrolo[3,4-*q*][1,7,13]triazacycloheneicosine-23,25(24*H*)-dione (9). To a solution of **8** (13 mg, 0.022 mmol) in CH_2Cl_2 (1.0 mL) was added TFA (0.2 mL). After the mixture was stirred at 20 °C for 1 h, solvent and excess TFA were removed under reduced pressure. Ammonium hydroxide was carefully added, and orange solids were precipitated out. The solids were collected by filtration and washed with water to give **9** (10 mg, 100%) after drying under vacuum: ^{1}H NMR (300 MHz, CD_3OD) δ 8.27 (dd, J = 4.7, 1.4 Hz, 2H), 7.66 (s, 2H), 7.56 (dd, J = 8.0, 1.4 Hz, 2H), 7.02 (dd, J = 8.0, 4.8 Hz, 2H), 4.33 (t, J = 5.9 Hz, 4H), 3.14 (s, 3H), 2.26 (t, J = 6.5 Hz, 4H), 1.84 (m, 4H), 1.40 (m, 4H), 0.96 (m, 4H); MS (ES) m/z: 497 (M + H$^+$).

8.1.9. 7,8,9,10,11,12,13,14,15,16-decahydro-(6*H*,23*H*-5,26:17,22-dimethenodipyrido[2,3-*n*:3′,2′-*t*]pyrrolo[3,4-*q*][1,7,13]triazacycloheneicosine-23,25(24*H*)-dione (10). A mixture of **9** (10 mg, 0.020 mmol) and KOH (198 mg, 3.53 mmol) in ethanol (2.0 mL) was heated under reflux for 18 h. It was cooled to 20 °C and concentrated under reduced pressure. The residue was dissolved in water (3.0 mL) and acidified with 10% citric acid (pH 4). The mixture was stirred at 20 °C for 10 min and concentrated. The resulting residue was mixed with ammonium acetate solids (2.4 g, 31.2 mmol), and heated to 140 °C for 3 h. The mixture was cooled to 20 °C, diluted with water (3.0 mL), basified with 20% aqueous NaOH till pH 10, and extracted with EtOAc (3 × 25 mL). The combined organic layers was dried (Na_2SO_4) and concentrated. The product was purified by column chromatography (eluting with MeOH/CH_2Cl_2) to give **10** (4 mg, 42%) as an orange solid: ^{1}H NMR (300 MHz, CD_3OD) δ 8.27 (dd, J = 4.7, 1.5 Hz, 2H), 7.65 (s, 2H), 7.55 (dd, J = 8.0, 1.5 Hz, 2H), 7.01 (dd, J = 8.0, 4.8 Hz, 2H), 4.32 (t, J = 5.9 Hz, 4H), 2.23 (t, J = 6.3 Hz, 4H), 1.81 (t, J = 5.9 Hz, 4H), 1.40 (m, 4H), 0.94 (t, J = 7.5 Hz, 4H); MS (ES) m/z: 483 (M + H$^+$); FAB-HRMS (M + H$^+$). calcd 483.2508, found 483.2513.

8.1.10. 11-Ethyl-7,8,9,10,11,12,13,14,15,16-decahydro-24-methyl-6*H*,23*H*-5,26:17,22-dimethenodipyrido[2,3-*n*:3′,2′-*t*]pyrrolo[3,4-*q*][1,7,13]triazacycloheneicosine-23,25(24*H*)-dione (11). A mixture of **9** (14 mg, 0.028 mmol) and iodoethane (0.004 mL, 0.063 mmol) in THF (1.0 mL) was heated to reflux for 2 days. The reaction mixture was concentrated and the product was purified by column chromatography eluting with MeOH/CH_2Cl_2/NH_4OH to give **11** (12 mg, 75%) as an orange solid: ^{1}H NMR (400 MHz, CD_3OD) δ 8.27 (dd, J = 4.7, 1.6 Hz, 2H), 7.64 (s, 2H), 7.62 (dd, J = 8.0, 1.6 Hz, 2H), 7.03 (dd, J = 8.0, 4.7 Hz, 2H), 4.31 (m, 4H), 3.14 (s, 3H), 2.44 (m, 2H), 2.11 (m, 4H), 1.84 (m, 4H), 1.25 (m, 4H), 0.98 (m, 7H); MS (ES) m/z: 525 (M + H$^+$).

8.1.11. 11-Ethyl-7,8,9,10,11,12,13,14,15,16-decahydro-6*H*,23H-5,26:17,22-dimethenodipyrido[2,3-*n*:3′,2′-*t*][pyrrolo[3,4-*q*][1,7,13]triazacycloheneicosine-23,25(24*H*)-dione (12). Compound **11** (12 mg, 0.023 mmol) was transformed into **12** (6 mg, 50%) using the same procedure described for covert **9** to **10**. Compound **12** was isolated as an orange solid: ^{1}H NMR (400 MHz, CD_3OD) δ 8.27 (dd, J = 4.7, 1.4 Hz, 2H), 7.62 (s, 2H), 7.60 (dd, J = 7.7, 1.5 Hz, 2H), 7.03 (dd, J = 8.0, 4.7 Hz, 2H), 4.30 (m, 4H), 2.44 (q, J = 7.1 Hz, 2H), 2.11 (m, 4H), 1.83 (m, 4H), 1.26 (m, 4H), 0.98 (m, 7H); MS (ES) m/z: 511 (M + H$^+$); FAB-HRMS (M + H$^+$). calcd 511.2821, found 511.2840.

8.1.12. (4-Hydroxy-butyl)-(5-hydroxy-pentyl)-carbamic acid *tert*-butyl ester (15). A mixture of δ-valerolactone (1.7 mL, 18.3 mmol), 4-amino-1-butanol (1.7 mL, 18.3 mmol) in *m*-xylene (50 mL) was heated to 120 °C for 20 h. After it was cooled to 20 °C, the lower layer was separated from the upper xylene layer, and concentrated under reduced pressure to give crude **13** (3.50 g, 99%). A solution of crude **13** (1.91 g, 10.1 mmol) in THF (50 mL) was heated to reflux. Borane dimethylsulfide complex (20 mL, 2 M in THF, 40.0 mmol) was added dropwise via addition funnel. After the addition was completed, the mixture was refluxed for another h. It was cooled to 20 °C and quenched with MeOH (4.0 mL). Hydrogen chloride (12 mL, 1 M in Et_2O, 12.0 mmol) was added, and after the mixture was stirred at 20 °C for 10 min, it was concentrated under reduced pressure to give crude diol salt **14**. The crude **14** was then mixed with MeOH (40 mL), Et_3N (5.7 mL, 40.4 mmol) and Boc_2O (2.7 g, 12.1 mmol). After the mixture was refluxed for 3 h, it was cooled to 20 °C, concentrated and taken up in CH_2Cl_2 (40 mL). It was quickly washed with cold 1 N HCl, dried (Na_2SO_4) and concentrated. The product was purified by column chromatography eluting with EtOAc to give **15** (1.90 g, 70%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.67 (m, 4H), 3.18 (m, 4H), 1.60 (m, 10H), 1.45 (s, 9H); MS (ES) m/z: 298 (M + Na).

