Structure−Activity Relationship of Triazafluorenone Derivatives as Potent and Selective mGluR1 Antagonists

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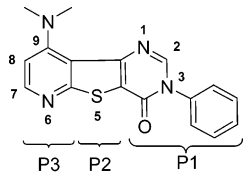
SAR (structure−activity relationship) studies of triazafluorenone derivatives as potent mGluR1 antagonists are described. The triazafluorenone derivatives are non-amino acid derivatives and noncompetitive mGluR1 antagonists that bind at a putative allosteric recognition site located within the seven-transmembrane domain of the receptor. These triazafluorenone derivatives are potent, selective, and systemically active mGluR1 antagonists. Compound **1n**, for example, was a very potent mGluR1 antagonist (IC_{50} = 3 nM) and demonstrated full efficacy in various in vivo animal pain models.

Introduction

Glutamate is the most abundant excitatory neurotransmitter in the central nervous system. Glutamate modulates activity of many types of synapses by activating G-protein coupled receptors (GPCR) called metabotropic glutamate receptors (mGluRs).[1] There are three different groups, groups I, II, and III, of mGluRs with a total of eight distinct subtypes, mGluR1 to mGluR8, based on their primary sequence similarity, signal transduction linkages, and pharmacological profile.[2] Group 1 mGluRs, mGluR1 and mGluR5, play key roles in the central sensitization of pain, in addition to a variety of functions with potential implications in neurological and psychiatric disorders.[3] Glutamate and other excitatory amino acids are released from nerve endings in the periphery under inflammatory conditions. Glutamate or mGluR group 1 agonists induce hyperalgesia when administrated peripherally.[4] mGluRs modulate pain transmission in the spinal cord, most likely via sensitization of dorsal horn neurons to sustain high-intensity C-fiber input.[2c] Normalization of glutamatergic neurotransmission in the spinal cord and nociceptive afferents via inhibition of the group I mGluRs is manifested in the attenuation of pain.[5] There is mounting evidence to support a role of peripheral mGluRs in nociception and pain.[6]

We have investigated mGluR1 antagonists as a therapy for the treatment of pain. The role of mGluR1 in the treatment of pain has not been validated, due to lack of potent, selective, and systemically active mGluR1 antagonists. Earlier efforts to evaluate amino acid antagonists, competing with the glutamate binding site, were not successful due to poor selectivity, weak antagonism, and lack of CNS availability.[3] The first reported noncompetitive, non-amino acid-like mGluR1 antagonist, CPCCOEt (7-(hydroxyimino)cyclopropa[b]chromen-1a-carboxylate ethyl ester), elucidated an allosteric binding site for the target.[7] Other noncompetitive antagonists, such as BAY 36-7620,[8] R214127,[9] dicarboxypyrroles,[10] and JNJ16259685,[11] were also published. Instead of competing at the glutamate binding N-terminal extracellular domain (ECD), the reported noncompetitive mGluR1 antagonists bind at the seven-transmembrane domain (7-TMD).[12] The allosteric modulation provides additional possibilities for non-amino acid-like small molecules of mGluR1 antagonists with superior physicochemical properties which could facilitate the target validation process for the treatment of pain.



1a

*h*mGluR1 = 20 nM

*h*mGluR5 > 100000 nM

Figure 1. Triazafluorenone derivative identified from HTS.

Triazafluorenone derivatives (Figure 1) were identified as potent group I mGluRs antagonists in our high-throughput screening (HTS) of this target. This type of tricyclic heterocycle was first synthesized by Kadushkin and co-workers.[13] The synthetic approach described in their paper was, however, lengthy, low yielding, and difficult to apply to high-throughput synthesis. Therefore, we needed to modify these procedures to suit high-throughput synthesis for variations at several moieties of the 5-thia-1,3,6-triazafluroenone pharmacophore. These triazafluorenone derivatives consist of three primary moieties: pyridine, thiophene, and pyrimidone (Figure 1). We report here new triazafluorenone derivatives **1a**−**ah** and their analogues, as potent and selective mGluR1 antagonists. These new mGlu1 receptor antagonists exhibited efficacy in several rat in vivo pain models.

Chemistry

Variation at the P1 moiety (*N3*-substitutent) of the triazafluorenone pharmacophore with both aryl and

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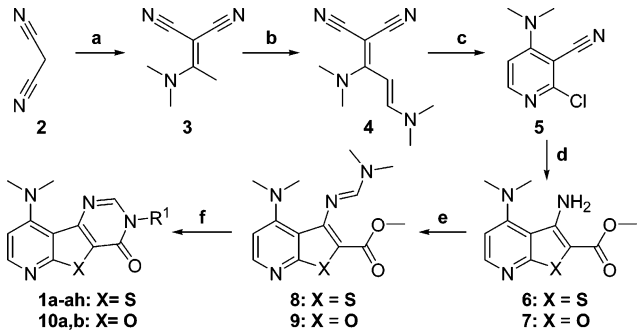
Scheme 1[a]



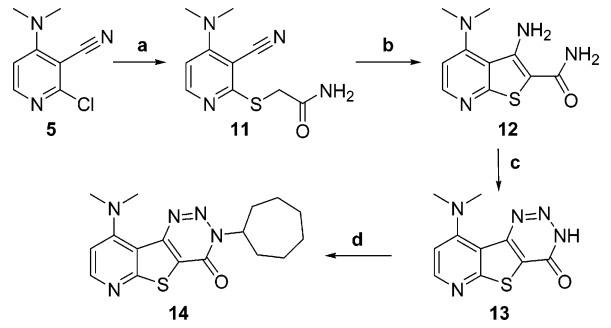
[a] (a) $Me_2NCMe(OMe)_2$, EtOH, 23 °C, 2 days, 96%. (b) $Me_2NCH(OMe)_2$, 100 °C, 1.5 h, 93%. (c) HCl, MeOH, 0 °C, 9 h, 98%. (d) NaH, THF, $HSCH_2CO_2Me$ (85%); $HOCH_2CO_2Me$ (76%). (e) $Me_2NCH(OMe)_2$, reflux, 5 h (X = S 99%, X = O 100%). (f) R^1NH_2, toluene, *p*-TsOH.

alkyl groups required synthetic methods to accommodate the SAR studies. The Ullmann reaction could be applied to aryl group attachment to the nitrogen atom (*N3*) of the pyrimidone ring.[14] Available aryl reagents suitable for the Ullmann coupling reactions only provided limited aryl groups using this approach. Moreover, products generated as mixtures of *O*-arylation and *N*-arylation using this strategy also complicated purification.

Scheme 1 shows a viable synthetic approach for the synthesis of triazafluorenone compounds with variation of the R^1 group for both aryl and alkyl substitutions (P1 moiety). A solution of malononitrile (**2**) in diethyl ether was reacted with *N*,*N*-dimethylacetamide dimethyl acetal in ethanol at 0 °C, and then warmed to room temperature to give 2-(1-dimethylaminoethylidene)-malononitrile (**3**) in quantitative chemical yield.[15] Compound **3** was then reacted with *N*,*N*-dimethylformamide dimethyl acetal by heating at reflux to yield 2-(1,3-bis-(dimethylamino)allylidene)malononitrile (**4**),[16] which could be purified through recrystallization in cold methanol five times to give a combined 93% yield. Intermediate **4** was then treated with anhydrous HCl at 0 °C and cyclized to form the desired product, 4-dimethylamino-2-chloro-3-cyanopyridine (**5**, 98%).[17] This product could be used for the next reaction without further purification. In the presence of sodium hydride, nucleophilic displacement of the 2-chloro atom of the pyridine compound **5**, followed by reaction with methyl thioglycolate and cyclization in THF, gave a bicyclic aminoester intermediate, 5-amino-4-dimethylaminothieno[8,9-*b*]pyridine-6-carboxylic acid methyl ester (**6**) (85%).[18] The formamidine derivative, 4-dimethylamino-5-(dimethylaminomethyleneamino)thieno-[8,9-*b*]pyridine-6-carboxylic acid methyl ester (**8**), was obtained in quantitative yield after condensation with *N*,*N*-dimethylformamide dimethyl acetal. This formamidine derivative **8** was a key intermediate, which was used to make a wide variety of R^1 substituted derivatives, from aryl to alkyl 5-thia-1,3,6-triazafluroenone derivatives **1a**−**ah** in parallel fashion, catalyzed by *p*-toluenesulfonic acid in toluene (Scheme 1).

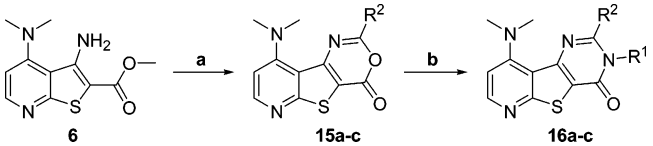
The furan analogues **10a**,**b** (X = O) were made from the corresponding furan aminoester intermediate, 5-amino-4-dimethylaminofuro[8,9-*b*]pyridine-6-carboxylic acid methyl ester (**7**). The latter was made using methyl glycolate under the same conditions as the

Scheme 2[a]



[a] (a) $HSCH_2CONH_2$, NaOMe, MeOH, 96%. (b) *t*-BuOK, MeOH−pyridine, 98%. (c) $NaNO_2$, 0 °C, 91%. (d) Cycloheptyl bromide, NaH, DMF.

Scheme 3[a]



[a] (a) $(R^2CO)_2O$, reflux, 2 h. (b) R^1NH_2, AcOH, reflux, 2 h.

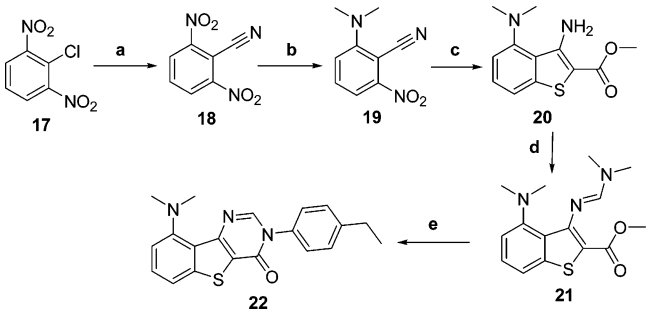
thiophene analogue, 5-amino-4-dimethylaminothieno-[8,9-*b*]pyridine-6-carboxylic acid methyl ester (**6**, Scheme 1).

Tetraazafluorenone **14** was prepared as described in Scheme 2. 2-Mercapto acetamide was reacted with intermediate **5** in the presence of sodium methoxide in methanol to generate the noncyclized intermediate, 2-(3-cyano-4-dimethylaminopyridin-2-ylsulfanyl)acetamide (**11**) in 96% yield. This intermediate was then treated with potassium *t*-butoxide in a mixture of methanol and pyridine to give the cyclized product **12** in quantitative yield. The amino amide, 5-amino-4-dimethylaminothieno-[8,9-*b*]pyridine-6-carboxylic acid amide (**12**), was oxidized with sodium nitrate at 0 °C to form the tricyclic heterocycle, 9-dimethylamino-*3H*-5-thia-1,2,3,6-tetraazafluoren-4-one (**13**) (91%).[19] With this tetraazafluorenone intermediate **13** in hand, the 3-substituted tetraazafluorenone **14** could be made by reaction of the corresponding alkyl halide using sodium hydride in dimethylformamide (DMF) with moderate chemical yield (Scheme 2) accompanied by small amounts of *O*-alkylation product.[20]

2-Substituted triazafluorenones **16a**−**c** were prepared as described in Scheme 3. The amino methylester **6** could be converted to another key intermediate oxazinones **15a**−**c**, in the presence of the desired carboxylic acid anhydride with good yields (Scheme 3).[21] The oxazinones **15a**−**c** were then treated with amine in acetic acid to generate the 2-substituted triazafluorenone derivatives **16a**−**c** with good yields.

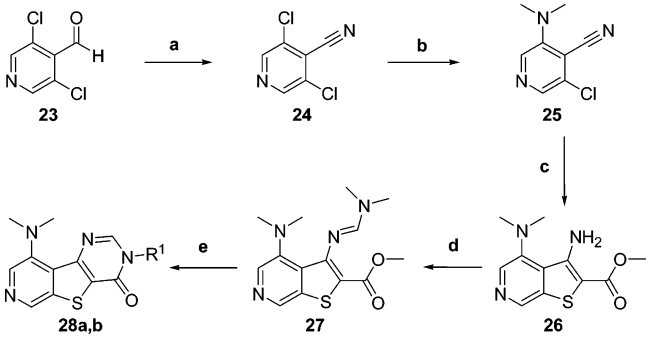
The diazofluorenone analogue **22** was made using a different route. 1,3-Dinitro-2-chlorobenzene (**17**) was reacted with cuprous cyanide in DMF to give 1,3-dinitro-2-cyanobenzene (**18**).[22] One of the nitro groups of dinitrocyanobenzene **17** was displaced with dimethylamine to generate 1-dimethylamino-2-cyano-3-nitrobenzene (**19**).[23] The other nitro group of the nitrobenzene **19** was reacted with methyl thioglycolate, followed by cyclization to form bicyclic aminoester, 5-amino-4-dimethylaminothieno[8,9-*b*]benzo-6-carboxylic acid methyl ester (**20**), in the presence of sodium methoxide. The ben-

Scheme 4[a]



[a] (a) CuCN, DMF, 145 °C, 2 h, 70%. (b) Me_2NH, DMF, 50 °C, 10 min, 95%. (c) $HSCH_2CO_2Me$, NaOMe, DMF, 23 °C, 16 h, 88%. (d) $Me_2NCH(OMe)_2$, EtOH, reflux, 16 h, 90%. (e) *p*-Ethylaniline, toluene, *p*-TsOH (cat.) reflux 16 h, 13%.

Scheme 5[a]



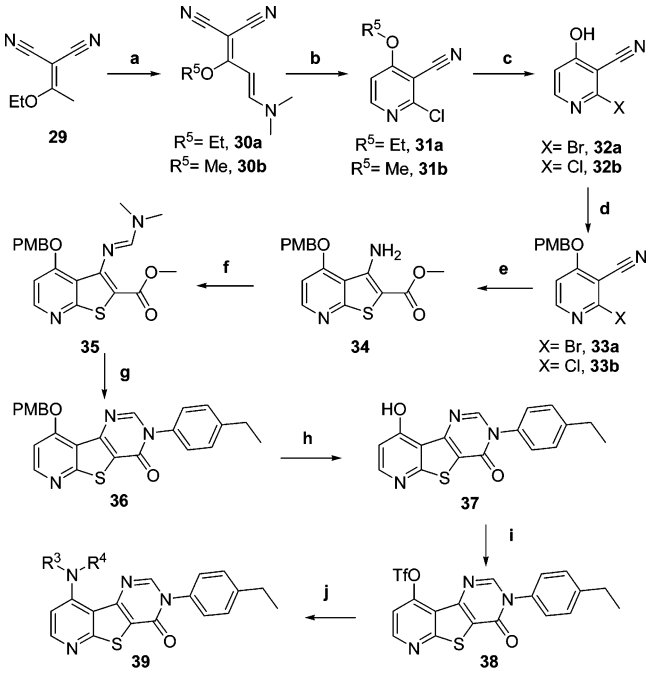
[a] (a) NH_2OH, CHO_2H, H_2SO_4 (cat.), reflux 6 h, 83%. (b) Me_2NH, DMF, 23 °C, 4 h, 75%. (c) $HSCH_2CO_2Me$, NaOMe, DMF, 23 °C, 16 h, 88%. (d) $Me_2NCH(OMe)_2$, EtOH, reflux 16 h, 90%. (e) R^1NH_2, toluene, *p*-TsOH (cat.), reflux 16 h.

zoaminoester **20** was converted to formamidine intermediate **21** by heating with *N*,*N*-dimethylformamide dimethyl acetal. Catalyzed by *p*-toluenesulfonic acid in toluene, the intermediate **21** formed the pyrimidone ring with an aryl or alkyl amine to produce **22** (Scheme 4).