8.1.13. Methanesulfonic acid 5-[*tert*-butoxycarbonyl-(4-methanesulfonyloxy-butyl)-amino]-pentyl ester (16). A solution of **15** (1.90 g, 6.91 mmol) in CH_2Cl_2 (20 mL) was cooled in an ice bath, pyridine (2.2 mL, 27.6 mmol) was added, followed by MsCl (2.1 mL, 27.6 mmol). The mixture was stirred at 20 °C for 1.5 h. It was diluted with Et_2O (15 mL), and washed with cold 5% HCl and 5% NaOH. The organic phase was dried (Na_2SO_4) and concentrated. The product was purified by column chromatography on silica gel (eluting with hexane/EtOAc) to give **16** (2.40 g, 82%) as colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.24 (m, 4H), 3.19 (m, 4H), 3.01 (s, 3H), 3.00 (s, 3H), 1.75 (m, 4H), 1.64 (m, 2H), 1.56 (m, 2H), 1.45 (s, 9H), 1.41 (m, 2H); MS (ES) *m*/*z*: 454 (M + Na).

8.1.14. 23-[(2,4-Dimethoxyphenyl)methyl]-6,7,8,9,12,13,14,15,23,24-decahydro-22,24-dioxo-5,25:16,21-dimetheno-22*H*-dipyrido[2,3-*m*:3′,2′-*s*]pyrrolo[3,4-*p*][1,6,12]triazacycloeicosine-10(11*H*)-carboxylic acid, 1,1-dimethylethyl ester (17). A mixture of **5** (38 mg, 0.079 mmol) and Cs_2CO_3 (300 mg, 0.92 mmol) in DMF (12 mL) was heated to 70 °C. A DMF solution (2 mL) of bismesylate **16** (60 mg, 0.14 mmol) was added via syringe pump over 1 h. After the addition was complete, it was stirred at 70 °C for 22 h. The mixture was cooled to 20 °C, quenched with saturated aqueous ammonium chloride (30 mL), and diluted with EtOAc (50 mL). The organic phase was separated, washed with water (3 × 20 mL) and brine (15 mL). It was then dried (Na_2SO_4) and concentrated. The crude product was chromatographed on silica gel column (eluting with hexane/EtOAc) to give **17** (27 mg, 48%) as an orange solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (dd, J = 4.8, 1.5 Hz, 2H), 8.29 (m, 2H), 7.78 (s, 1H), 7.18 (dd, J = 8.0, 4.7 Hz, 2H), 7.10 (s, 1H), 6.85 (m, 1H), 6.46 (s, 1H), 6.43 (d, J = 2.4 Hz, 1H), 4.85 (s, 2H), 4.44 (m, 2H), 4.14 (m, 2H), 3.86 (s, 3H), 3.78 (s, 3H), 3.18 (m, 2H), 2.90 (m, 2H), 2.56 (m, 2H), 1.90 (m, 2H), 1.64 (m, 2H), 1.39 (s, 9H), 1.13 (m, 2H), 0.74 (m, 2H); MS (ES) *m*/*z*: 719 ($M + H^+$).

8.1.15. 23-[(2,4-Dimethoxyphenyl)methyl]-6,7,8,9,10,11,12,13,14,15-decahydro-5,25:16,21-dimetheno-22*H*-dipyrido[2,3-*m*:3′,2′-*s*]pyrrolo[3,4-*p*][1,6,12]triazacycloeicosine-22,24(23*H*)-dione (18). To a solution of **17** (27 mg, 0.037 mmol) in CH_2Cl_2 (2 mL) was added TFA (1.0 mL). The mixture was stirred at 20 °C for 30 min. Ammonium hydroxide was carefully added to adjust the pH of the mixture to 10. It was extracted with EtOAc (3 × 10 mL). The organic layers were combined, washed with water (10 mL), brine (5 mL), dried (Na_2SO_4) and concentrated to give **18** (22 mg, 100%) as an orange solid: ^{1}H NMR (300 MHz, CD_3OD) δ 8.27 (m, 2H), 7.77 (d, J = 8.0 Hz, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.61 (s, 1H), 7.52 (s, 1H), 7.13 (d, J = 8.3 Hz, 1H), 7.07 (m, 2H), 6.53 (s, 1H), 6.45 (d, J = 8.5 Hz, 1H), 4.77 (s, 2H), 4.26 (m, 4H), 3.84 (s, 3H), 3.83 (s, 3H), 2.44 (t, J = 7.1 Hz, 2H), 2.15 (t, J = 6.8 Hz, 2H), 1.78 (m, 4H), 1.31 (m, 2H), 1.20 (m, 2H), 1.01 (m, 2H); MS (ES) *m*/*z*: 619 ($M + H^+$).

8.1.16. 6,7,8,9,10,11,12,13,14,15-decahydro-22*H*-5,25:16,21-dimetheno-22*H*-dipyrido[2,3-*m*:3′,2′-*s*]pyrrolo[3,4-*p*][1,6,12]triazacycloeicosine-22,24(23*H*)-dione (19). To a solution of **18** (5 mg, 0.008 mmol) in CH_2Cl_2 (1.0 mL) was added methanesulfonic acid (0.5 mL). After the mixture was stirred at 20 °C for 6 h, ammonium hydroxide was added carefully to basify the mixture. It was extracted with EtOAc (2 × 10 mL), and the organic layers were combined, washed with water (5 mL), brine (5 mL), dried (Na_2SO_4) and concentrated. The product was purified by column chromatography on silica gel (eluting with $MeOH/CH_2Cl_2/NH_4OH$) to give **19** (5 mg, 100%) as an orange solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (m, 2H), 7.96 (d, J = 7.9 Hz, 1H), 7.55 (s, 1H), 7.53 (d, J = 8.1 Hz, 1H), 7.42 (s, 1H), 7.09 (dd, J = 8.0, 4.7 Hz, 1H), 6.97 (dd, J = 8.0, 4.7 Hz, 1H), 4.33 (t, J = 6.0 Hz, 2H), 4.22 (t, J = 6.6 Hz, 2H), 2.45 (t, J = 6.4 Hz, 2H), 2.32 (t, J = 6.3 Hz, 2H), 1.87 (m, 2H), 1.73 (m, 2H), 1.35 (m, 2H), 1.25 (m, 2H), 1.13 (m, 2H); MS (ES) *m*/*z*: 469 ($M + H^+$).

8.1.17. 23-[(2,4-Dimethoxyphenyl)methyl]-10-ethyl-6,7,8,9,10,11,12,13,14,15-decahydro-5,25:16,21-dimetheno-22*H*-dipyrido[2,3-*m*:3′,2′-*s*]pyrrolo[3,4-*p*][1,6,12]triazacycloeicosine-22,24(23*H*)-dione (20). A mixture of **18** (17 mg, 0.027 mmol), and iodoethane (5 μL, 0.062 mmol) in THF (0.8 mL) was refluxed for 2 days. It was cooled and concentrated under reduced pressure. The product was purified by column chromatography (eluting with $MeOH/CH_2Cl_2$) to give **20** (6 mg, 35%) as an orange solid: ^{1}H NMR (400 MHz, CD_3OD) δ 8.32 (dd, J = 4.7, 1.5 Hz, 1H), 8.25 (m, 2H), 7.85 (s, 1H), 7.32 (s, 1H), 7.27 (d, J = 7.8 Hz, 1H), 7.22 (dd, J = 8.0, 4.8 Hz, 1H), 7.14 (d, J = 8.4 Hz, 1H), 6.93 (dd, J = 8.0, 4.8 Hz, 1H), 6.54 (d, J = 2.3 Hz, 1H), 6.46 (dd, J = 8.4, 2.3 Hz, 1H), 4.79 (s, 2H), 4.45 (m, 2H), 4.15 (m, 2H), 3.84 (s, 3H), 3.77 (s, 3H), 2.83 (m, 4H), 2.27 (m, 2H), 1.99 (m, 2H), 1.65 (t, J = 6.4 Hz, 2H), 1.27 (m, 4H), 1.15 (m, 2H), 0.88 (t, J = 7.3 Hz, 3H); MS (ES) *m*/*z*: 647 ($M + H^+$).

8.1.18. 10-Ethyl-6,7,8,9,10,11,12,13,14,15-decahydro-22*H*-5,25:16,21-dimetheno-22*H*-dipyrido[2,3-*m*:3′,2′-*s*]pyrrolo[3,4-*p*][1,6,12]triazacycloeicosine-22,24(23*H*)-dione (21). To a solution of **20** (6 mg, 0.009 mmol) in CH_2Cl_2 (1.0 mL) was added methanesulfonic acid (0.2 mL). After the mixture was stirred at 20 °C for 2 h, ammonium hydroxide was added carefully to basify the mixture. It was extracted with EtOAc (2 × 10 mL), and the organic layers were combined, washed with water (5 mL), brine (5 mL), dried (Na_2SO_4) and concentrated. The product was purified by column chromatography on silica gel (eluting with $MeOH/CH_2Cl_2/NH_4OH$) to give **21** (4 mg, 90%) as an orange solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (m, 2H), 7.90 (m, 1H), 7.71 (m, 1H), 7.54 (s, 1H), 7.35 (s, 1H), 7.07 (dd, J = 7.8, 4.9 Hz, 1H), 7.00 (dd, J = 7.3, 4.7 Hz, 1H), 4.24 (m, 4H), 2.37 (m, 2H), 2.30 (m, 2H), 2.04 (m, 2H), 1.73 (t, J = 6.2 Hz, 4H), 1.24 (m, 4H), 0.95–1.02 (m, 5H); MS (ES) *m*/*z*: 497 ($M + H^+$); FAB-HRMS ($M + H^+$). calcd 497.2665, found 497.2682.

8.1.19. 5-Oxo-undecanedioic acid diethyl ester (22). A mixture of 4-oxo-1,9-nonanedicarboxylic acid (240 mg, 1.04 mmol), absolute ethanol (3.0 mL) and concentrated HCl (1.0 mL) was heated under reflux for 20 h. After

the mixture was cooled to 20 °C, it was diluted with EtOAc (25 mL), and neutralized with saturated aqueous $NaHCO_3$. The organic layer was separated, washed with water (5 mL), brine (5 mL), dried (Na_2SO_4) and concentrated to give **22** (270 mg, 91%) as colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.09–4.16 (m, 4H), 2.10–2.50 (m, 10H), 1.89 (q, J = 7.0 Hz, 2H), 1.54–1.68 (m, 4H), 1.25 (t, J = 7.1 Hz, 6H); MS (ES) m/z: 309 (M + Na).

8.1.20. 6-[2-(3-Ethoxycarbonyl-propyl)-[1,3]dioxolan-2-yl]-hexanoic acid ethyl ester (23). A mixture of **22** (270 mg, 0.94 mmol), ethylene glycol (0.24 mL, 4.30 mmol), triethyl orthoformate (0.48 mL, 2.89 mmol) and TsOH monohydrate (14 mg, 0.074 mmol) was refluxed for 45 min, and cooled to 20 °C. It was diluted with saturated aqueous $NaHCO_3$, and extracted with diethyl ether (2 × 20 mL). The organic layers were combined, washed again with $NaHCO_3$, dried (Na_2SO_4) and concentrated to give **23** (310 mg, 100%) as colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.08–4.16 (m, 4H), 3.92 (s, 4H), 2.26–2.33 (m, 4H), 1.58–1.72 (m, 8H), 1.30–1.35 (m, 4H), 1.25 (t, J = 7.2 Hz, 6H); MS (ES) m/z: 353 (M + Na).

8.1.21. 6-[2-(4-Hydroxy-butyl)-[1,3]dioxolan-2-yl]-hexan-1-ol (24). To a THF solution of $LiAlH_4$ (1.0 M, 1.50 mmol) was added **23** (330 mg, 0.94 mmol) in THF (5.0 mL) solution. After the mixture was stirred at 20 °C for 2 h, it was quenched with water, extracted with diethyl ether (3 × 20 mL). The organic layers were combined, dried (Na_2SO_4) and concentrated to give **24** (210 mg, 91%) as colorless liquid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.93 (s, 4H), 3.62–3.67 (m, 4H), 1.34–1.67 (m, 16H); MS (ES) m/z: 269 (M + Na).

8.1.22. 1,11-Dihydroxy-undecan-5-one (25). A mixture of **24** (210 mg, 0.85 mmol), water (3.4 mL), H_2SO_4 (6 M, 0.5 mL) and acetone (0.3 mL) was refluxed for 1.5 h. After the mixture was concentrated, the residue was extracted with CH_2Cl_2 (3 × 15 mL). The organic extracts were combined, dried (Na_2SO_4) and concentrated to give **25** (121 mg, 71%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.64 (s, 2H), 3.63 (t, J = 6.5 Hz, 4H), 2.39–2.46 (m, 4H), 1.25–1.78 (m, 12H); MS (ES) m/z: 225 (M + Na).