Isomeric fused pyridine analogues **28a**,**b** were prepared as described in Scheme 5. Dichloropyridinecarboxaldehyde **23** was condensed with hydroxylamine, which after dehydration formed 3,5-dichloro-4-cyanopyridine (**24**).[24] One of the chloro groups of **24** was easily displaced by aqueous dimethylamine to generate 3-chloro-4-cyano-5-dimethylaminopyridine (**25**). A nucleophilic displacement of the 3-chloro group of **25** by thioglycolate, followed by cyclization, afforded aminoester analogue, 3-amino-4-dimethylaminothieno[2,3-*c*]pyridine-2-carboxylic acid methyl ester (**26**).[25] The aminoester **26** was heated to reflux with *N*,*N*-dimethylformamide dimethyl acetal yielding formamidine intermediate **27** quantitatively. The formamidine intermediate **27** was then reacted with either 4-methyl- or 4-ethylanaline in the presence of catalyst, *p*-toluenesulfonic acid, to form the final triazafluorenone products **28a** and **28b**, respectively (Scheme 5).

Variations of R^3 and R^4 at the 4-amino group of the pyridine moiety (P3 moiety) were achieved via triflate intermediate **38** (Scheme 6). 2-(1-Ethoxyethylidene)-malononitrile (**29**) was reacted with *N*,*N*-dimethylformamide dimethyl acetal and generated a mixture of desired products **30** (**30a**, R^5 = ethyl; **30b**, R^5 = methyl).[26] The mixture of **30a**,**b** was then treated with anhydrous HCl and converted to a mixture of pyridines,

Scheme 6[a]



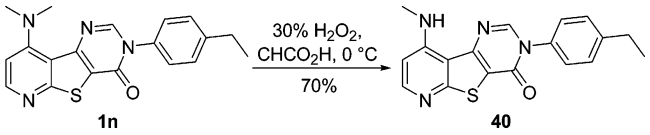
[a] (a) $Me_2NCH(OMe)_2$, 100 °C, 1 h, 78%. (b) HCl, MeOH, 0 °C, 14.5 h, 98%. (c) HBr (30% in AcOH), 100 °C, 2 h, 100%. (d) PMBnCl, NaH, DMF, 60 °C, 2.5 h, 37%. (e) $HSCH_2CO_2Me$, NaOMe, DMF, 12 h, 86%. (f) $Me_2NCH(OMe)_2$, reflux 10 h, 100%. (g) *p*-ethylaniline, toluene, *p*-TsOH (cat.), microwave 160 °C, 1 h, 39%. (h) TFA, 0 °C, 1.2 h, 100 %. (i) Tf_2NPh, $EtN(^iPr)_2$, 80%. (j) R^3R^4NH, CH_2Cl_2, 23 °C.

2-chloro-3-cyano-4-ethoxypyridine (**31a**) and 2-chloro-3-cyano-4-methoxypyridine (**31b**). The pyridine mixture of **31a**,**b** was deprotected with HBr (30%), giving the desired product, 2-bromo-3-cyano-4-hydroxypyridine (**32a**), with a small amount of 2-chloro-3-cyano-4-hydroxypyridine (**32b**).[27] Protecting the 4-hydroxy group of **32a** and **32b** with *p*-methoxybenzylchloride (PMBCl) in the presence of NaH yielded protected pyridine analogues **33a** (37%) and **33b** (8%). The protected pyridines **33a** and **33b** were then condensed with methyl thioglycolate, followed by cyclization to form aminoester intermediate, 5-amino-4-(4′-methoxybenzyloxy)thieno[8,9-*b*]pyridine-6-carboxylic acid methyl ester (**34**, 89%). This aminoester **34** was converted to formamidine derivative **35** in the presence of *N*,*N*-dimethylformamide dimethyl acetal in quantitative yield. The triazafluorenone analogue **36** was generated in the presence of *p*-ethylaniline and catalyst, *p*-toluenesulfonic acid, in toluene (39%). 9-(4′-Methoxybenzyloxy)-3-(4′-ethylphenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (**36**) was then deprotected with trifluoroacetic acid (TFA) to give 9-hydroxy-3-(4′-ethylphenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (**37**, 100%).[28] The hydroxy group of **37** was converted to a triflate group in the presence of *N*,*N*-bis-(trifluoromethanesulfonyl)phenylamide.[29] The key triflate intermediate **38** was used to prepare analogues **39a**−**f** by addition of the appropriate amine in dichloromethane at room temperature (Scheme 6).

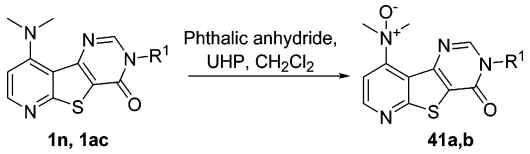
The 9-methylamino triazafluorenone derivative **40** was obtained by demethylation of **1n** using hydrogen peroxide and formic acid in excellent chemical yield (79%, Scheme 7).

The 9-dimethylamino substituent of triazafluorenone derivatives **1n** and **1ac** could be converted to *N*-oxides

Scheme 7[a]



Scheme 8[a]



41a and **41b** respectively when treated with phthalic anhydride and hydrogen peroxide−urea complex (Scheme 8).[30]

Results and Discussion

Both binding affinity (K_i) and functional antagonism (IC_{50}) were used to measure the biochemical potency of mGluR1 antagonists for SAR studies.

Binding assays have been reported for both mGluR1 and 5 subtypes using radioligands that have been shown to be noncompetitive to the extracellular *N*-terminal glutamate binding site.[9] The triazafluroenone derivatives reported here compete with these allosteric binding radioligands. Binding assays were used to generate affinities for ligands at both mGluR1 and 5 subtypes. In addition to affinity assays, functional antagonism and potency (IC_{50}) were measured using a calcium mobilization assay (vide infra), measuring the ability of ligands to block glutamate activation in engineered 1321 cells expressing the mGlu1 or 5 receptor.

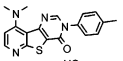
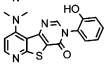
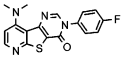
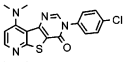
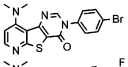
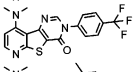
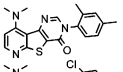
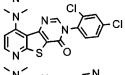
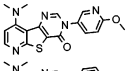
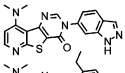
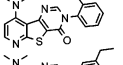
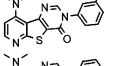
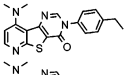
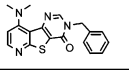
The first SAR table shown (Table 1) that similar SAR trends were observed using both K_i values in the binding assay and IC_{50} values in the calcium mobilization assay within each subtype (mGluR1 and 5). Functional IC_{50} values in the calcium mobilization assays were therefore used for additional SAR studies for measurement of potency and subtype (mGluR1 vs mGluR5) selectivity of antagonists (Table 1).

The triazafluorenone derivatives consisted of three submoieties: aminopyridine ring (P3), thiophene ring (P2), and pyrimidone ring (P1) (Figure 1). From Table 1, variation at the P1 moiety of the pyrimidone indicated that the *N3*-aryl substituent of the pyrimidone affected both in vitro potency and selectivity (mGluR1 vs mGluR5). With a viable synthetic route to access derivatives with different substituents at the *N3* position of the pyrimidone ring, we investigated SAR of this key substituent. This substituent (P1) tolerated both aryl and cyclic alkyl variations. Table 1 summarizes typical aryl substitution. Various aryl groups, from alkylaryl to haloaryl, hydroxyaryl, and heteroaryl, all produced potent mGluR1 antagonists. Compound **1b**, 4-methylphenyl triazafluorenone was a very potent mGluR1 antagonist (IC_{50} = 5 nM). This compound exhibited weaker mGluR5 antagonism (IC_{50} = 890 nM). The 4-ethylphenyl triazafluorenone **1n** maintained high mGluR1 potency (IC_{50} = 3 nM), and the selectivity of this compound (mGluR1 vs mGluR5) was also high (mGluR5 IC_{50} = 442 nM) (entry 11). Similar results were observed for the *p*-halide substituted phenyl analogues. *N3*-4-Bromophenyl triazafluorenone **1f** (mGluR1 IC_{50} = 4 nM; mGluR5 IC_{50} = 1250 nM) was the most potent mGluR1 antagonist among 4-halophenyl derivatives (entries 3−5). The strong electron withdrawing *p*-CF_3 group substituted phenyl analogue (**1g**) was also a potent mGluR1 antagonist devoid of mGluR5 activity. Disubstituted aryl triazafluorenone derivative, **1h**, was only slightly less potent (IC_{50} = 17 nM) compared to the monosubstituted aryl derivative **1b** and now lacked completely mGluR5 potency. Similar results were observed for dichlorophenyl triazafluorenone derivative **1i**, which was more subtype selective than the monochlorophenyl derivative **1e** (entries 4 and 6). Heteroaryl substituted triazafluorenone derivatives, **1j** (IC_{50} = 39 nM) and **1k** (IC_{50} = 122 nM), were quite potent mGluR1 antagonists with little activity at mGluR5 (entries 7 and 8). Benzyl substitution disfavored mGluR1 potency slightly (**1o**, mGluR1 IC_{50} = 281 nM, entry 12).

In general, *para*-substitution on the *N3*-aryl pyrimidone analogues boosted mGluR1 potency. This was exemplified by the *o*-, *m*-, *p*-ethyl substituted aryl analogues (**1l**, **1m**, and **1n**). This effect of the *para*-substitution often improved mGluR5 potency of triazafluorenone analogues as well.

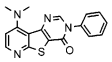
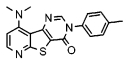
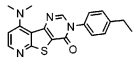
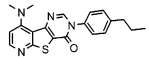
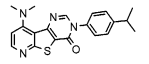
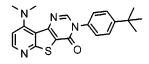
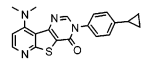
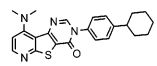
Additional SAR studies examined the scope of *para*-substituted phenyl analogues in more detail and are

Table 1. *N*-Aryl Substituted Triazafluroenone Derivatives Affect Both Potency and Selectivity[a]

entry	structure		FLIPR IC_{50} ± SEM (nM)[a] mGluR1	FLIPR mGluR5	Binding Ki ± SEM (nM)[b] mGluR1[c]	Binding mGluR5[d]
1		**1b**	5 (± 3)	890 (± 59)	2 (± 0.4)	500 (± 59)
2		**1c**	50 (± 22)	>100000	99 (± 9)	>100000
3		**1d**	23 (± 9)	11300 (± 8300)	8 (± 2)	>100000
4		**1e**	16 (± 3)	1810 (± 240)	4 (± 0.5)	1030 (± 260)
5		**1f**	4 (± 1)	1250 (± 50)	1 (± 0.2)	>100000
6		**1g**	32 (± 7)	>100000	8 (± 3)	>100000
5		**1h**	17 (± 7)	>100000	4 (± 0.6)	2440 (± 400)
6		**1i**	45 (± 8)	>100000	11 (± 3)	2300 (± 600)
7		**1j**	39 (± 15)	>100000	261 (± 52)	>100000
8		**1k**	122 (± 14)	>100000	62 (± 18)	1180 (± 94)
9		**1l**	251 (± 59)	>100000	142 (± 45)	>100000
10		**1m**	9 (± 2)	>100000	6 (± 0.4)	613 (± 150)
11		**1n**	3 (± 0.9)	442 (± 93)	5 (± 0.6)	194 (± 27)
12		**1o**	281 (± 65)	>100000	87 (± 15)	>100000

[a] (a) 1321N1 cells expressing either human mGluR1 or mGluR5, mean of multiple results with standard error of mean. (b) Rat mGluR1 binding (cerebellum) and rat mGluR5 binding (cortex), mean of multiple results with standard error of mean. (c) Radioligand [^{3}H]R214127 was used (ref 9). (d) Radioliagnd [^{3}H]MPEP was used (ref 33).

Table 2. Para Substitution Affects Both Potency and Selectivity[a]

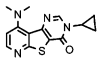
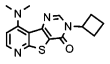
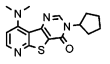
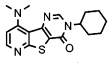
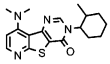
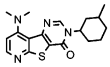
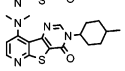
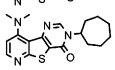
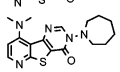
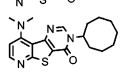
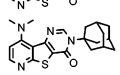
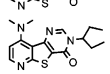
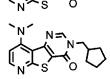
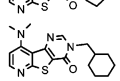
entry	Structure		FLIPR IC_{50} ± SEM (nM)† mGluR1	mGluR5
1		**1a**	20 (± 7)	>100000
2		**1b**	5 (± 3)	890 (± 59)
3		**1n**	3 (± 0.9)	442 (± 93)
4		**1p**	180 (± 72)	2040 (± 140)
5		**1q**	407 (± 200)	3540 (± 1080)
6		**1r**	8500 (± 500)	>100000
7		**1s**	87 (± 55)	4620 (± 430)
8		**1t**	>100000	>100000

[a] (†) 1321N1 cells expressing either human mGluR1 or mGluR5, mean of multiple results with standard error of mean.

shown in Table 2. Replacing hydrogen (**1a**) with *p*-methyl (**1b**) or *p*-ethyl (**1n**) at the *para*-position of the *N3*-phenyl moiety, as stated, demonstrated a clear trend in boosting mGluR1 potency (Table 2). There were limits to size or lipophilicity of this *para*-substituent that were tolerated. Substituted phenyl pyrimidone derivatives containing larger alkyl groups, such as *n*-propyl or isopropyl, , **1p** and **1q**, became less potent mGluR1 antagonists (IC_{50} = 180 and 407 nM, respectively, entries 4 and 5). When a bulky *tert*-butyl group was incorporated at the *para*-position of the phenyl triazafluorenone (**1r**), a dramatic loss of potency was observed (IC_{50} = 8.5 μM, entry 6). Cyclohexyl substituted phenyl triazafluorenone **1t** was also inactive in the calcium influx assay (entry 8).