8.1.23. Methanesulfonic acid 11-methanesulfonyloxy-7-oxo-undecyl ester (26). To a methylene chloride (2.5 mL) solution of **25** (120 mg, 0.59 mmol) was added triethylamine (0.41 mL, 2.97 mmol) and MsCl (0.23 mL, 2.97 mmol) at 0 °C. The mixture was stirred at 20 °C for 2 h, and quenched with water. The layers were separated, and the aqueous phase was extracted with CH_2Cl_2 (2 × 20 mL). The organic phases were combined, washed sequentially with 5 mL of 5% HCl, water and 5% $NaHCO_3$. It was dried (Na_2SO_4) and concentrated to give **26** (169 mg, 80%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.20–4.25 (m, 4H), 3.64 (m, 2H), 3.01 (s, 3H), 3.00 (s, 3H), 2.34–2.49 (m, 4H), 1.32–1.78 (m, 10H); MS (ES) m/z: 381 (M + Na).

8.1.24. 8,9,11,12,13,14,15,16 - Octahydro - 24 - methyl - 6*H*,23*H*-5,26:17,22-dimethenodipyrido[2,3-*b*:3′,2′-*h*]pyrrolo[3,4-*e*][1,10]diazacycloheneicosine-10,23,25(7*H*,24*H*)-trione (27). A mixture of **3** (55 mg, 0.13 mmol), Cs_2CO_3 (370 mg, 1.13 mmol) and DMF (25 mL) was heated to 100 °C. A DMF (5 mL) solution of **26** (84 mg, 0.23 mmol) was added via syringe pump over 1.5 h. After the addition was complete, the mixture was stirred at 20 °C for 2 h. It was quenched with saturated ammonium chloride (30 mL), and extracted with methylene chloride (2 × 30 mL). The organic phases were combined, washed with water (3 × 20 mL), brine (30 mL), dried (Na_2SO_4) and concentrated. The product was purified by column chromatography (eluting with EtOAc/hexane) to give **27** (11 mg, 16%) as an orange solid: ^{1}H NMR (300 MHz, CD_3OD) δ 8.24–8.30 (ddd, J = 6.0, 4.7, 1.2 Hz, 2H), 7.82–7.85 (dd, J = 8.0, 1.3 Hz, 1H), 7.80 (s, 1H), 7.58 (s, 1H), 7.40 (dd, J = 8.1, 1.3 Hz, 1H), 7.09 (dd, J = 8.0, 4.7 Hz, 1H), 6.96 (dd, J = 8.1, 4.8 Hz, 1H), 4.34 (t, J = 5.8 Hz, 2H), 4.20 (t, J = 6.2 Hz, 2H), 3.14 (s, 3H), 2.32 (t, J = 7.1 Hz, 2H), 2.11 (t, J = 6.8 Hz, 2H), 1.69–1.84 (m, 4H), 1.37–1.41 (m, 2H), 1.18–1.31 (m, 2H), 1.07–1.16 (m, 2H), 0.90–1.04 (m, 2H); MS (ES) m/z: 510 ($M + H^+$).

8.1.25. 8,9,11,12,13,14,15,16 - Octahydro - 6*H*,23*H* - 5,26:17,22 - dimethenodipyrido[2,3 - *b*:3′,2′ - *h*]pyrrolo[3,4 - *e*][1,10]diazacycloheneicosine-10,23,25(7*H*,24*H*)-trione (28). Compound **27** (12 mg, 0.023 mmol) was converted into **28** (2 mg, 10%) using the same procedure described for converting **9** to **10**. Compound **28** was isolated as an orange solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (d, J = 5.0 Hz, 1H), 8.32 (d, J = 4.6 Hz, 1H), 7.84 (d, J = 8.0 Hz, 1H), 7.70 (s, 1H), 7.49 (s, 1H), 7.42 (m, 1H), 7.08 (dd, J = 8.0, 4.6 Hz, 1H), 6.95 (dd, J = 8.0, 4.5 Hz, 1H), 4.34 (t, J = 6.1 Hz, 2H), 4.20 (t, J = 6.2 Hz, 2H), 2.30 (t, J = 7.1 Hz, 2H), 2.12 (t, J = 6.7 Hz, 2H), 1.73–1.84 (m, 4H), 1.34–1.41 (m, 2H), 1.10–1.22 (m, 4H), 0.85–1.08 (m, 2H); MS (ES) m/z: 496 ($M + H^+$); FAB-HRMS ($M + H^+$). calcd 495.2805, found 495.2810.

8.1.26. 4-[2-(4-Hydroxy-butyl)-[1,3]dioxolan-2-yl]-butan-1-ol (30). A mixture of diethyl 5-oxoazelate (318 mg, 1.23 mmol), TsOH monohydrate (19 mg, 0.10 mmol), ethylene glycol (0.35 mL, 6.20 mmol) and triethyl orthoformate (0.62 mL, 3.72 mmol) was heated to reflux for 1 h, and cooled to 20 °C. It was diluted with saturated aqueous $NaHCO_3$ (5 mL), extracted with diethyl ether (3 × 15 mL). The organic layers were combined, washed with saturated $NaHCO_3$, dried (Na_2SO_4) and concentrated to give crude **29** (370 mg, 100%): MS (ES) m/z: 325 (M + Na). To a THF solution of $LiAlH_4$ (2.9 mL, 1 M in THF, 2.90 mmol) was added a solution of crude **29** (370 mg, 1.23 mmol) in THF (6 mL). The mixture was stirred at 20 °C for 2 h, and water was added to quenched excess $LiAlH_4$. It was extracted with diethyl ether (3 × 20 mL) and the combined diethyl ether extracts were dried over Na_2SO_4 and concentrated. The crude product was purified by column chromatography eluting with EtOAc to give **30** (168 mg, 63%) as colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.94 (s, 4H), 3.65 (t, J = 6.3 Hz, 4H), 1.43–1.67 (m, 12H); MS (ES) m/z: 241 (M + Na).

8.1.27. Methanesulfonic acid 9-methanesulfonyloxy-5-oxo-nonyl ester (31). To a solution of **30** (151 mg, 0.69

mmol) in methylene chloride (2 mL) was added triethylamine (0.48 mL, 3.45 mmol) and MsCl (0.27 mL, 3.45 mmol) at 0 °C. The mixture was stirred at 20 °C for 3 h, and quenched with water. The layers were separated, and the organic phase was washed with 5% HCl, water, 5% $NaHCO_3$ and brine sequentially. It was then dried over Na_2SO_4 and concentrated. The product was purified by column chromatography eluting with EtOAc/hexane to give **31** (192 mg, 84%) as a light brown oily solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.21 (m, 4H), 3.01 (s, 6H), 2.48 (m, 2H), 1.43–1.77 (m, 10H); MS (ES) *m*/*z*: 353 (M + Na).

8.1.2.8. 7,8,9,11,12,13,14-heptahydro-6*H*,21*H*-5,24:15,20-dimethenodipyrido[2,3-*b*:3′,2′-*h*]pyrrolo[3,4-*e*][1,10]diazacyclononadecine-10,21,23(22*H*)-trione (33). To a mixture of **5** (19 mg, 0.040 mmol), Cs_2CO_3 (160 mg, 0.50 mmol) and DMF (6 mL) was added dropwise a solution of **31** (24 mg, 0.072 mmol) in DMF (3 mL) at 70 °C. After stirring at 70 °C for 4 h, it was cooled in an ice bath, and quenched with aqueous NH_4Cl. It was extracted with EtOAc (2 × 30 mL), and the organic extracts were combined, washed with water (3 × 15 mL), brine (15 mL), dried (Na_2SO_4) and concentrated to give the crude **32**. The crude **32** was mixed with methylene chloride (1 mL), and methanesulfonic acid (0.3 mL) was added. The mixture was stirred at 20 °C for several hours until no **32** was detected by MS. It was cooled in an ice bath, and carefully quenched with ammonium hydroxide. The mixture was extracted with EtOAc (3 × 15 mL), and the extracts were washed with water (10 mL), brine (10 mL), dried (Na_2SO_4) and concentrated. The product was purified by column chromatography on silica gel (eluting with MeOH/CH_2Cl_2) to give **33** (12 mg, 67% from **31**) as an orange solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (d, *J* = 3.9 Hz, 2H), 7.80 (d, *J* = 7.9 Hz, 2H), 7.63 (s, 2H), 7.05 (dd, *J* = 8.0, 4.7 Hz, 2H), 4.26 (t, *J* = 6.0 Hz, 4H), 2.10 (t, *J* = 7.0 Hz, 4H), 1.71–1.80 (m, 4H), 1.32–1.39 (m, 4H); MS (ES) *m*/*z*: 468 (M + H$^+$). Anal. calcd for $C_{27}H_{25}N_5O_3 \cdot 2HCl \cdot 2H_2O$: C, 56.45; H, 5.09; N, 12.19. Found: C, 56.20; H, 5.23; N, 11.81.

8.1.29. Methanesulfonic acid 5-hydroxy-9-methanesulfonyloxy-nonyl ester (34). To a mixture of $NaBH_4$ (33 mg, 0.88 mmol) in ethanol (0.8 mL) was added a solution of **31** (48 mg, 0.14 mmol) in THF (1 mL) and ethanol (1 mL). The mixture was stirred at 20 °C for 40 min, and quenched with water (1 mL) and acetic acid (1 mL). The mixture was then extracted with CH_2Cl_2 (3 × 10 mL), and the combined organic layers were washed with water (10 mL), saturated aqueous $NaHCO_3$ (10 mL), dried (Na_2SO_4) and concentrated. The product was purified by column chromatography (eluting with EtOAc/hexane) to give **34** (38 mg, 79%) as colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.25 (t, *J* = 6.4 Hz, 4H), 3.62 (m, 1H), 3.01 (s, 6H), 1.74–1.82 (m, 4H), 1.47–1.62 (m, 8H); MS (ES) *m*/*z*: 355 (M + Na).

8.1.30. 22-[(2,4-Dimethoxyphenyl)methyl]-7,8,9,10,11,12,13,14-octahydro-10-hydroxy-6*H*,21*H*-5,24:15,20-dimethenodipyrido[2,3-*b*:3′,2′-*h*]pyrrolo[3,4-*e*].[1,10]diazacyclononadecine-21,23(22*H*)-dione (35)** To a mixture of **5** (33 mg, 0.069 mmol), Cs_2CO_3 (450 mg, 1.38 mmol) and DMF (12 mL) was added a DMF (4 mL) solution of **34** (33 mg, 0.10 mmol) at 60 °C over 30 min. After the addition was completed, the mixture was stirred at 80 °C for 3 h, and then 20 °C for 17 h. It was quenched with aqueous NH_4Cl (10 mL), and extracted with EtOAc (2 × 45 mL). The organic layers were combined, washed with water (3 × 20 mL), brine (20 mL), dried (Na_2SO_4) and concentrated. Purification with column chromatography eluting with acetone/CH_2Cl_2 gave **35** (27 mg, 64%) as an orange solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (dd, *J* = 4.6, 1.3 Hz, 2H), 7.69 (d, *J* = 7.9 Hz, 2H), 7.39 (s, 2H), 7.25 (s, 1H), 6.99 (dd, *J* = 8.0, 4.7 Hz, 2H), 6.46 (s, 1H), 6.44 (d, *J* = 2.3 Hz, 1H), 4.85 (s, 2H), 4.47–4.51 (m, 2H), 4.08–4.14 (m, 2H), 3.86 (s, 3H), 3.79 (s, 3H), 3.49 (d, *J* = 4.8 Hz, 1H), 3.26 (m, 1H), 1.73–1.83 (m, 4H), 0.93–1.04 (m, 8H); MS (ES) *m*/*z*: 620 (M + H$^+$).

8.1.31. 7,8,9,10,11,12,13,14-Octahydro-10-hydroxy-6*H*,21*H*-5,24:15,20-dimethenodipyrido[2,3-*b*:3′,2′-*h*]pyrrolo[3,4-*e*].[1,10]diazacyclononadecine-21,23(22*H*)-dione (36)** To a solution of **35** (18 mg, 0.029 mmol) in CH_2Cl_2 (2 mL) was added methanesulfonic acid (0.2 mL). The mixture was stirred at 20 °C for 15 h, and carefully quenched with ammonium hydroxide. It was then extracted with EtOAc (2 × 20 mL), and the combined organic extracts were washed with water (10 mL), brine (10 mL), dried (Na_2SO_4) and concentrated. Purification with column chromatography on silica gel (eluting with acetone/CH_2Cl_2) gave **36** (5 mg, 38%) as an orange solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (dd, *J* = 4.7, 1.5 Hz, 2H), 7.69 (dd, *J* = 7.9, 1.1 Hz, 2H), 7.49 (s, 1H), 7.40 (s, 2H), 7.03 (dd, *J* = 8.0, 4.7 Hz, 2H), 4.48–4.56 (m, 2H), 4.10–4.18 (m, 2H), 3.28 (m, 1H), 1.40–1.87 (m, 4H), 0.79–1.11 (m, 8H); MS (ES) *m*/*z*: 470 (M + H$^+$). Anal. calcd for $C_{27}H_{27}N_5O_3 \cdot 2HCl \cdot 1.7H_2O$: C, 56.59; H, 5.70; N, 12.22. Found: C, 56.63; H, 5.47; N, 12.13.