A lipophilic cycloalkyl substituent was an effective replacement for the *N3*-aryl substituent. The size of the cyclic alkyl group modulated in vitro potencies of these cyclic alkyl triazafluorenone derivatives (Table 3). The selectivity of these new mGlu1 receptor antagonists also depended on the size of the cyclic alkyl ring. The trend was that increasing the size of cyclic ring favored in vitro potency for both mGluR1 and mGluR5. Once the ring size reached seven-membered ring, the optimum in vitro potency was obtained (**1ab**, IC_{50} = 1 nM, entry 8). Compound **1ab** was also one of the most potent mGluR5 antagonists from this series (mGluR5 IC_{50} = 147 nM). The eight-membered cyclic alkyl substituted pyrimidone, **1ad** (IC_{50} = 13 nM), was not as potent as the seven-membered alkyl pyrimidone derivative **1ab** (entries 8 and 10). Bulky adamantyl substituted pyrimidone, **1ae**, was a mixed mGluR1 and mGluR5 antagonist (mGluR1 IC_{50} = 76 nM, mGluR5 IC_{50} = 154 nM, entry 12) exhibiting some drop in potency. Incorporating a nitrogen atom into the cyclic alkyl ring, such as compound **1ac**, was also well tolerated (IC_{50} = 10 nM, entry 9). A different substitution pattern on the cyclic ring did not greatly affect in vitro potency or selectivity

Table 3. Alkyl Substituted Triazafluroenone Derivatives[a]

entry	structure		FLIPR IC_{50} ± SEM (nM)† mGluR1	mGluR5
1		**1u**	632 (± 140)	>100000
2		**1v**	167 (± 50)	>100000
3		**1w**	5 (± 2)	1160 (±460)
4		**1x**	50 (± 24)	650 (±130)
5		**1y**	130 (± 4)	>100000
6		**1z**	482 (± 98)	2160 (± 550)
7		**1aa**	140 (± 38)	592 (± 270)
8		**1ab**	1 (± 0.5)	147 (± 16)
9		**1ac**	10 (± 4)	342 (± 60)
10		**1ad**	13 (± 6)	179 (± 42)
12		**1ae**	76 (± 23)	154 (± 10)
13		**1af**	335 (± 110)	>100000
14		**1ag**	26 (± 8)	>100000
15		**1ah**	139 (± 29)	>100000

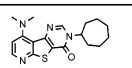
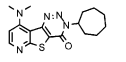
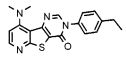
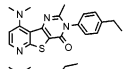
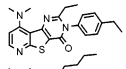
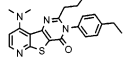
[a] (†) 1321N1 cells expressing either human mGluR1 or mGluR5, mean of multiple results with standard error of mean.

to the degree that had been observed with the aryl analogues (compounds **1y**, **1z**, and **1aa**, entries 6−8). Acyclic alkyl substituted triazafluorenone, **1af**, was not as potent as the corresponding cyclic compound **1w** (entries 3 and 13). Insertion of a methylene (CH_2) linkage between cyclic alkyl group and *N3* atom of the pyrimidone ring resulted in weaker mGluR1 antagonists (entries 14 and 15). Compound **1ag** (mGluR1 IC_{50} = 26 nM) was 5 times weaker than its parent derivative **1w** (mGluR1 IC_{50} = 5 nM). The elongated cyclohexyl triazafluorenone, **1ah**, was also less potent than its truncated parent compound **1x**, similar to the results observed with the aryl triazafluorenone derivatives (**1a** vs **1o**). These methylene inserted derivatives, **1ag** and **1ah**, could however be argued to be more selective mGluR1 antagonists (mGluR5 IC_{50} > 100 000 nM) than analogues **1w** and **1x** since they showed no mGluR5 activity at the highest concentration tested (entires 14 to 15).

The pyrimidone ring could also be converted to a triazinone ring. Analogue **14** was still a very potent mGluR1 antagonist (IC_{50} = 19 nM), comparable to its parent compound, **1ab** (entry 2, Table 4).

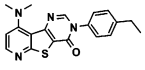
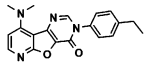
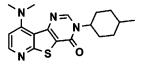
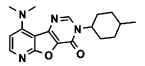
The C2 position of the triazafluorenone analogues could be substituted with an alkyl group to produce potent mGluR1 antagonists. Table 4 shows that there

Table 4. Tetraazafluroenone and C2-Substituted Triazafluroenone Derivative[a]

entry	structure		FLIPR IC_{50} ± SEM (nM)† mGluR1	mGluR5
1		**1ab**	1 (± 0.5)	147 (± 16)
2		**14**	19 (± 2)	816 (± 210)
3		**1n**	3 (± 0.9)	442 (± 93)
4		**16a**	44 (± 13)	344 (± 69)
5		**16b**	84 (± 11)	4530 (± 3100)
6		**16c**	101 (± 20)	901 (± 160)

[a] (†) 1321N1 cells expressing either human mGluR1 or mGluR5, mean of multiple results with standard error of mean.

Table 5. 5-Oxa-1,3,6-triazafluroenone Derivatives[a]

entry	structure		FLIPR IC_{50} ± SEM (nM)† mGluR1	mGluR5
1		**1n**	3 (± 0.9)	442 (± 93)
2		**10a**	1460 (± 290)	>100000
3		**1aa**	84 (± 38)	4530 (± 270)
4		**10b**	82 (± 11)	901 (± 37)

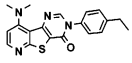
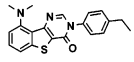
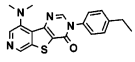
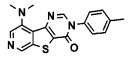
[a] (†) 1321N1 cells expressing either human mGluR1 or mGluR5, mean of multiple results with standard error of mean.

was also some size limitation for this alkyl substitution. C2-methyl incorporated triazafluorenone derivative, **16a**, was a 10-fold weaker mGluR1 antagonist (mGluR1 IC_{50} = 44 nM, entry 4) than **1n**. This compound was also less selective (mGluR1 vs mGluR5) when compared with its parent non-C2 substituted analogue, **1n**. Larger alkyl groups at C2 position of the triazafluorenone derivatives further disfavored in vitro potency (entries 5 and 6).

The thieno moiety of the triazafluorenone analogues could be replaced by a furan moiety (Table 5). The *N3*-aryl substituted 5-oxa-1,3,6-triazafluorenone analogues were weaker mGluR1 antagonists compared with the 5-thia-1,3,6-triazafluorenone analogues. Compound **10a** (IC_{50} = 1460 nM), for example, was much weaker than thieno analogue **1n** (IC_{50} = 3 nM). On the other hand, the *N3*-cyclic alkyl 5-oxa-1,3,6-triazafluorenone analogues were potent mGluR1 antagonists. Compound **10b** (IC_{50} = 82 nM) retained the potency of the 5-thia-1,3,6-triazafluorenone analogue **1aa**, but lost selectivity (vs mGluR5) (entry 4, Table 5).

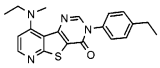
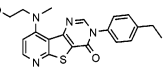
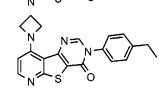
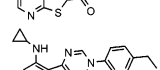
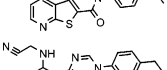
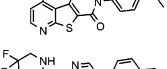
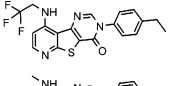
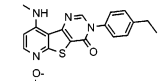
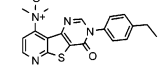
Replacement of the pyridine ring of the triazafluorenone derivatives by a phenyl ring resulted in analogue **22**, a far less potent mGluR1 antagonist (IC_{50} = 949 nM) compared with the corresponding pyridine analogue **1n** (entry 2, Table 6). Moving the nitrogen group from the

Table 6. Diazafluorenone and Triazafluorenone Derivatives[a]

entry	structure		FLIPR IC_{50} ± SEM (nM)† mGluR1	mGluR5
1		**1n**	3 (± 0.9)	442 (± 93)
2		**22**	949 (± 220)	>100000
3		**28a**	183 (± 110)	2160 (± 210)
4		**28b**	43 (± 14)	4040 (± 2100)

[a] (†) 1321N1 cells expressing either human mGluR1 or mGluR5, mean of multiple results with standard error of mean.

Table 7. 9-Alkylamino Triazafluorenone Derivatives[a]

entry	structure		FLIPR IC_{50} ± SEM (nM)† mGluR1	mGluR5
1		**39a**	37 (± 4)	2570 (± 460)
2		**39b**	71 (± 5)	>100000
3		**39c**	113 (± 34)	>100000
4		**39d**	41 (± 6)	>100000
5		**39e**	49 (± 10)	2100 (±700)
6		**39f**	99 (± 13)	>100000
7		**40**	98 (± 10)	5960 (± 760)
9		**41a**	306 (± 35)	>100000
10		**41b**	167 (± 15)	>100000

[a] (†) 1321N1 cells expressing either human mGluR1 or mGluR5, mean of multiple results with standard error of mean.

C6-position of compound **1n** to the C7-position (Figure 1) produced derivative **28a**, which was still a potent mGluR1 antagonist (IC_{50} = 183 nM, entry 3). The IC_{50} value of the 1,3,7-triazafluorenone analogues **28a** could be improved by reoptimization of the P1 moiety. Compound **28b**, the *p*-methylphenyl analogue for example, was a more potent and selective mGluR1 antagonist (IC_{50} = 43 nM, entry 4) than the *p*-ethylphenyl analogue (**28a**).

The dimethylamino group of the pyridine ring could also be replaced by various mono- or diamino groups (Table 7). Small monoalkylamino group substituted derivatives, such as cyclopropylamino derivative **39d** (IC_{50} = 41 nM, entry 4), were potent mGluR1 antagonists. Similarly, small dialkylamino group substituted triazafluorenone derivatives were also potent mGlu1 receptor antagonists. Ethylmethylaminopyridine de-

Table 8. Triazafluorenone Compound **1n** in Animal Pain Models[a]

	FLIPR IC_{50} ± SEM (nM)[a]		Pain models ED_{50} (μmol/kg, i.p.)[b]			Rotorod ED_{50}
	mGluR1	mGluR5	CFA	Carr	Formalin	(μmol/kg, i.p.)[b]
 **1n**	3 ± 0.9	442 ± 93	15	11	19	>300

[a] (a) 1321N1 cells expressing either human mGluR1 or mGluR5, mean of multiple results with standard error of mean. (b) Test performed 30 min after intraperitoneal administration of compound in rats (6 rats per group). Vehicle was 10% DMSO/PEG (5 mL/kg) (ref 35).

rivative, **39a**, was a potent example (mGluR1 IC_{50} = 37 nM, entry 1). In general, the monoalkylamino substituted pyrimidone derivatives were slightly less potent than dialkylamino substituted derivatives. Monomethylaminopyridine derivative **40** (IC_{50} = 98 nM, entry 7), for example, was weaker than the dimethylaminopyridine derivative **1n** (IC_{50} = 3 nM). Various alkyl, alkoxyalkyl, and halide alkyl amino groups could be used to replace the original dimethylamino group and maintained good in vitro potency. It was interesting to note that even a polar *N*-oxide group was tolerated. Converting the dimethylamino group of the triazafluorenone derivative **1n** to the corresponding *N*-oxide moiety gave a new polar mGluR1 antagonist **41a** with reasonable in vitro potency (IC_{50} = 306 nM, entry 8). Better in vitro potency of the *N*-oxide pyrimidone derivatives was achieved by modifying *N*-substituents at triazafluorenone ring (P1 moiety). Compound **41b**, for example, was a potent polar mGluR1 antagonist (IC_{50} =167 nM, entry 9).

To test the hypothesis that mGluR1 antagonists could be useful analgesics, we conducted initial in vivo pharmacology studies with selected triazafluorenone antagonists. Compound **1n** (PK profile in rat: $t_{1/2}$ = 1.9 h, V_d = 4.8 L/kg, $C_{max(ip)}$ = 137.5 ng/mL, $F_{(ip)}$ = 45%, $F_{(oral)}$ = 12%), for example, was fully efficacious in CFA (complete Freund's adjuvant-induced thermal hyperalgesia, ED_{50} = 15 μmol/kg, ip), carrageenan-induced hyperalgesia (carrageenan ED_{50} = 11 μmol/kg, ip), and formalin (phase II, ED_{50} = 19 μmol/kg, ip) rat models of pain. No rotorod side effect was observed at doses up to 300 μmol/kg (rotorod ED_{50} > 300 μmol/kg), and no sedative effect was observed at effective doses indicated above (Table 8).

In summary, we have identified a new triazafluorenone series of mGluR1 antagonists. These novel triazafluorenone derivatives are non-amino acid-like, potent, and subtype selective mGluR1 antagonists that interact with a well-defined allosteric recognition site of the metabotropic glutamate receptor. Therefore these novel triazafluorenone mGluR1 antagonists are noncompetitive with glutamate. SAR studies have identified the *N3*-phenyl substituent as a key moiety contributing to potency and selectivity. A specific site demonstrating this substituent effect was the *para*-position of this phenyl ring, and this site was optimized by introduction of small alkyl groups. One of these optimized antagonists, **1n**, is a single digit nanomolar mGluR1 antagonist with at least 40-fold selectivity over the mGluR5 subtype. Analogue **1n** also exhibits efficacy in preclinical rodent pain models without showing any rotorod side effect up to 300 μmol/kg. These data are a preliminary indication that mGluR1 antagonism may be a valid therapeutic target for treatment of pain.

Biology

Rat mGluR1 and Rat mGluR5 binding assays were carried out as described by El-Kouhen et al.[31] Briefly, mGluR1 binding was performed using rat cerebellum membrane preparation using [^{3}H]R214127 (9 Ci/mmol) as radioligand, and nonspecific binding was determined in the presence of 1 μM LY-456066.[32] Rat mGluR5 binding was performed using rat cortex preparation and the selective mGluR5 radioligand. Nonspecific binding was determined using 10 μM MTEP.[33] Specific binding was obtained by calculating the difference between total binding and nonspecific. Radioligand saturation binding data were analyzed using Prism GraphPad software (San Diego, CA). Competition binding data were analyzed by nonlinear regression curve fitting. K_i values were determined by the method of Cheng and Prusoff.[34]

The Ca^{2+} mobilization assay was performed using Fluorometric Imaging Plate Reader (FLIPR) assays. Calcium mobilization induced by L-glutamate was modulated by an mGlu1 receptor antagonist. Cloning of human mGluR1 and human mGluR5 is described by El-Kouhen et al.,[31] as well as FLIPR assays. Human mGluR1 and human mGluR5 receptor-transfected 1321N1 cells (coexpressing rat GLAST) were plated into 96-well biocoat black-wall/clear bottom microplates (Becton Dickinson, Boston, MA) at a density of 30 000 cells per well, in DMEM glutamate-free media (containing 10% FBS, 1% GluMax, 500 μg/mL G418, 200 μg/mL hygromycin B). After 2 days of culture, the culture medium was removed and replaced by 100 μL per well of D-PBS containing 0.04% Pluronic F127 and 4 μM Fluo-4 AM (fluorescent calcium indicator dye). After incubation for 1 h at room temperature, the cells were washed four times with D-PBS in a plate washer (Molecular Devices, Sunnyvale, CA), and 150 μL of D-PBS was left in each well. To assess the effect of antagonists on 10 μM L-glutamate-induced calcium released, antagonists were preincubated for 3 min before the addition of the agonist. FLIPR instrument made fluorescent reading for 5 min (every second for the first minute of compound addition (antagonist and agonist), then every 5 s for the remaining time). The instrument software normalizes the fluorescent reading to give equivalent initial reading at time zero, and all data were normalized with the response of 10 μM L-glutamate.