8.1.32. 2,6-Bis-[4-(*tert*-butyl-dimethyl-silanyloxy)-butyl]-pyridine (37). To a solution of 2,6-lutidine (0.5 mL, 4.30 mmol) in THF (15 mL) was added *n*-BuLi (6.4 mL, 1.6 M in hexane, 10.3 mmol) at −78 °C. The deep red solution was kept stirring at −78 °C for 30 min, and 3-bromo-propoxy-*tert*-butyldimethylsilane (2.4 mL, 10.3 mmol) was added. The mixture was warmed to 20 °C for 18 h, quenched with water (2 mL), and concentrated under reduced pressure. The residue was diluted with water (15 mL), extracted with hexane (3×20 mL). The organic extracts were combined, dried (Na_2SO_4) and concentrated. Purification by column chromatography (eluting with hexane/EtOAc) gave **37** (0.55 g, 30%) as colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (m, 1H), 6.91 (m, 2H), 3.59 (t, *J* = 6.4 Hz, 4H), 2.73 (m, 4H), 1.70 (m, 4H), 1.57 (m, 4H), 0.85 (s, 18H), 0.00 (s, 12H); MS (ES) *m*/*z*: 452 (M + H$^+$).

8.1.33. 4-[6-(4-Hydroxy-butyl)-pyridin-2-yl]-butan-1-ol (38). To a mixture of **37** (0.55 g, 1.20 mmol) in THF (3.0 mL) was added TBAF (2.6 mL, 1 M in THF, 2.60 mmol). It was stirred at 20 °C for 3 h, and concentrated under reduced pressure. Purification by column chromatography on silica gel (eluting with ethyl acetate containing 5% Et_3N) gave **38** (254 mg, 95%) as color-

less oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (t, J = 7.6 Hz, 1H), 6.98 (d, J = 7.6 Hz, 2H), 3.70 (t, J = 6.0 Hz, 4H), 2.83 (t, J = 7.4 Hz, 4H), 1.85 (m, 4H), 1.64 (m, 4H); MS (ES) m/z: 224 ($M+H^+$).

8.1.34. Methanesulfonic acid 4-[6-(4-methanesulfonyloxy-butyl)-pyridin-2-yl]-butyl ester (39). To a solution of diol **38** (254 mg, 1.14 mmol) in CH_2Cl_2 (4 mL) was added triethylamine (0.95 mL, 6.84 mmol) at 0 °C, followed by MsCl (0.35 mL, 4.56 mmol). After the mixture was stirred at 20 °C for 1.5 h, it was diluted with diethyl ether (20 mL) and washed with 5% HCl (5 mL). The layers were separated, and the organic phase was discarded. The aqueous phase was diluted with CH_2Cl_2 (10 mL), and treated with 5% NaOH (5 mL) till it turned basic. The mixture was extracted with CH_2Cl_2 (3×20 mL), and the organic extracts were combined, washed with brine (10 mL), dried (Na_2SO_4) and concentrated. Purification with column chromatography eluting with hexane/EtOAc gave **39** (162 mg, 38%) as light brown liquid: MS (ES) m/z: 380 ($M+H^+$).

8.1.35. 6,7,8,9,15,16,17,18-Octahydro-26-methyl-25*H*-5,28:19,24-dimetheno-10,14-nitrilodipyrido[2,3-*b*:3′,2′-*h*]pyrrolo[3,4-*e*][1,10]diazacyclotricosine-25,27(26*H*)-dione (40). A mixture of **3** (74 mg, 0.21 mmol), Cs_2CO_3 (290 mg, 0.89 mmol) and DMF (30 mL) was heated to 100 °C. A solution of **39** (100 mg, 0.26 mmol) in DMF (7 mL) was added via syringe pump over 2 h. After the addition was completed, the mixture was stirred at 20 °C for 18 h. It was quenched with saturated ammonium chloride, and extracted with ethyl acetate (3×50 mL). The organic extracts were combined, washed with water (3×30 mL), brine (30 mL), dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (eluting with acetone/methylene chloride) to give **40** (14 mg, 22%) as an orange solid and recover **39** (21 mg): ^{1}H NMR (300 MHz, CD_3OD) δ 8.14 (d, J = 4.6 Hz, 1H), 7.54–7.73 (m, 8H), 6.97 (m, 2H), 4.30 (t, J = 5.6 Hz, 4H), 3.14 (s, 3H), 2.65 (m, 4H), 1.73 (m, 4H), 1.31 (m, 4H); MS (ES) m/z: 531 ($M+H^+$).

8.1.36. 6,7,8,9,15,16,17,18-Octahydro-25*H*-5,28:19,24-dimetheno-10,14-nitrilodipyrido[2,3-*b*:3′,2′-*h*]pyrrolo[3,4-*e*][1,10]diazacyclotricosine-25,27(26*H*)-dione (41). A mixture of **40** (14 mg, 0.026 mmol), KOH (360 mg, 6.43 mmol) and ethanol (3 mL) was refluxed for 2 days. After it was cooled to 20 °C, the solvent was removed under reduced pressure. The residue was dissolved in water (5 mL), acidified with 10% citric acid and stirred at 20 °C for 10 min. It was extracted with methylene chloride (3×20 mL). The organic extracts were combined, dried (Na_2SO_4) and concentrated. The residue was mixed with ammonium acetate (2.5 g), and heated to 140 °C for 3 h. After it was cooled to 20 °C, water (10 mL) was added, and it was then basified with 20% NaOH. The mixture was extracted with EtOAc (3×20 mL), and the organic extracts were combined, washed with water (20 mL), brine (10 mL), dried (Na_2SO_4) and concentrated. The product was purified by column chromatography (eluting with acetone/CH_2Cl_2) to give **41** (4 mg, 30%) as a yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.28 (d, J = 4.0 Hz, 2H), 7.68 (m, 2H), 7.43–7.54 (m, 3H), 6.99 (dd, J = 7.9, 4.7 Hz, 2H), 6.87 (d, J = 7.4 Hz, 2H), 4.28 (t, J = 6.2 Hz, 4H), 2.65 (m, 4H), 1.81 (m, 4H), 1.46 (m, 4H); MS (ES) m/z: 517 ($M+H^+$); FAB-HRMS ($M+H^+$). calcd 517.2352, found 517.2343.