Detailed experimental procedures for animal pain models are described by Lehto et al.[35] Male Sprague−Dawley rats (Charles River, Wilmington, MA) weighing 200−300 g were utilized in all experiments.

In the formalin-induced spontaneous pain model (Formalin), following a 30-min acclimation period to individual observation cages, 50 μL of a 5% formalin solution was injected (sc) into the dorsal aspect of the right hind paw and the rats were then returned to the clear observation cages. Rats were observed for periods of time corresponding to phase 1 (0−10 min) and phase 2 (30−50 min) of the formalin test. Nociceptive behaviors were recorded from animals during the session by observing each animal for one 60-s observation period during each 5-min interval. Nociceptive behaviors recorded included flinching, licking, or biting the injected paw.

Carrageenan- and Complete Freund's Adjuvant-Induced Thermal Hyperalgesia. Unilateral inflammation was induced by injecting 100 μL of a 1% solution of λ-carrageenan or 150 μL of a 50% solution of complete Freund's adjuvant (CFA) (Sigma Chemical Co., St. Louis, MO) in physiological saline into the plantar surface of the right hind paw of the rat. The hyperalgesia to thermal stimulation was determined 2 or 48 h following carrageenan or CFA injection.

Rotorod performance was measured using an accelerating rotorod apparatus (Omnitech Electronics, Inc., Columbus, OH). For the rotorod assay, rats were allowed a 30 min acclimation period in the testing room and then placed on a 9 cm diameter rod that increased in speed from 0 to 20 rpm over a 60 s period. The time required for the rat to fall from the rod was recorded, with a maximum score of 60 s. Each rat was given 3 training sessions. Latencies to fall from the rotorod were determined 30 (ip) or 60 (po) min following compound injection.

All tests were performed 30 min after intraperitoneal administration of compound **1n**, using 10%DMSO/PEG as vehicle (5 mL/kg).

Experimental Section

General Information. Unless otherwise noted, all solvents, including anhydrous solvents, and chemicals were purchased from Aldrich Co. and/or Acros Organics, and used without further purification. NMR spectra were obtained at either 300 or 500 MHz using deuterated solvent and TMS (tetramethylsilane) as the internal standard. The mass spectra were recorded on a Finnigin-4000 instrument using EI or CI. Elemental analyses were performed by Robertson Microlit Laboratories with ±0.4% of theoretical value. Column chromatography purifications used silica gel 60 (230−400 mesh) from E. Merck. Thin-layer chromatography analyses were performed on silica gel plate 60 F254 with a thickness of 0.25 mm.

LY-456066 and [^{3}H]R214127 were synthesized at Abbott Laboratories (Abbott Park, IL). [^{3}H]MPEP was from Tocris (Bristol, U.K.). Rat cerebellum and rat cortex were purchased from Pel-Freez Biologicals (Rogers, AR). Pluronic F127 and 4 μM Fluo-4 AM were bought from Molecular Probes (Eugene, OR). D-PBS, neomycin (G418), hygromycin B, GluMax, and tissue culture reagents were from Invitrogen/Life Technology. All other chemicals were purchased from Sigma unless otherwise noted. Male Sprague−Dawley rats (Charles River, Wilmington, MA) weighing 200−300 g were utilized in all experiments.

2-(1-Dimethylaminoethylidene)malononitrile (3). To a solution of *N*,*N*-dimethylacetamide dimethyl acetal (90%, 125.0 g, 845 mmol) in ethanol (75 mL), a solution of malononitrile (**2**, 56.0 g, 847.2 mmol) in diethyl ether (500 mL) was added slowly at 0 °C. The reaction mixture was then gradually warmed to room temperature and stirred for 2 days. Solvent was removed, and the solid was redissolved in ethyl acetate, and was purified via a short column chromatography (SiO_2, ethyl acetate) to give a pale yellow solid product (109.2 g, 96%).

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.32 ppm (s, br, 6 H); 2.28 (s, 3H). MS (ESI/NH_3): m/z = 135.9 (M + H$^+$).

2-[1,3-Bis(dimethylaminoallylidene)]malononitrile (4). 2-(1-Dimethylaminoethylidene)malononitrile (**3**, 100.0 g, 740 mmol) was dissolved in *N*,*N*-dimethylformamide dimethyl acetal (94%, 210 mL, 1.48 mol), and the reaction mixture was heated to reflux at 100 °C for 1 h 20 min and then cooled down to room temperature. Solid was collected, and washed with cold methanol (10 × 3 mL) to give a yellow solid product. The mother liquor was concentrated, and the solid was collected again, and washed with cold methanol. This procedure was repeated 5 times, and all the solid products were combined (113.7 g, 81%). The final residue was purified by a short column chromatography (SiO_2, ethyl acetate) to give an additional 17.2 g of product (12%).

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.42 ppm (d, J = 12.2 Hz, 1H); 4.36 (d, J = 12.2 Hz, 1H); 3.15 (s, 6H), 3.05 (s, br, 6H). MS (ESI/NH_3): m/z = 191.1 (M + H$^+$).

4-Dimethylamino-2-chloro-3-cyanopyridine (5). To a slurry of 2-(1,3-bisdimethylaminoallylidene)malononitrile (**4**, 120.0 g, 632 mmol) in methanol (1500 mL), HCl gas was introduced gently at 0 °C. The reaction mixture became homogeneous in about 1 h, and was allowed to stir under constant HCl flow for additional 9 h at 0 °C. N_2 was bubbled though the reaction mixture for 2 h, and all the solvent was removed. The residue solid was redissolved in CH_2Cl_2, and washed with water/K_2CO_3/water. The organic layer was separated and dried over Na_2SO_4. Removal of salt and solvent gave a pure product (113.0 g, 98%).

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.96 ppm (d, J = 6.4 Hz, 1H); 6.59 (d, J = 6.4 Hz, 1H); 3.30 (s, 6H). MS (ESI/NH_3): m/z = 181.9 (M + H$^+$).

5-Amino-4-dimethylaminothieno[8,9-*b*]pyridine-6-carboxylic Acid Methyl Ester (6). To a solution of methyl thioglycolate (40 mL, 442 mmol) in anhydrous THF (500 mL), sodium hydride (60%, 20.0 g, 500 mmol) was added in small portions at 0 °C. A solution of 4-dimethylamino-2-chloro-3-cyanopyridine (**5**, 80.0 g, 442 mmol) in THF (1000 mL) was added. The reaction mixture was allowed to stir for 1 day at room temperature. Additional NaH (60%, 11.0 g, 276 mmol) was added, and the reaction mixture was then heated to reflux for 1 h 50 min. After being cooled down to room temperature, the reaction mixture was quenched with saturated NH_4Cl. Organic solvent was removed under vacuum, and the aqueous layer was extracted with dichloromethane (500 mL). The organic layer was separated and washed with water and brine, then dried over Na_2SO_4. The solvent was concentrated, and a pure product was precipitated. Solid was collected and washed with cold methanol (3 × 5 mL), then ether, and dried under vacuum (82.3 g, 74%). Additional solid was collected via the same, repeated procedures (12.1 g, 11%).

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.43 ppm (d, J = 5.2 Hz, 1H), 6.83 (d, J = 5.2 Hz, 1H), 6.74, s, br, 2H), 3.86 (s, 3H), 2.84 (s, 6H). MS (ESI/NH_3): m/z = 251.9 (M + H$^+$).

4-Dimethylamino-5-(dimethylaminomethyleneamino)-thieno[8,9-*b*]pyridine-6-carboxylic Acid Methyl Ester (8). 5-Amino-4-dimethylaminothieno[8,9-*b*]pyridine-6-carboxylic acid methyl ester (**6**, 32.0 g, 127 mmol) was dissolved in ethanol (150 mL) and *N*,*N*-dimethylformamide dimethyl acetal (100 mL), and heated to reflux for 4.5 h. Excess of solvent and reagent were removed to give a yellow solid product (38.5 g, 99%).

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.30 ppm (d, J = 5.4 Hz, 1H), 7.41 (s, 1H), 6.60 (d, J = 5.4 Hz, 1H), 3.82 (s, 3H), 3.17 (s, br, 3H), 3.07 (s, br, 3H), 2.99 (s, 6H). MS (ESI/NH_3): m/z = 307.0 (M + H$^+$).

General Procedure for the Synthesis of Tricyclic Pyrimidone Derivatives 1a−ah. 9-Dimethylamino-3-(*p*-tolyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (1b). 4-Dimethylamino-5-(dimethylaminomethyleneamino)thieno[8,9-*b*]pyridine-6-carboxylic acid methyl ester (**8**, 1.0 g, 3.2 mmol), *p*-toluenesulfonic acid (25 mg, 0.13 mmol), and *p*-toluidine (512 μL, 4.8 mmol) were placed in a flask with toluene (25 mL) and then heated to 130 °C overnight. The mixture was cooled down to room temperature. Solvent was removed under vacuum, and the residue was treated with cold methanol and sonicated. White precipitate was formed. The product was then collected, washed with cold methanol (2 × 2 mL), and dried under vacuum to give a pure product (451 mg, 42%).

^{1}H NMR ($CDCl_3$/MeOD, 300 MHz): δ = 8.43 ppm (d, J = 5.9 Hz, 1H), 8.29 (s, 1H), 7.37 (d, J = 8.4 Hz, 2H), 7.34 (d, J = 8.4 Hz, 2H), 6.84 (d, J = 5.9 Hz, 1H), 3.26 (s, 6H), 2.46 (s, 3H). MS (ESI/NH_3): m/z = 337.0 (M + H$^+$). Anal. ($C_{18}H_{16}N_4OS\cdot 0.2H_2O$) C, H, N.

9-Dimethylamino-3-(*o*-hydroxyphenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (1c): prepared using the same procedure

as for **1b**, but employing *o*-hydroxyaniline as the amine source (15% yield). ^{1}H NMR ($CDCl_3$/MeOD, 300 MHz): δ = 8.41 ppm (d, J = 5.9 Hz, 1H), 8.27 (s, 1H), 7.39 (m, 1H), 7.28 (m, 1H), 7.11 (m, 1H), 7.05 (m, 1H), 6.83 (d, J = 5.9 Hz, 1H), 3.26 (s, 6H). MS (ESI, M+1): 338.9 (M + H$^+$). Anal. ($C_{17}H_{14}N_4O_2S\cdot 0.3H_2O$) C, H, N.

9-Dimethylamino-3-(*p*-fluorophenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (1d): prepared using the same procedure as for **1b**, but employing *p*-fluoroaniline as the amine source (81% yield). ^{1}H NMR ($CDCl_3$/MeOD, 300 MHz): δ = 8.43 ppm (d, J = 5.9 Hz, 1H), 8.28 (s, 1H), 7.46 (m, 2H), 7.27 (m, 2H), 7.36 (m, 2H), 6.84 (d, J = 5.9 Hz, 1H), 3.25 (s, 6H). MS (ESI/NH_3): 340.9 (M + H$^+$). Anal. ($C_{17}H_{13}FN_4OS$) C, H, N.

9-Dimethylamino-3-(*p*-chlorophenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (1e): prepared using the same procedure as for **1b**, but employing *p*-chloroaniline as the amine source (72% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.43 ppm (d, J = 5.9 Hz, 1H), 8.27 (s, 1H), 7.55 (m, 2H), 7.42 (m, 2H), 6.84 (d, J = 5.9 Hz, 1H), 3.24 (s, 6H). MS (ESI/NH_3): 356.9 (M + H$^+$). Anal. ($C_{17}H_{13}ClN_4OS$) C, H, N.

9-Dimethylamino-3-(*p*-bromophenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (1f): prepared using the same procedure as for **1b**, but employing *p*-bromoaniline as the amine source (62% yield). ^{1}H NMR ($CDCl_3$/MeOD, 300 MHz): δ = 8.43 ppm (d, J = 5.9 Hz, 1H), 8.26 (s, 1H), 7.72 (d, J = 8.7 Hz, 2H), 7.36 (d, J = 8.7 Hz, 2H), 6.84 (d, J = 5.9 Hz, 1H), 3.26 (s, 6H). MS (ESI/NH_3): 400.0, 402.8 (M + H$^+$). Anal. ($C_{17}H_{13}BrN_4OS$) C, H, N.

9-Dimethylamino-3-(*p*-trifluoromethylphenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (1g): prepared using the same procedure as for **1b**, but employing *p*-trifluoromethylaniline as the amine source (21% yield). ^{1}H NMR ($CDCl_3$/MeOD, 300 MHz): δ = 8.45 ppm (s, br, 1H), 8.29 (s, 1H), 7.87 (d, J = 8.4 Hz, 2H), 7.64 (d, J = 8.4 Hz, 2H), 6.85 (d, J = 5.3 Hz, 1H), 3.23 (s, 6H). MS (ESI/NH_3): 390.9 (M + H$^+$). Anal. ($C_{18}H_{13}F_3N_4OS$) C, H, N.

9-Dimethylamino-3-(2,4-dimethylphenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (1h): prepared using the same procedure as for **1b**, but employing 2,4-dimethylaniline as the amine source (85% yield). ^{1}H NMR ($CDCl_3$/MeOD, 300 MHz): δ = 8.44 ppm (d, J = 5.9 Hz, 1H), 8.17 (s, 1H), 7.23 (s, 1H), 7.18 (d, J = 8.1 Hz, 1H), 7.15 (d, J = 8.1 Hz, 1H), 6.85 (d, J = 5.9 Hz, 1H), 3.28 (s, 6H), 2.42 (s, 3H), 2.17 (s, 3H). MS (ESI/NH_3): 351.0 (M + H$^+$). Anal. ($C_{19}H_{18}N_4OS$) C, H, N.