8.1.37. 3-[5-(3-Hydroxy-propyl)-thiophen-2-yl]-propan-1-ol (43). To a mixture of thiophene (0.50 mL, 6.25 mmol), anhydrous hexane (2.0 mL), TMEDA (2.2 mL, 14.4 mmol) was added *n*-BuLi (9 mL, 1.6 M in hexane, 14.4 mmol) at 20 °C. After it was refluxed for 30 min, the mixture was cooled to −40 °C and diluted with anhydrous THF (5 mL). A THF (3 mL) solution of (3-bromopropoxy)-*tert*-butyldimethylsilane (3.3 mL, 14.4 mmol) was added. The mixture was warmed slowly to 20 °C overnight, quenched with water (3 mL) and concentrated. The residue was diluted with hexane (50 mL), washed with water (15 mL), brine (15 mL), dried (Na_2SO_4) and concentrated under reduced pressure to give crude **42** (2.60 g): MS (ES) e/z: 451 ($M+Na$). The crude **42** was dissolved in THF (6 mL), and TBAF (18 mL, 1 M in THF, 18.0 mmol) was added. After the mixture was stirred at 20 °C for 2 h, it was concentrated under reduced pressure. Purification with column chromatography on silica gel eluting with ethyl acetate/hexane gave **43** (0.44g, 30%) as colorless oil: ^{1}HNMR (300 MHz, $CDCl_3$) δ 6.60 (s, 2H), 3.71 (t, J = 6.3 Hz, 4H), 2.87 (t, J = 7.4 Hz, 4H), 1.87–1.96 (m, 4H); MS (ES) e/z: 223 ($M+Na$).

8.1.38. Methanesulfonic acid 3-[5-(3-methanesulfonyloxy-propyl)-thiophen-2-yl]-propyl ester (44). A solution of **43** (0.44 g, 2.20 mmol) in CH_2Cl_2 (5 mL) was cooled in an ice bath, and triethylamine (3.0 mL, 22.0 mmol) and MsCl (1.7 mL, 22.0 mmol) were added. The mixture was stirred at 20 °C for 30 min, and then quenched with water (2 mL). It was extracted with CH_2Cl_2 (15 mL), and the combined organic extracts were combined, dried (Na_2SO_4) and concentrated. Purification with column chromatography on silica gel eluting with ethyl acetate/hexane gave **44** (245 mg, 31%) as light brown oil: ^{1}HNMR (300 MHz, $CDCl_3$) δ 6.63 (s, 2H), 4.26 (t, J = 6.2 Hz, 4H), 3.00 (s, 6H), 2.91 (t, J = 7.3 Hz, 4H), 2.07-2.14 (m, 4H); MS (ES) e/z: 379 ($M+Na$).

8.1.39. 23-[(2,4-Dimethoxyphenyl)methyl]-6,7,8,13,14,15-hexahydro-9,12-epithio-5,25:16,21-dimethenodipyrido[2,3-*b*:3′,2′-*h*]pyrrolo[3,4-*e*][1,10]diazacyclotricosine-22,24(23*H*)-dione (45). To a mixture of **5** (42 mg, 0.088 mmol), Cs_2CO_3 (550 mg, 1.68 mmol) and DMF (15 mL) at 70 °C was added a DMF (3 mL) solution of **44** (56 mg, 0.15 mmol). The mixture was stirred at 90 °C for 3.5 h, and then 20 °C for 18 h. It was quenched with aqueous NH_4Cl (5 mL), diluted with water (10 mL) and extracted with EtOAc (3×20 mL). The organic layers were combined, washed with water (3×10 mL), brine (10 mL), dried (Na_2SO_4) and concentrated. Purification with column chromatography eluting with acetone/CH_2Cl_2 gave **45** (11 mg, 39%) as an orange solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (dd, J = 4.7, 1.4 Hz, 2H), 7.79 (d, J = 7.9 Hz, 2H), 7.33 (s, 2H), 6.97 (dd, J = 8.0, 4.7 Hz, 2H), 6.59 (s, 1H), 6.47 (m, 2H), 6.33 (s, 2H), 4.85 (s, 2H), 4.33 (t, J = 5.4 Hz, 4H), 3.87 (s, 3H),

3.78 (s, 3H), 2.82 (m, 4H), 2.27 (m, 4H); MS (ES) m/z: 644 ($M+H^+$).

8.1.40. 6,7,8,13,14,15-Hexahydro-9,12-epithio-5,25:16,21-dimethenodipyrido[2,3-*b*:3′,2′-*h*]pyrrolo[3,4-*e*][1,10]diazacyclotricosine-22,24(23*H*)-dione (46). To a solution of **45** (11 mg, 0.017 mmol) in CH_2Cl_2 (2 mL) was added methanesulfonic acid (0.2 mL). The mixture was stirred at 20 °C for 15 h, and carefully quenched with ammonium hydroxide. It was then extracted with EtOAc (2×15 mL), and the combined organic extracts were washed with water (3×10 mL), brine (10 mL), dried (Na_2SO_4) and concentrated. Purification with column chromatography on silica gel (eluting with acetone/CH_2Cl_2) gave **46** (3.5 mg, 50%) as an orange solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.32 (dd, $J=4.7$, 1.5 Hz, 2H), 7.76 (m, 2H), 7.33 (s, 2H), 7.00 (dd, $J=8.0$, 4.7 Hz, 2H), 6.34 (s, 2H), 4.35 (t, $J=5.6$ Hz, 4H), 2.83 (t, $J=5.7$ Hz, 4H), 2.28 (m, 4H); MS (ES) m/z: 494 ($M+H^+$). Anal. calcd for $C_{28}H_{23}N_5O_2S\cdot0.39H_2O$: C, 66.91; H, 4.81; N, 13.93. Found: C, 67.25; H, 4.57; N, 13.54.

8.2. Biology

8.2.1. GSK-3β kinase assay. Compounds were tested for the ability to inhibit recombinant rabbit GSK-3β (New England Biolabs) using the following protocol. Protein phosphatase inhibitor-2 (PPI-2, Calbiochem) phosphorylation was measured using a standard filtration assay (MultiScreen-DV/Millipore). Briefly, the test compounds were added to a reaction mixture containing PPI-2 (45 ng), GSK-3β (0.75 units) and ^{33}P-ATP (1 μCi) in 50 mM Tris–HCl (pH 8.0), 10 mM $MgCl_2$, 0.1% BSA, 1 mM DTT, and 100 μM activated Sodium Orthovanadate. The total ATP concentration was 25 μM. After 90 min incubation at 30 °C, the phosphorylated PPI-2 was precipitated using one volume of 20% trichloroacetic acid (TCA). Filter plates were subsequently washed with 10% TCA and radioactivity was quantified using a TopCount Scintillation Counter (Packard). IC_{50} values were determined from at least three separate experiments and the K_i values ± SEM were calculated using the Cheng–Prussof equation.

8.2.2. PKCßII assays. Compounds were tested for the ability to inhibit recombinant human PKCßII using the following protocol. Histone H1 phosphorylation was measured using a standard filtration assay. Briefly, the test compounds were added to a reaction mixture containing histone H1 (0.1 mg/mL), the PKCßII (5-10 mU) and ^{33}P-ATP (1 μCi) in 20 mM Hepes (pH 7.4), 0.03% Triton X-100, 0.1 mM $CaCl_2$, 10 mM MgAcetate, 0.1 mg/mL phosphatidylserine, and 10 μg/mL diacylglycerol. The total ATP concentration was 100 μM. After 40 min incubation at room temperature, the reaction was stopped by the addition of 5 μL of a 3% phosphoric acid solution. Reaction mix was then spotted onto a P30 filtermat and the filters were washed three times for 5 min with 75 mM phosphoric acid. The filtermat was then washed one time with MeOH and dried prior to counting.