9-Dimethylamino-3-(2,4-dichlorophenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (1i): prepared using the same procedure as for **1b**, but employing 2,4-dichloroaniline as the amine source (77% yield). ^{1}H NMR ($CDCl_3$/MeOD, 300 MHz): δ = 8.42 ppm (d, J = 6.2 Hz, 1H), 8.12 (s, 1H), 7.67 (d, J = 2.2 Hz, 1H), 7.48 (dd, J = 8.7, 2.2 Hz, 1H), 7.44 (d, J = 8.7 Hz, 1H), 6.87 (d, J = 6.2 Hz, 1H), 3.33 (s, 6H). MS (ESI/NH_3): 390.9 (M + H$^+$). Anal. ($C_{17}H_{12}Cl_2N_4OS\cdot 0.1H_2O$) C, H, N.

9-Dimethylamino-3-(2′-methoxy-5′-pyridyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (1j): prepared using the same procedure as for **1b**, but employing 5-amino-2-methoxypyridine as the amine source (57% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.44 ppm (d, J = 5.8 Hz, 1H), 8.25 (s, 1H), 8.23 (d, J = 2.7 Hz, 1H), 7.75 (dd, J = 8.8, 2.7 Hz, 1H), 6.92 (d, J = 8.8 Hz, 1H), 6.81 (d, J = 5.8 Hz, 1H), 4.01 (s, 3H), 3.18 (s, 6H). MS (ESI/NH_3): m/z = 354.0 (M + H$^+$). Anal. ($C_{17}H_{15}N_5O_2S$) C, H, N.

9-Dimethylamino-3-(*1H*-indazol-6-yl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (1k): prepared using the same procedure as for **1b**, but employing 6-amino-*1H*-indazole as the amine source (65% yield). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 13.42 ppm (s, 1H), 8.65 (s, 1H), 8.40 (d, J = 5.8 Hz, 1H), 8.22 (s, 1H), 7.95 (d, J = 8.5 Hz, 1H), 7.82 (s, 1H), 7.28 (dd, J = 8.5, 1.7 Hz, 1H), 6.95 (d, J = 5.8 Hz, 1H), 3.13 (s, 6H). MS (ESI/NH_3): m/z = 363.0 (M + H$^+$). Anal. ($C_{18}H_{14}N_6OS$) C, H, N.

9-Dimethylamino-3-(2-ethylphenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (1l): prepared using the same procedure as for **1b**, but employing *o*-ethylaniline as the amine source (51% yield). ^{1}H NMR ($CDCl_3$/MeOD, 300 MHz): δ = 8.45 ppm (d, J = 6.6 Hz, 1H), 8.19 (s, 1H), 7.52 (m, 1H), 7.48 (m, 1H), 7.40 (m, 1H), 7.26 (m, 1H), 6.87 (d, J = 6.6 Hz, 1H), 2.52 (m, 2H), 2.48 (s, 6H), 1.17 (t, J = 7.5 Hz, 3H). MS (ESI/NH_3): m/z = 351.0 (M + H$^+$). Anal. ($C_{19}H_{18}N_4OS$) C, H, N.

9-Dimethylamino-3-(3-ethylphenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (1m): prepared using the same procedure as for **1b**, but employing *m*-ethylaniline as the amine source (41% yield). ^{1}H NMR ($CDCl_3$/MeOD, 300 MHz): δ = 8.44 ppm (d, J = 6.2 Hz, 1H), 8.30 (s, 1H), 7.48 (t, J = 7.8 Hz, 1H), 7.37 (d, J = 7.8 Hz, 1H), 7.26 (m, 2H), 6.84 (d, J = 6.2 Hz, 1H), 2.76 (q, J = 7.8 Hz, 2H), 2.28 (s, 3H), 1.30 (t, J = 7.8 Hz, 3H), 1.17 (t, J = 7.5 Hz, 3H). MS (ESI/NH_3): m/z = 351.0 (M + H$^+$). Anal. ($C_{19}H_{18}N_4OS$) C, H, N.

9-Dimethylamino-3-(4-ethylphenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (1n): prepared using the same procedure as for **1b**, but employing *p*-ethylaniline as the amine source (73% yield). ^{1}H NMR ($CDCl_3$/MeOD, 300 MHz): δ = 8.43 ppm (d, J = 5.9 Hz, 1H), 8.29 (s, 1H), 7.40 (d, J = 8.4 Hz, 2H), 7.36 (d, J = 8.4 Hz, 2H), 6.84 (d, J = 5.9 Hz, 1H), 3.27 (s, 6H), 2.76 (q, J = 7.8 Hz, 2H), 1.30 (t, 7.8 Hz, 3H). MS (ESI/NH_3): m/z = 351.0 (M + H$^+$). Anal. ($C_{19}H_{18}N_4OS$) C, H, N.

9-Dimethylamino-3-benzyl-*3H*-5-thia-1,3,6-triazafluoren-4-one (1o): prepared using the same procedure as for **1b**, but employing benzylamine as the amine source (65% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.34 ppm (d, J = 6.6 Hz, 1H), 8.33 (s, 1H), 7.38 (m, 3H), 7.36 (m, 2H), 6.86 (d, J = 6.6 Hz, 1H), 5.28 (s, 2H), 3.34 (s, 6H). MS (ESI/NH_3): m/z = 337.0 (M + H$^+$). Anal. ($C_{18}H_{16}N_4OS\cdot 0.1H_2O$) C, H, N.

9-Dimethylamino-3-(4-*n*-propylphenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (1p): prepared using the same procedure as for **1b**, but employing a mixture of 4-*n*-propylaniline as the amine source (63% yield). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 8.58 ppm (s, 1H), 8.41 (d, J = 6.1 Hz, 1H), 7.48 (m, 2H), 7.40 (m, 2H), 6.97 (d, J = 6.1 Hz, 1H), 3.16 (s, 6H), 2.66 (t, J = 7.5 Hz, 2H), 1.65 (m, 2H), 0.95 (t, J = 7.1 Hz, 3H). MS (ESI/NH_3): m/z = 365.0 (M + H$^+$). Anal. ($C_{20}H_{20}N_4OS$) C, H, N.

9-Dimethylamino-3-(4-isopropylphenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (1q): prepared using the same procedure as for **1b**, but employing a mixture of 4-isopropylaniline as the amine source (58% yield). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 8.59 ppm (s, 1H), 8.40 (d, J = 5.8 Hz, 1H), 7.49 (m, 2H), 7.46 (m, 2H), 6.97 (d, J = 5.8 Hz, 1H), 3.16 (s, 6H), 3.01 (m, 1H), 1.27 (d, J = 7.1 Hz, 6H). MS (ESI/NH_3): m/z = 365.1 (M + H$^+$). Anal. ($C_{20}H_{20}N_4OS\cdot 0.1H_2O$) C, H, N.

9-Dimethylamino-3-(4-*tert*-butylphenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (1r): prepared using the same procedure as for **1b**, but employing a mixture of 4-*tert*-butylaniline as the amine source (66% yield). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 8.59 ppm (s, 1H), 8.40 (d, J = 5.8 Hz, 1H), 7.62 (m, 2H), 7.50 (m, 2H), 6.97 (d, J = 5.8 Hz, 1H), 3.15 (s, 6H), 1.35 (s, 9H). MS (ESI/NH_3): m/z = 379.1 (M + H$^+$). Anal. ($C_{21}H_{22}N_4OS$) C, H, N.

9-Dimethylamino-3-(4-cyclopropylphenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (1s): prepared using the same procedure as for **1b**, but employing a mixture of 4-cyclopropylaniline as the amine source (55% yield). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 8.55 ppm (s, 1H), 8.38 (d, J = 5.8 Hz, 1H), 7.44 (m, 2H), 7.26 (m, 2H), 6.94 (d, J = 5.8 Hz, 1H), 3.13 (s, 6H), 2.02 (m, 1H), 1.03 (m, 2H), 0.76 (m, 2H). MS (ESI/NH_3): m/z = 363.1 (M + H$^+$). Anal. ($C_{20}H_{18}N_4OS$) C, H, N.

9-Dimethylamino-3-(4-cyclohexylphenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (1t): prepared using the same procedure as for **1b**, but employing a mixture of 4-cyclohexylaniline as the amine source (64% yield). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 8.59 ppm (s, 1H), 8.41 (d, J = 6.1 Hz, 1H), 7.46 (m, 2H), 7.42 (m, 2H), 6.98 (d, J = 6.1 Hz, 1H), 3.17 (s, 6H), 2.62 (m, 1H), 1.20−1.90 (m, 10H), 0.76 (m, 2H). MS (ESI/NH_3): m/z = 405.1 (M + H$^+$). Anal. ($C_{23}H_{24}N_4OS\cdot 0.1H_2O$) C, H, N.

9-Dimethylamino-3-cyclopropyl-*3H*-5-thia-1,3,6-triazafluoren-4-one (1u): prepared using the same procedure as for **1b**, but employing cyclopropylamine as the amine source (21% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.40 ppm (d, J = 6.2 Hz, 1H), 8.23 (s, 1H), 6.84 (d, J = 6.2 Hz, 1H), 3.35 (m,

1H), 3.27 (s, 6H), 1.20−1.40 (m, 4H). MS (ESI/NH_3): *m/z* = 287.0 (M + H^+). Anal. ($C_{14}H_{14}N_4OS·0.1H_2O$) C, H, N.

9-Dimethylamino-3-cyclobutyl-*3H*-5-thia-1,3,6-triazafluoren-4-one (1v): prepared using the same procedure as for **1b**, but employing cyclobutylamine the amine source (51% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.42 ppm (d, *J* = 6.2 Hz, 1H), 8.34 (s, 1H), 6.82 (d, *J* = 6.2 Hz, 1H), 5.12 (m, 1H), 3.31 (s, 6H), 2.64 (m, 2H), 2.42 (m, 2H), 1.98 (m, 2H). MS (ESI/NH_3): *m/z* = 300.7. Anal. ($C_{15}H_{16}N_4OS$) C, H, N.

9-Dimethylamino-3-cyclopentyl-*3H*-5-thia-1,3,6-triazafluoren-4-one (1w): prepared using the same procedure as for **1b**, but employing cyclopentylamine as the amine source (24% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.39 ppm (d, *J* = 6.9 Hz, 1H), 8.30 (s, 1H), 6.86 (d, *J* = 6.9 Hz, 1H), 5.22 (m, 1H), 3.37 (s, 6H), 2.30 (m, 2H), 1.95 (m, 2H), 1.84 (m, 4H). MS (ESI/NH_3): *m/z* = 315.0 (M + H^+). Anal. ($C_{16}H_{18}N_4OS$) C, H, N.

9-Dimethylamino-3-cyclohexyl-*3H*-5-thia-1,3,6-triazafluoren-4-one (1x): prepared using the same procedure as for **1b**, but employing cyclohexylamine as the amine source (16% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.39 ppm (d, *J* = 6.6 Hz, 1H), 8.30 (s, 1H), 6.85 (d, *J* = 6.6 Hz, 1H), 4.84 (m, 1H), 3.34 (s, 6H), 1.30−2.10 (m, 10H). MS (ESI/NH_3): *m/z* = 329.0 (M + H^+). Anal. ($C_{17}H_{20}N_4OS·0.3H_2O$) C, H, N.

9-Dimethylamino-3-(2-methylcyclohexyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (1y): prepared using the same procedure as for **1b**, but employing a mixture of *cis* and *trans* 2-methylcyclohexylamine as the amine source (27% yield). ^{1}H NMR ($CDCl_3$/MeOD, 300 MHz): δ = 8.42 ppm (m, 1H), 8.18 (m, 1H), 6.80 (m, 1H), 3.50 (m, 1H), 2.92 (s, 6H), 2.80 (m, 1H), 1.00−2.00 (m, 8H), 0.80 (m, 3H). MS (ESI/NH_3): *m/z* = 343.1 (M + H^+). Anal. ($C_{18}H_{22}N_4OS·0.1H_2O$) C, H, N.

9-Dimethylamino-3-(2-methylcyclohexyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (1z): prepared using the same procedure as for **1b**, but employing a mixture of *cis* and *trans* 3-methylcyclohexylamine as the amine source (33% yield). ^{1}H NMR ($CDCl_3$/MeOD, 300 MHz): δ = 8.22 ppm (m, 1H), 8.20 (m, 1H), 6.78 (m, 1H), 5.00 (m, 1H), 3.22 (m, 6H), 3.02 (m, 1H), 1.00−2.00 (m, 8H), 0.90 (m, 3H). MS (ESI/NH_3): *m/z* = 343.1 (M + H^+). Anal. ($C_{18}H_{22}N_4OS$) C, H, N.

9-Dimethylamino-3-(4-methylcyclohexyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (1aa): prepared using the same procedure as for **1b**, but employing a mixture of *cis* and *trans* 4-methylcyclohexylamine as the amine source (27% yield). ^{1}H NMR ($CDCl_3$/MeOD, 300 MHz): δ = 8.25 ppm (m, 1H), 8.20 (m, 1H), 6.75 (m, 1H), 4.42 (m, 1H), 3.30 (m, 6H), 1.00−2.00 (m, 9H), 0.90 (m, 3H). MS (ESI/NH_3): *m/z* = 343.1 (M + H^+). Anal. ($C_{18}H_{22}N_4OS·0.2H_2O$) C, H, N.

9-Dimethylamino-3-cycoheptyl-*3H*-5-thia-1,3,6-triazafluoren-4-one (1ab): prepared using the same procedure as for **1b**, but employing cycloheptylamine as the amine source (54% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.40 ppm (d, *J* = 6.6 Hz, 1H), 8.29 (s, 1H), 6.85 (d, *J* = 6.6 Hz, 1H), 4.96 (m, 1H), 3.35 (s, 6H), 1.50−2.10 (m, 12H). MS (ESI/NH_3): *m/z* = 343.0 (M + H^+). Anal. ($C_{18}H_{22}N_4OS·0.2H_2O$) C, H, N.

9-Dimethylamino-3-(*N*-hexamethyleneiminyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (1ac): prepared using the same procedure as for **1b**, but employing 1-aminohomopiperidine as the amine source (45% yield). ^{1}H NMR ($CDCl_3$/MeOD, 300 MHz): δ = 8.40 ppm (d, *J* = 5.8 Hz, 1H), 8.37 (s, 1H), 6.78 (d, *J* = 5.8 Hz, 1H), 3.88 (m, 4H), 3.15 (s, 6H), 1.78 (m, 8H). MS (ESI/NH_3): *m/z* = 344.1 (M + H^+). Anal. ($C_{17}H_{21}N_5OS·0.1H_2O$) C, H, N. **9-Dimethylamino-3-cyclooctyl-*3H*-5-thia-1,3,6-triazafluoren-4-one (1ad):** prepared using the same procedure as for **1b**, but employing cyclooctylamine as the amine source (48% yield). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 8.69 ppm (s, 1H), 8.39 (d, *J* = 6.1 Hz, 1H), 6.97 (d, *J* = 6.1 Hz, 1H), 4.90 (m, 1H), 3.18 (s, 6H), 1.50−2.15 (m, 14H). MS (ESI/NH_3): *m/z* = 357.1 (M + H^+). Anal. ($C_{19}H_{24}N_4OS·0.1H_2O$) C, H, N.

9-Dimethylamino-3-(1-adamantyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (1ae): prepared using the same procedure as for **1b**, but employing 1-adamantylamine as the amine source (55% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.41 ppm (d, *J* = 6.6 Hz, 1H), 8.41 (s, 1H), 6.82 (d, *J* = 6.6 Hz, 1H), 3.30 (s, 6H), 2.49 (m, 6H), 2.30 (m, 3H), 1.81 (m, 6H). MS (ESI/NH_3): *m/z* = 381.0 (M + H^+). Anal. ($C_{21}H_{24}N_4OS·0.1H_2O$) C, H, N.

9-Dimethylamino-3-(3-*n*-pentyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (1af): prepared using the same procedure as for **1b**, but employing 3-*n*-pentylamine as the amine source (35% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.42 ppm (d, *J* = 6.9 Hz, 1H), 8.16 (s, 1H), 6.87 (d, *J* = 6.9 Hz, 1H), 4.82 (m, 1H), 3.36 (s, 6H), 1.95 (m, 2H), 1.85 (m, 2H), 0.92 (t, *J* = 7.5 Hz, 6H). MS (ESI/NH_3): *m/z* = 317.0 (M + H^+). Anal. ($C_{16}H_{20}N_4OS·0.1H_2O$) C, H, N.

9-Dimethylamino-3-cyclopentylmethyl-*3H*-5-thia-1,3,6-triazafluoren-4-one (1ag): prepared using the same procedure as for **1b**, but employing cyclopentylmethylamine as the amine source (54% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.40 ppm (d, *J* = 6.9 Hz, 1H), 8.23 (s, 1H), 6.86 (d, *J* = 6.9 Hz, 1H), 4.04 (d, *J* = 7.5 Hz, 2H), 3.35 (s, 6H), 2.45 (m, 1H), 1.30−1.8 (m, 8H). MS (ESI/NH_3): *m/z* = 329.0 (M + H^+). Anal. ($C_{17}H_{20}N_4OS·0.2H_2O$) C, H, N.

9-Dimethylamino-3-cyclohexylmethyl-*3H*-5-thia-1,3,6-triazafluoren-4-one (1ah): prepared using the same procedure as for **1b**, but employing cyclohexylmethylamine as the amine source (15% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.40 ppm (d, *J* = 6.2 Hz, 1H), 8.16 (s, 1H), 6.85 (d, *J* = 6.2 Hz, 1H), 3.92 (d, *J* = 7.5 Hz, 2H), 3.30 (s, 6H), 1.00−2.00 (m, 11H). MS (ESI/NH_3): *m/z* = 343.1 (M + H^+). Anal. ($C_{18}H_{22}N_4OS·0.1H_2O$) C, H, N.

5-Amino-4-dimethylaminofuro[8,9-*b*]pyridine-6-carboxylic Acid Methyl Ester (7). To a solution of methyl glycolate (2.7 mL, 35 mmol) in anhydrous THF (100 mL), sodium hydride (60%, 2.8 g, 70 mmol) was added in small portions at 0 °C. A solution of 4-dimethylamino-2-chloro-3-cyanopyridine (**5**, 5.0 g, 27.6 mmol) in THF (25 mL) was added. The reaction mixture was allowed to stir for 3 days at room temperature. The reaction mixture was then heated to reflux for 2 h. After being cooled down to room temperature, the reaction mixture was quenched with saturated NH_4Cl. Organic solvent was removed under vacuum, and the aqueous layer was extracted with dichloromethane (500 mL). The organic layer was separated and washed with water and brine, then dried over Na_2SO_4. The solvent was concentrated, and purified via column chromatography (SiO_2, ethyl acetate:hexanes = 1:9 then 1:4) to give 4.9 g product (76%).

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.22 ppm (d, *J* = 5.8 Hz, 1H), 7.27 (d, *J* = 5.8 Hz, 1H), 5.27 (s, br, 2H), 3.94 (s, 3H), 3.01 (s, 6H). MS (ESI/NH_3): *m/z* = 236.0 (M + H^+).

4-Dimethylamino-5-(dimethylaminomethyleneamino)-furo[8,9-*b*]pyridine-6-carboxylic Acid Methyl Ester (9). 5-Amino-4-dimethylaminofuro[8,9-*b*]pyridine-6-carboxylic acid methyl ester (**7**, 2.0 g, 8.5 mmol) was dissolved in ethanol (12 mL) and *N*,*N*-dimethylformamide dimethyl acetal (6 mL), and heated to reflux for 4.5 h. Excess of solvent and reagent were removed under vacuum to give a yellow solid product (2.5 g, 100%).

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.12 ppm (d, *J* = 5.8 Hz, 1H), 7.68 (s, 1H), 6.44 (d, *J* = 5.8 Hz, 1H), 3.87 (s, 3H), 3.16 (s, 6H), 3.14 (s, br, 3H), 3.09 (s, br, 3H). MS (ESI/NH_3): *m/z* = 291.1 (M + H^+).

9-Dimethylamino-3-(4′-methyl-1′-cyclohexyl)-*3H*-1,3,6-triazafluoren-4-one (10b). Dimethylamino-5-(dimethylaminomethyleneamino)furo[8,9-*b*]pyridine-6-carboxylic acid methyl ester (**9**, 150 mg, 0.52 mmol), *p*-toluenesulfonic acid (10 mg, 0.05 mmol), and 4-methylcyclohexylamine (88 mg, 0.78 mmol) were placed in a flask with toluene (10 mL) and then heated to 130 °C for overnight. The mixture was cooled down to room temperature. Solvent was removed under vacuum, and the residue was purified via column chromatography (SiO_2, ethyl acetate:hexanes = 1:9 then 1:4) to give a pure product (75 mg, 44%).

^{1}H NMR ($CDCl_3$/MeOD, 300 MHz): δ = 8.19 ppm (m, 1H), 8.16 (m, 1H), 6.50 (m, 5.8 Hz, 1H), 4.90 (m, 1H), 3.42 (m, 6H), 1.50−2.10 (m, 8H), 1.30 (m, 1H), 1.00 (m, 3H). MS (ESI/NH_3): *m/z* = 327.1 (M + H^+). Anal. ($C_{18}H_{22}N_4O_2$) C, H, N.

9-Dimethylamino-3-(4′-ethylphenyl)-*3H*-1,3,6-triazafluoren-4-one (10a): prepared using the same procedure as for **10b**, but employing *p*-ethylaniline as the amine source (18% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.21 ppm (d, J = 6.1 Hz, 1H), 8.15 (s, 1H), 7.37 (m, 4H), 6.54 (d, J = 6.1 Hz, 1H), 3.43 (s, 6H), 2.75 (q, J = 7.8 Hz, 2H), 1.30 (t, J = 7.8 Hz, 3H). MS (ESI/NH_3): m/z = 335.0 (M + H$^+$). Anal. ($C_{19}H_{18}N_4O_2$) C, H, N.

2-(3-Cyano-4-dimethylaminopyridin-2-ylsulfanyl)acetamide (11). 2-Mercapto acetamide (in methanolic ammonia) (1.0 g, 10.0 mmol) in methanol (15 mL) was added sodium methoxide (2.28 g, 25.0 mmol) and then 2-chloro-4-dimethylaminonicotinonitrile (**5**, 1.6 g, 9.0 mmol). The reaction mixture was refluxed for 20 h. After cooling to room temperature, the mixture was evaporated to dryness, water was added to the residue, and solid was collected and dried in the drying oven to give the desired product (2.1 g, 96%) as a white solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ = 3.32 ppm (s, 6H), 3.85 (s, 2H), 6.60 (d, J = 6.4 Hz, 1H), 7.10 (s, 1H), 7.50 (s, 1H), 8.06 (d, J = 6.4 Hz, 1H). MS (DCI/NH_3): m/z = 237.0 (M + H$^+$).

5-Amino-4-dimethylaminothieno[8,9-*b*]pyridine-6-carboxylic Acid Amide (12). 2-(3-Cyano-4-dimethylaminopyridin-2-ylsulfanyl)acetamide (**11**, 2.1 g, 9.0 mmol) in THF (20 mL)/methanol (5 mL)/pyridine (0.5 mL) was treated with potassium *t*-butoxide (2.3 g, 18.5 mmol), and the reaction mixture was refluxed for 20 h. After cooling to room temperature, the mixture was evaporated to dryness, water was added to the residue, and yellow solid was collected and dried in the drying oven to give the desired product (1.9 g, 98%).

^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.81 ppm (s, 6H), 6.82−7.21 (m, 5H), 8.38 (d, J = 5.4 Hz, 1H). MS (DCI/NH_3): m/z = 237.0 (M + H$^+$).

9-Dimethylamino-*3H*-5-thia-1,2,3,6-tetraazafluoren-4-one (13). 5-Amino-4-dimethylaminothieno[8,9-*b*]pyridine-6-carboxylic acid amide (**12**, 1.9 g, 8.0 mmol), in 5*N* HCl (20 mL) was cooled to 0 °C and treated dropwise with a solution of $NaNO_2$ (1.1 g, 16.0 mmol) in water (15 mL). The reaction mixture was vigorously stirred for 30 min. Then a solution of 5% aq $NaHCO_3$ was added until pH = 7 was reached, whereupon a solid precipitated. The solid was collected by filtration and washed with cold water to give the desired product (1.8 g, 91% yield) as an off-white solid.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.01−3.25 ppm (m, 6H), 7.03 (d, J = 5.76 Hz, 1H), 8.44 (d, J = 5.76 Hz, 1H), 15.54 (s, 1H). MS (DCI/NH_3): m/z = 248.0 (M + H$^+$).

3-Cycloheptyl −9-dimethylamino-*3H*-5-thia-1,2,3,6-tetraazafluoren-4-one (14). 9-Dimethylamino-*3H*-5-thia-1,2,3,6-tetraazafluoren-4-one (**13**, 70 mg, 0.28 mmol) in DMF (4 mL) was treated with NaH 60% disp. in oil (18 mg, 0.4 mmol) and then with cycloheptyl bromide (0.04 mL, 0.29 mmol). The reaction mixture was heated at 80 °C for 18 h. After cooling to room temperature, the mixture was poured into water (25 mL) and extracted with ethyl acetate (30 mL). The organic layer was washed with water (3 × 25 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by chromatography (silica gel, 1:1 hexane:EtOAc) to provide the desired product (55 mg, 57% yield).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.47−1.80 ppm (m, 4H), 1.83−2.02 (m, 4H), 2.03−2.46 (m, 4H), 3.23 (s, 6H), 4.99−5.59 (m, 1H), 6.85 (d, J = 5.8 Hz, 1H) 8.43 (d, J = 5.8 Hz, 1 H). MS (DCI/NH_3): m/z = 344.0 (M + H$^+$). Anal. ($C_{17}H_{21}N_5OS$) C, H, N.

General Procedure To Make Oxazinone 15. 9-Dimethylamino-2-methyl-3-oxa-5-thia-1,6-diazafluoren-4-one (15a). To a flask containing the aminomethyl ester intermediate **6** (1.0 g, 4.0 mmol) was added acetic anhydride (15 mL), and the reaction mixture was refluxed for 2 h. After cooling the reaction to room temperature and standing for 24 h, the desired material crystallized and was filtered off. The product was recrystallized from toluene to give white needles (0.80 g) in 76% yield.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.58 ppm (s, 3H), 3.17 (s, 6H), 6.75 (d, J = 3.0 Hz, 1H), 8.39 (d, J = 3.0 Hz, 1H). MS (DCI/NH_3): m/z = 262.0 (M + H$^+$).

9-Dimethylamino-2-ethyl-3-oxa-5-thia-1,6-diazafluoren-4-one (15b): prepared using the same procedure as for **15a**, but employing propanoic acid anhydride (64% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.67 ppm (s, 3H), 2.94−3.05 (m, 2H), 3.19 (s, 6H), 6.78 (d, J = 3.0 Hz, 1H), 8.40 (d, J = 3.0 Hz, 1H). MS (DCI/NH_3): m/z = 276.0 (M + H$^+$).

9-Dimethylamino-2-*n*-butyl-3-oxa-5-thia-1,6-diazafluoren-4-one (15c): prepared using the same procedure as for **15a**, but employing *n*-pentanoic acid anhydride (62% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.00 (t, J = 11.0 Hz, 2H), 1.28−1.42 (m, 3H), 1.75−2.05 (m, 4H), 3.21 (s, 6H), 6.95 (d, J = 4.0 Hz, 1H), 8.43 (d, J = 4.0 Hz, 1H). MS (DCI/NH_3): m/z = 316.0 (M + H$^+$).

General Procedure To Make 9-Dimethylamino-2-methyl-3H-5-thia-1,3,6-triazafluoren-4-one (16). 9-Dimethylamino-2-methyl-*3H*-5-thia-1,3,6-triazafluoren-4-one (16a). To a round-bottom flask containing AcOH (10 mL) was added the oxazinone intermediate **15a** (1.0 g, 4.0 mmol), and the reaction mixture was heated at reflux for 2 h. The reaction mixture was cooled and extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated. The mixture was purified via column chromatography (SiO_2, CH_2Cl_2 to 10%MeOH/CH_2Cl_2) to give 0.68 g of the desired product in 49% yield as a white solid/foam.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.30 ppm (t, J = 9.0 Hz, 3H), 2.37 (s, 3H), 2.75 (dd, J = 6.0, 12.0 Hz, 2H), 3.20 (s, 6H), 6.87 (d, J = 6.0 Hz, 1H), 7.18 (d, J = 9.0 Hz, 2H), 7.39 (d, J = 9.0 Hz, 2H), 8.40 (d, J = 6.0 Hz, 1H). MS (DCI/NH_3): m/z = 365.0 (M + H$^+$). Anal. ($C_{20}H_{20}N_4OS$) C, H, N.

9-Dimethylamino-2-ethyl-*3H*-5-thia-1,3,6-triazafluoren-4-one (16b): prepared using the same procedure as for **16a**, but employing intermediate **15b** and propanoic acid anhydride (52% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.35−1.39 ppm (m, 6H), 2.60 (dd, J = 10.9, 22.0 Hz, 2H), 2.78−2.84 (m, 2H), 3.24 (s, 6H), 6.78 (d, J = 5.8 Hz, 1H), 7.18 (d, J = 8.5 Hz, 2H), 7.37 (d, J = 8.5 Hz, 2H), 8.40 (d, J = 5.76 Hz, 1H). MS (DCI/NH_3): m/z = 378.0 (M + H$^+$). Anal. ($C_{21}H_{22}N_4OS$) C, H, N.