8.2.3. CDK2 kinase assay. A kinase reaction mixture was prepared containing 8 mM MOPS (pH 7.0), 0.2 mM EDTA, 0.1 mg/mL histone-H1, 10 mM MgAcetate, and 0.2 μCuries per well ^{33}P-γ-ATP (2000–3000 Ci/mmol). After incubation for 40 min at room temperature, the reaction was terminated by the addition of 5 μL of a 3% phosphoric acid solution. 10 μL of the reaction mixture was then spotted onto a P30 filtermat and washed three times for 5 min in 75 mM phosphoric acid and once in methanol prior to drying and counting on a scintillation counter.

8.2.4. Rsk3 kinase assay. A kinase reaction mixture was prepared containing 8 mM MOPS (pH 7.0), 0.2 mM EDTA, 30 μM KKKNRTLSVA, 10 mM MgAcetate, and 0.2 μCuries per well ^{33}P-γ-ATP (2000–3000 Ci/mmol). After incubation for 40 min at room temperature, the reaction was terminated by the addition of 5 μL of a 3% phosphoric acid solution. 10 μL of the reaction mixture was then spotted onto a P30 filtermat and washed three times for 5 min in 75 mM phosphoric acid and once in methanol prior to drying and counting on a scintillation counter.

8.2.5. Protein kinase selectivity panel (Upstate Biotech Inc.). Protein kinase selectivity assays were performed as previously described.[31,40] Briefly, protein kinases were assayed for their ability to phosphorylate the appropriate peptide/protein substrates in the presence of 10 μM compound. Assays were done using 100 μM ATP and were linear with respect to time. IC_{50} values were determined from at least three separate experiments and the K_i values ± SEM were calculated using the Cheng–Prussof equstion.

8.2.6. Glycogen synthase assay. Compounds were tested for the ability to increase glycogen synthase (GS) activity in living cells (HEK293 cells). To do this, cell extracts were prepared from cells treated with compounds or vehicle and GS activity measured using a modified protocol.[17] Briefly, cells were serum (and glucose) starved for 3 h and treated with the appropriate compounds for 90 min at 37 °C. Cells were then washed, scraped and collected by centrifugation prior to lysis using three freeze/thaw cycles. Lysates were then clarified by centrifugation and the supernatants assayed for GS activity. To do this, ^{14}C-UDP glucose incorporation into glycogen was measured in the absence or presence of glucose 6-phosphate. The EC_{50} for GS activation was then determined and compared with lithium.

8.2.7. Molecular docking procedures. The X-ray structure of unbound GSK-3β (1 h8f.pdb)[35] was used as the target structure for docking. Its ATP-binding site was determined by superimposition to the X-ray structure of CDK2 and staurosporine complex (1aq1.pdb)[36] and defined as the spatial region occupied by staurosporine. Conformational searching was first conducted for compound **46**, using the Monte Carlo Multiple Minimum (MCMM)[41] method implemented in MacroModel,[42] leading to 260 low-energy and unique conformers. The cutoff of energy window was set as 50 kj/mol, and the Monte Carlo steps were set to 10,000 times. Conformational structures were not considered unique unless the least squares superimposition of heavy atoms found one

or more pairs of equivalent atoms separated by more than 0.25 Å. All of the 260 conformational structures were then individually docked into the ATP binding-site of GSK-3β, using the program Glide.[43] Both the compound **46** conformational structure and the GSK-3β protein structure were held rigid during the docking. The docking poses were evaluated by the empirical scoring function, GlideScore.[44] The GSK-3β structure complexed with the best-scoring pose of compound **46** was further optimized by full energy minimization, and then treated as the binding structure of GSK-3β and compound **46** (Supporting Information). All of the molecular mechanism calculations were done with the program MacroModel,[42] using OPLS_AA force field.[45] The effect of aqueous solution was treated by GB/SA model.[46]

9. Abbreviations

GSK-3β, glycogen synthase kinase-3β; AMPK, AMP-activated protein kinase; Blk, B lymphocyte kinase; CAMKII, calmodulin-dependent protein kinase II; CAMKIV, calmodulin-dependent protein kinase IV; CDK1/cyclinB, cycline-dependent protein kinase 1; CDK2/cycA, cyclin-dependent protein kinase 2; CDK5/p35, cyclin-dependent protein kinase 5; CDK6/cyclin D3, cyclin-dependent protein kinase 6; CDK7/cyclin H, cyclin-dependent protein kinase 7; CHK1, checkpoint kinase 1; CHK2, checkpoint kinase 2; CK1, casein kinase 1; CK2, casein kinase 2; CSK, carboxy-terminal Src kinase; Fes, cellular product of the *fes* proto-oncogene; Fyn, product of *fyn* proto-oncogene; FGFR3, FGF receptor kinase 3; IGF-1R, insulin-like growth factor-1 receptor kinase; IKK, I kappa B kinase; IR, insulin receptor kinase; JNK1α1, c-Jun N-terminal kinase 1α1; JNK2α2, c-Jun N-terminal kinase 2α2; JNK3, c-Jun N-terminal kinase 3; Lck, lymphocyte kinase; Lyn, Lck/Yes-related tyrosine kinase; MAPK1, mitogen-activated protein kinase 1; MAPK2, mitogen-activated protein kinase 2; MAPKAP-K2, MAPK-activated protein kinase 2; MEK1, MAPK/ERK kinase 1; MKK4, MAPK kinase 4; MKK6, MAPK kinase 6; MKK7β, MAPK kinase 7β; MSK1, mitogen- and stress-activated protein kinase 1; PAK2, p21-activated protein kinase-2; PDGFR, platelet-derived growth factor receptor; PDK1, 3-phosphoinositide-dependent protein kinase 1; PKA, camp-dependent protein kinase; PKBα, protein kinase B α (also called Akt); PKBβ, protein kinase B β; PKC, protein kinase C; PRAK, p38-regulated/activated kinase; c-Raf, cellular product of *raf* proto-oncogene; RsK, ribosomal S6 kinase; SAPK2b, stress-activated protein kinase 2b (also known as p38β2); P70S6K, p70 ribosomal protein S6 kinase; c-SRC, cellular product of *src* oncogene, Syk, splenic tyrosine kinase; Yes, cellular product of the *yes* proto-oncogene; ZAP-70, zeta-associated protein kinase-70.

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