9-Dimethylamino-2-*n*-butyl-*3H*-5-thia-1,3,6-triazafluoren-4-one (16c): prepared using the same procedure as for **16a**, but employing intermediate **15c** and *n*-pentanoic acid anhydride (42% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.85 ppm (t, J = 11.2 Hz, 2H), 1.22−1.38 (m, 6H), 1.70−1.82 (m, 2H), 2.55 (t, J = 11.2 Hz, 2H), 2.75 (dd, J = 15.3 Hz, 2H), 3.21 (s, 6H), 6.77 (d, J = 5.8 Hz, 1H), 7.18 (d, J = 8.5 Hz, 2H), 7.39 (d, J = 8.5 Hz, 2H), 8.40 (d, J = 5.8 Hz, 1H). MS (DCI/NH_3): m/z = 407.0 (M + H$^+$). Anal. ($C_{23}H_{26}N_4OS$) C, H, N.

1-Cyano-2,6-dinitrobenzene (18). A mixture of 2,6-ddinitro-1-chlorobenzene (**17**, 14.7 g, 73 mmol), copper cyanide (8.0 g, 90.0 mmol), and DMF (75 mL) was heated at 145 °C under N_2 for 2 h. The mixture was cooled to room temperature and poured into ice water. The solid was filtered off, and then the solid was extracted with hot EtOAc (2 × 200 mL). Combined extracts were evaporated. The residue was suspended in 25 mL of hot EtOH, filtered off, and dried to give the desired 1-cyano-2,6-dinitrobenzene (**18**, 9.8 g, 70%).

^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.22 ppm (t, J = 9.0 Hz, 1H), 8.67 (d, J = 9.0 Hz, 2H). MS (DCI/NH_3): m/z 194 (M + H)$^+$.

2-Dimethylamino-1-cyano-6-nitrobenzene (19). To the nitrile intermediate **18** from above (1.0 g, 5.2 mmol) dissolved in DMF (6 mL) was added 40% aqueous dimethylamine (1.5 mL, 14.0 mmol). The mixture was heated to 50 °C for 10 min and then cooled to room temperature. The mixture was poured into ice water. The precipitate formed was filtered off and vacuum-dried to obtain 0.93 g (95%) of desired product as a brown color solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.15 ppm (s, 6H), 7.22 (m, 2H), 7.55 (m, 2H). MS (DCI,/NH_3): m/z = 191.0 (M + H$^+$).

5-Amino-4-dimethylaminothieno[8,9-*b*]benzo-6-carboxylic Acid Methyl Ester (20). A mixture of nitrile intermediate **19** (1.9 g, 10.4 mmol) and methyl thioglycolate (1.2 g, 10.4 mmol) was dissolved in dry DMF (20 mL). The mixture was cooled to 0 °C. Sodium methoxide (1.2 g, 22.0 mmol) was then added under N_2. The reaction mixture was allowed to warm

up to room temperature and stirred for 16 h, it was poured into ice water (250 mL), and a yellow precipitate that formed was filtered off and vacuum-dried to obtain 2.3 g (88%) of the desired 5-amino-4-dimethylaminothieno[8,9-*b*]benzo-6-carboxylic acid methyl ester (**20**) as a yellow amorphous solid.

^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 2.71$ ppm (s, 6H), 3.78 (s, 3H), 7.19 (d, $J = 9.0$ Hz, 1H), 7.38 (bs, 2H), 7.44 (t, $J = 9.0$ Hz, 1H), 7.54 (d, $J = 9$ Hz, 1H). MS (DCI/NH_3): m/z 251 $(M + H)^+$.

9-Dimethylamino-3-(4-ethylphenyl)-*3H*-5-thia-1,3-diazafluoren-4-one (22). A mixture of intermediate **20** (1.9 g, 7.6 mmol), dimethylformamide dimethylacetal (6.0 mL), and EtOH (6 mL) was refluxed under N_2 for 16 h, cooled to room temperature, and concentrated under vacuum. The solid obtained was recrystallized from EtOH−water to obtain 2.1 g (90%) of the desired 4-dimethylamino-5-(dimethylaminomethyleneamino)thieno[8,9-*b*]benzene-6-carboxylic acid methyl ester (**21**).

A mixture of 4-dimethylamino-5-(dimethylaminomethyleneamino)thieno[8,9-*b*]benzene-6-carboxylic acid methyl ester (**21**, 0.306 g, 1.0 mmol), 4-ethylaniline (0.181 g, 1.5 mmol), *p*-toluenesulfonic acid (0.02 g, 0.1 mmol), and toluene (7 mL) was refluxed under N_2 for 16 h. The reaction mixture was cooled to room temperature and concentrated under vacuum, and the residue was purified by flash column chromatography (SiO_2, 1:1 hexane:EtOAc) to give 0.11 g (13%) of the desired diazafluorenone as yellow color solid.

^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 1.25$ ppm (t, $J = 9$ Hz, 3H), 2.72 (q, $J = 9.0$ Hz, 2H), 2.95 (s, 6H), 7.07 (d, $J = 9.0$ Hz, 1H), 7.51 (m, 5H), 7.65 (d, $J = 9$ Hz, 1H), 8.58 (s, 1H). MS (DCI/NH_3): $m/z = 350.0$ $(M + H^+)$. Anal. ($C_{20}H_{19}N_3OS \cdot 0.1H_2O$) C, H, N.

3,5-Dichloroisonicotinonitrile (24). A mixture of 3,5-dichloro-4-pyridinecarboxaldehyde (**23**, 10.0 g, 57.1 mmol), hydroxylamine hydrochloride (5.25 g 76 mmol), formic acid (50 mL) and concentrated H_2SO_4 (5 drops) was refluxed under N_2 for 6 h. The mixture was concentrated under vacuum. The solids were taken in Et_2O (250 mL), washed with $NaHCO_3$ and brine, and the organic layer was dried over $MgSO_4$, filtered, concentrated, and recrystallized from hexane to give 8.2 g (83%) of the desired nitrile **24** (mp 114 °C).

^{1}H NMR (300 MHz, CDCl3): $\delta = 8.70$ ppm (s, 2H). MS (DCI/NH_3): m/z 173 $(M + H)^+$.

3-Chloro-5-dimethylaminoisonicotinonitrile (25). The nitrile intermediate **24** from above (3.0 g, 17.2 mmol) was dissolved in DMF (25 mL). The mixture was cooled to 0 °C. To this mixture was added 40% aqueous dimethylamine (7.0 mL, 35 mmol). The mixture was allowed to warm up room temperature, stirred at that temperature for 4 h, and then poured into ice water. The precipitate that formed was filtered off and vacuum-dried to obtain 2.3 g (75%) of the desired 3-chloro-4-cyano-5-dimethylaminopyridine (**25**) as a beige color solid (mp114 °C).

^{1}H NMR (300 MHz, CDCl3): $\delta = 3.11$ ppm (s, 6H), 8.05 (s, 1H), 8.10 (s, 1H); MS (DCI/NH_3) m/z 182 $(M + H)^+$.

3-Amino-4-dimethylaminothieno[2,3-*c*]pyridine-2-carboxylic Acid Methyl Ester (26). A mixture of **25** (1.9 g, 10.4 mmol) and methyl thioglycolate (1.2 g, 10.4 mmol) was dissolved in dry DMF (20 mL). The mixture was cooled to 0 °C. Sodium methoxide (1.2 g, 22 mmol) was then added under N_2. The reaction mixture was allowed to warm up to room temperature and allowed to stir for 16 h, it was poured into ice water (250 mL), and a yellow precipitate that formed was filtered off and vacuum-dried to obtain 2.3 g (88%) of 5-amino-4-dimethylamino-2-aza-thieno[8,9-*b*]pyridine-6-carboxylic acid methyl ester (**26**) as a yellow amorphous solid.

^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 2.80$ ppm (s, 6H), 3.81 (s, 3H), 7.18 (bs, 2H), 8.35 (s, 1H), 8.83 (s, 1H). MS (DCI/NH_3): m/z 252 $(M + H)^+$.

4-Dimethylamino-3-(dimethylaminomethyleneamino)-thieno[2,3-*c*]pyridine-2-carboxylic Acid Methyl Ester (27). A mixture of aminocarboxylate intermediate **26** (1.9 g, 7.6 mmol), dimethyl-formamide dimethylacetal (6.0 mL), and EtOH (6 mL) was refluxed under N_2 for 16 h, cooled to room temperature, and concentrated under vacuum. The solid obtained was recrystallized from EtOH−water to obtain 2.1 g (90%) of the desired 4-dimethylamino-5-(dimethylaminomethyleneamino)-2-aza-thieno[8,9-*b*]pyridine-6-carboxylic acid methyl ester (**27**).

^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 2.81$ ppm (s, 6H), 3.04 (s, 6H), 3.76 (s, 3H), 7.52 s, 1H), 8.04 (s, 1H), 8.65 (s, 1H). MS (DCI/NH_3): m/z 307 $(M + H)^+$.

9-Dimethylamino-3-(4-ethylphenyl)-*3H*-5-thia-1,3,7-triazafluoren-4-one (28a). A mixture of **27** (0.306 g, 1.0 mmol), 4-ethylaniline (0.181 g, 1.5 mmol), *p*-toluenesulfonic acid (0.02 g, 0.1 mmol), and toluene (7 mL) was refluxed under N_2 for 16 h. The reaction mixture was cooled to room temperature and concentrated under vacuum, and the residue was purified by flash column chromatography (silica gel, 1:1 hexane:EtOAc) to give 0.11 g (38%) of the desired pyrimidone as a yellow color solid.

^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 1.24$ ppm (t, $J = 9.0$ Hz, 3H), 2.71 (q, $J = 9.0$ Hz, 2H), 3.01 (s, 6H), 7.42 (d, $J = 9.0$ Hz, 2H), 7.50 (d, $J = 9$ Hz, 2H), 8.27 (s, 1H), 8.63 (s, 1H), 8.96 (s, 1H). MS (DCI/NH_3): $m/z = 351.0$ $(M + H^+)$. Anal. ($C_{19}H_{18}N_4OS$) C, H, N.

9-Dimethylamino-3-(4-methylphenyl)-*3H*-5-thia-1,3,7-triazafluoren-4-one (28b): prepared using the same procedure as for **28a**, but employing *p*-toluidine as the amine source (58% yield). ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 2.42$ ppm (s, 3H), 3.02 (s, 6H), 7.40 (d, $J = 9.0$ Hz, 2H), 7.46 (d, $J = 9.0$ Hz, 2H), 8.27 (s, 1H), 8.63 (s, 1H), 8.97 (s, 1H). MS (DCI/NH_3): $m/z = 337.0$ $(M + H^+)$. Anal. ($C_{18}H_{16}N_4OS$) C, H, N.

2-(3-Dimethylamino-1-ethoxyallylidene)malononitrile (30). 2-(1-Ethoxyethylidene)malononitrile (**29**, 110.0 g, 808 mmol) was dissolved in *N*,*N*-dimethylformamide dimethyl acetal (94%, 230 mL, 1.62 mol), and the reaction mixture was heated to reflux at 100 °C for 1 h. The mixture was cooled down to room temperature. Solid was collected and washed with cold methanol to give an orange solid product. The mother liquor was concentrated, and the solid was collected again, and washed with cold methanol. This procedure was repeated a couple of times, and all the solid products were combined. The final residue was purified by a short column chromatography (SiO_2, ethyl acetate). The combined yield of the reaction is 78% (120.0 g).

^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 7.47$ ppm (d, $J = 12.6$ Hz, 1H); 5.16 (d, $J = 12.6$ Hz, 1H); 4.43 (q, $J = 7.1$ Hz, 2H), 3.19 (s, br. 3H), 2.94 (s, br 3H), 1.41 (t, $J = 7.1$ Hz, 3H). MS (ESI/NH_3): $m/z = 192.1$ $(M + H^+)$.

2-Chloro-3-cyano-4-ethoxypyridine (31). To a slurry of 2-(3-dimethylamino-1-ethoxyallylidene)malononitrile (**30**, 85.0 g, 445.0 mmol) in methanol (1200 mL), HCl gas was introduced gently at 0 °C. The reaction mixture became homogeneous in about 1 h, and was allowed to stir under constant HCl flow for additional 14.5 h at 0 °C. N_2 was bubbled though the reaction mixture overnight, and all the solvent was removed. The residue solid was redissolved in CH_2Cl_2 and washed with water/K_2CO_3/water. The organic layer was separated and dried over Na_2SO_4. Removal of salt and solvent gave a pure product (113.0 g, 98%).

^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 7.96$ ppm (d, $J = 6.4$ Hz, 1H); 6.59 (d, $J = 6.4$ Hz, 1H); 3.30 (s, 6H). MS (ESI/NH_3): $m/z = 181.9$ $(M + H^+)$.

2-Bromo-3-cyano-4-hydroxypyridine (32). A mixture of 2-chloro-3-cyano-4-ethoxypyridine (**31**, 8.5 g, 46.7 mmol) and HBr in acetic acid (30%, 85 mL) was heated to 100 °C for 2 h. The mixture was cooled down to room temperature, and solid was collected, washed with cold water, and dried under vacuum to give a white solid with 91.6% bromonation product, and 8.4% chlorination product.

2-Bromo-3-cyano-4-hydroxypyridine (32a). ^{1}H NMR (DMSO-d_6, 300 MHz): $\delta = 8.20$ ppm (d, $J = 5.9$ Hz, 1H); 6.95 (d, $J = 5.9$ Hz, 1H). MS (ESI/NH_3): $m/z = 200.9$ $(M + H^+)$.

2-Chloro-3-cyano-4-hydroxypyridine (32b). ^{1}H NMR (DMSO-d_6, 300 MHz): $\delta = 8.27$ ppm (d, $J = 6.3$ Hz, 1H); 6.98 (d, $J = 6.3$ Hz, 1H). MS (ESI/NH_3): $m/z = 172.0$ $(M + H^+)$.

2-Bromo-3-cyano-4-(4′-methoxybenzyloxy)pyridine (33). A mixture of 2-bromo-3-cyano-4-hydroxypyridine (**32a**) and 2-chloro-3-cyano-4-hydroxypyridine (**32b**) (10.0 g, 35.7 mmol) was dissolved in DMF (50 mL), followed by NaH in portions (60%, 2.86 g, 71.5 mmol). 4-Methoxybenxyl chloride (6.85 mL, 50.3 mmol) was added. The reaction mixture was then heated to 60 °C for 2.5 h and quenched with NH_4Cl (saturated). The mixture was then extracted with ethyl acetate (5 × 20 mL). The combined organic layers were washed with water, and dried over Na_2SO_4. Column chromatographic purification (SiO_2, ethyl acetate:hexanes = 1:9) gave an off-white solid product (4.21 g, 37%) with 92% the title product and 8% of 2-chloro-3-cyano-4-(4′-methoxybenzyloxy)pyridine.

2-Bromo-3-cyano-4-(4′-methoxybenxyloxy) pyridine (33a). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.33 ppm (d, J = 5.7 Hz, 1H); 7.34 (d, J = 8.8 Hz, 2H), 6.94 (d, J = 8.8 Hz, 2H), 5.23 (s, 2H), 3.83 (s, 3H). MS (ESI/NH_3): m/z = 320.0 (M + H^+).

2-Chloro-3-cyano-4-(4′-methoxybenzyloxy)pyridine (33b). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.36 ppm (d, J = 5.8 Hz, 1H); 7.31 (d, J = 8.8 Hz, 2H), 6.89 (d, J = 8.8 Hz, 2H), 4.63 (s, 2H), 3.82 (s, 3H). MS (ESI/NH_3): m/z = 275.0 (M + H^+).

5-Amino-4-(4′-methoxybenzyloxy)thieno[8,9-*b*]pyridine-6-carboxylic Acid Methyl Ester (34). 2-Bromo-3-cyano-4-(4′-methoxybenzyloxy)pyridine (**33a**, 4.0 g, 12.5 mmol) was dissolved in DMF (20 mL), followed by methyl thioglycolate (1.2 mL, 13.2 mmol), and then sodium methoxide (95%, 1.56 g, 27.4 mmol) in portions at room temperature. The reaction mixture was allowed to stir overnight, and then quenched with water. Solid was collected and washed with water several times, then dried under vacuum to give a pure product (3.7 g, 86%).

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.48 ppm (d, J = 5.8 Hz, 1H), 7.39 (m, 2H), 6.96 (m, 2H), 6.76 (d, J = 5.8 Hz, 1H), 6.53 (s, br. 1H), 5.19 (s, 2H), 3.85 (s, 3H), 3.84 (s, 3H). MS (ESI/NH_3): m/z = 344.9 (M + H^+).

4-(4′-Methoxybenzyloxy)-5-(dimethylaminomethyleneamino)thieno[8,9-*b*]pyridine-6-carboxylic Acid Methyl Ester (35). 5-Amino-4-(4′-methoxybenzyloxy)thieno[8,9-*b*]pyridine-6-carboxylic acid methyl ester (**34**, 3.7 g, 10.8 mmol) was dissolved in *N*,*N*-dimethylformamide dimethyl acetal (30 mL), and heated to reflux for 10 h. Excess of reagent was removed to give a yellow solid product (4.3 g, 100%).

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.47 ppm (d, J = 5.8 Hz, 1H), 7.44 (s, 1H), 7.40 (m, 2H), 6.91 (m, 2H), 6.75 (d, J = 5.8 Hz, 1H), 5.09 (s, 2H), 3.84 (s, 3H), 3.82 (s, 3H), 2.83 (s, br, 6H). MS (ESI/NH_3): m/z = 400.0 (M + H^+).

9-(4′-Methoxybenzyloxy)-3-(4′-ethylphenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (36). 4-(4′-Methoxybenzyloxy)-5-(dimethylaminomethyleneamino)thieno[8,9-*b*]pyridine-6-carboxylic acid methyl ester (**35**, 4.0 g, 10.0 mmol), 4-ethylanaline (2.42 g, 20.0 mmol), and a catalytic amount of *p*-toluenesulfonic acid (200 mg, 1.1 mmol) in toluene (50 mL) were heated under microwave to 160 °C for 1 h. The reaction mixture was then cooled, and solvent was removed. The residue was purified via column chromatography (SiO_2, ethyl acetate:hexanes = 1:4, then 1:1) to give a solid product, which was recrystallized from cold methanol (1.7 g, 39%).

^{1}H NMR (DMSO-d_6, 300 MHz): δ = 8.64 ppm (d, J = 5.5 Hz, 1H), 8.56 (s, 1H), 7.53 (d, J = 8.6 Hz, 2H), 7.48 (d, J = 8.3 Hz, 2H), 7.40 (d, J = 8.3 Hz, 2H), 7.33 (d, J = 5.5 Hz, 1H), 6.97 (d, J = 8.6 Hz, 2H), 5.45 (s, 2H), 3.75 (s, 3H), 3.71 (q, J = 7.4 Hz, 2H), 1.25 (t, J = 7.4 Hz, 3H). MS (ESI/NH_3): m/z = 444.0 (M + H^+).

9-Hydroxy-3-(4′-ethylphenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (37). 9-(4′-Methoxybenzyloxy)-3-(4′-ethylphenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (**36**, 1.6 g, 3.6 mmol) was added to cold (0 °C) trifluoroactic acid (10 mL) in portions, and then allowed to stir for 1.2 h. Excess of TFA was removed under vacuum. The residue was allowed to sit overnight, then treated with ethyl acetate (20 mL). Ethyl acetate was removed and the procedure was repeated five times until a yellow solid was obtained. Hexanes were added to the solid, and the mixture was sonicated. Solid was then collected to give the desired product (1.61 g, 100%).

^{1}H NMR (DMSO-d_6, 300 MHz): δ = 8.54 ppm (s, 1H), 8.42 (d, J = 5.9 Hz, 1H), 7.48 (d, J = 6.6 Hz, 2H), 7.43 (d, J = 6,6 Hz, 2H), 6.92 (d, J = 5.9 Hz, 2H), 2.72 (q, J = 7.5 Hz, 2H), 1.25 (t, J = 7.5 Hz, 3H). MS (ESI/NH_3): m/z = 324.0 (M + H^+).

9-(Trifluromethanesulfonyl)-3-(4′-ethylphenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (38). 9-Hydroxy-3-(4′-ethylphenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (**37**, 1.66 g, 3.82 mmol), *N*-phenyltrifluoromethanesulfonimide (3.42 g, 9.57 mmol), and *N*-ethyl-diisopropylamine (1.7 mL, 9.76 mmol) were dissolved in 1,4-dioxane (80 mL) at room temperature. The reaction mixture was allowed to stir for 3 days. Solvent was removed, and the residue was purified via column chromatography (SiO_2, ethyl acetate:hexanes = 1:9) to give a white solid product (1.42 g, 82%).

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.88 ppm (d, J = 5.1 Hz, 1H), 8.35 (s, 1H), 7.39 (m, 5H), 2.74 (q, J = 7.5 Hz, 2H), 1.31 (t, J = 7.5 Hz, 3H). MS (ESI/NH_3): m/z = 455.9 (M + H^+).

General Procedure for Making Mono and Disubstituted Triazafluorenone Analogues 39. 9-*N*-Azetidinyl-3-(4′-ethylphenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (39c). 9-(Trifluromethanesulfonyl)-3-(4′-ethylphenyl)-3*H*-5-thia-1,3,6-triazafluoren-4-one (**38**, 60 mg, 0.13 mmol) was dissolved in dichloromethane (2 mL). Azetidine hydrochloride (50 mg, 0.52 mmol) was neutralized with sodium hydroxide (1 N, 1 mL, 1.0 mmol) in dichloromethane. The organic layer was separated and dried over sodium sulfate. The solution of azetidine in dichloromethane was then filtrated and added to the reaction mixture. The reaction mixture was then allowed to stir at room temperature for 1 day. Solvent was removed, and the crude mixture was purified via a short column chromatography (SiO_2, ethyl acetate:hexanes = 1:4) to give a white solid product (42 mg, 89%).

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.26 ppm (d, J = 6.1 Hz, 1H), 8.17 (s, 1H), 7.37 (m, 4H), 6.25 (d, J = 6.1 Hz, 2H), 4.54 (m, 4H), 2.75 (q, J = 7.8 Hz, 2H), 2.47 (m, 2H), 1.30 (t, J = 7.8 Hz, 3H). MS (ESI/NH_3): m/z = 363.0 (M + H^+). Anal. ($C_{20}H_{18}N_4OS$) C, H, N.

9-(*N*,*N*-Ethylmethylamino)-3-(4′-ethylphenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (39a): prepared using the same procedure as for **39c**, but employing ethylmethylamine hydrochloride as the amine source (92% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.43 ppm (d, J = 6.6 Hz, 1H), 8.27 (s, 1H), 7.40 (d, J = 8.4 Hz, 2H), 7.35 (d, J = 8.4 Hz, 2H), 6.84 (d, J = 6.6 Hz, 1H), 3.82 (q, J = 7.2 Hz, 2H), 3.33 (s, 3H), 2.75 (q, J = 7.8 Hz, 2H), 1.38 (t, 7.2 Hz, 3H), 1.30 (t, J = 7.8 Hz, 3H). MS (ESI/NH_3): m/z = 365.0 (M + H^+). Anal. ($C_{20}H_{20}N_4OS \cdot 0.1H_2O$) C, H, N.

9-(2′-Methoxyethylmethylamino)-3-(4′-ethylphenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (39b): prepared using the same procedure as for **39c**, but employing 2-methoxyethylmethylamine as the amine source (78% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.42 ppm (d, J = 5.8 Hz, 1H), 8.26 (s, 1H), 7.37 (m, 4H), 6.85 (d, J = 5.8 Hz, 1H), 3.91 (t, J = 5.4 Hz, 2H), 3.68 (t, J = 5.4 Hz, 2H), 3.27 (s, 3H), 3.21 (s, 3H), 2.75 (q, J = 7.8 Hz, 2H), 1.30 (t, J = 7.8 Hz, 3H). MS (ESI/NH_3): m/z = 395.0 (M + H^+). Anal. ($C_{21}H_{22}N_4O_2S \cdot 0.3H_2O$) C, H, N.

9-Cyclopropylamino-3-(4′-ethylphenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (39d): prepared using the same procedure as for **39c**, but employing cyclopropylamine as the amine source (74% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.67 ppm (s, 1H), 8.49 (d, J = 6.6 Hz, 1H), 8.27 (s, 1H), 7.40 (d, J = 8.0 Hz, 2H), 7.34 (d, J = 8.0 Hz, 2H), 7.05 (d, J = 6.6 Hz, 1H), 2.80 (m. 1H), 2.76 (q, J = 7.8 Hz, 2H), 1.31 (t, J = 7.8 Hz, 3H), 1.09 (m, 2H), 0.83 (m, 2H). MS (ESI/NH_3): m/z = 363.1 (M + H^+). Anal. ($C_{20}H_{18}N_4OS \cdot 0.1H_2O$) C, H, N.

9-(1′-Cyanomethylamino)-3-(4′-ethylphenyl)-*3H*-5-thia-1,3,6-triaza-fluoren-4-one (39e): prepared using the same procedure as for **39c**, but employing cyanomethylamine as the amine source (82% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.71 ppm (t, J = 5.9 Hz, 1H), 8.62 (d, J = 6.5 Hz, 1H), 8.29 (s,

1H), 7.41 (d, $J = 8.4$ Hz, 2H), 7.35 (d, $J = 8.4$ Hz, 2H), 6.79 (d, $J = 6.5$ Hz, 1H), 4.47 (d, $J = 5.9$ Hz, 2H), 2.76 (q, $J = 7.5$ Hz, 2H), 1.31 (t, $J = 7.5$ Hz, 3H). MS (ESI/NH_3): $m/z = 361.9$ (M + H^+). Anal. ($C_{19}H_{15}N_5OS \cdot 0.2H_2O$) C, H, N.

9-(2′,2′,2′-Trifluoroethylamino)-3-(4′-ethylphenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (39f): prepared using the same procedure as for **39c**, but employing 2,2,2-trifluorethylamine as the amine source (67% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 8.94$ ppm (t, $J = 6.9$ Hz, 1H), 8.55 (d, $J = 6.5$ Hz, 1H), 8.32 (s, 1H), 7.97 (s, br. 1H), 7.40 (d, $J = 8.4$ Hz, 2H), 7.34 (d, $J = 8.4$ Hz, 2H), 6.82 (d, $J = 6.5$ Hz, 1H), 4.16 (m, 2H), 2.76 (q, $J = 7.5$ Hz, 2H), 1.31 (t, $J = 7.5$ Hz, 3H). MS (ESI/NH_3): $m/z = 404.9$ (M + H^+). Anal. ($C_{19}H_{15}F_3N_4OS$) C, H, N.

9-(Methylamino)-3-(4′-ethylphenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (40). To a solution of **1n** (53 mg, 0.15 mmol) in formic acid (5 mL) at 0 °C was added 30% H_2O_2 (1 mL), and the mixture was allowed to warm to room temperature for 24 h. Solid $NaHCO_3$ was added to pH = 8, and the mixture was extracted with ethyl acetate, washed with brine, and dried with anhydrous $MgSO_4$. The ethyl acetate was removed under reduced pressure and the residue was chromatographed (SiO_2, hexanes:EtOAc 1:1) to provide 35 mg (70%) of the desired product.

^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 1.24$ ppm (t, $J = 7.0$ Hz, 3H), 2.70 (q, $J = 7.0$ Hz, 2H), 3.03 (d, $J = 4.5$ Hz, 3H), 6.66 (d, $J = 6.0$ Hz, 1H), 7.41 (d, $J = 9.0$ Hz, 2H), 7.48 (d, $J = 9.0$ Hz, 2H), 7.71 (q, $J = 4.5$ Hz, 1H), 8.31 (d, $J = 6.0$ Hz, 1H), 8.59 (s, 1H). MS (DCI/NH_3): $m/z = 337.0$ (M + H^+). Anal. ($C_{18}H_{16}N_4OS \cdot 0.25H_2O$) C, H, N.

9-(*N*,*N*-Dimethyl-*N*-oxyamino)-3-(4′-ethylphenyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (41a). Hydrogen peroxide−urea complex (UHP) (1.7 g, 9.0 mmol) was added to phthalic anhydride (0.88 g, 6.0 mmol) in methylene chloride (50 mL). After 5 min was added **1n** (1.0 g, 2.86 mmol), and the reaction was continued at room temperature until disappearance of starting material (~30 min). A 2 N Solution of Na_2CO_3 (10 mL) was added, and the layers were separated. The water layer was extracted twice with methylene chloride, and all extracts were combined, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, CH_2Cl_2:EtOAc = 9:1) to afford 200 mg (20%) of the desired product.

^{1}H NMR (300 MHz, DMSO-d_6) $\delta = 1.23$ ppm (t, $J = 7.0$ Hz, 3H), 2.71 (q, $J = 7.0$ Hz, 2H), 2.91 (s, 6H), 7.42 (d, $J = 9.0$ Hz, 2H), 7.48 (d, $J = 9.0$ Hz, 2H), 7.55 (d, $J = 6.0$ Hz, 1H), 8.58 (s, 1H), 8.65 (d, $J = 6$ Hz, 1H). MS (DCI/NH_3): $m/z = 367.0$ (M + H^+). Anal. ($C_{19}H_{18}N_4O_2S \cdot 0.2H_2O$) C, H, N.

9-(*N*,*N*-Dimethyl-*N*-oxyamino)-3-(*N*-hexamethyleneiminyl)-*3H*-5-thia-1,3,6-triaza-fluoren-4-one (41b). 9-Dimethylamino-3-(*N*-hexamethyleneiminyl)-*3H*-5-thia-1,3,6-triazafluoren-4-one (**1ac**, 170 mg, 0.5 mmol) in dichloromethane (5 mL) was added to a mixture of urea−hydrogen peroxide complex (141 mg, 1.5 mmol) and phthalic anhydride (75 mg, 0.5 mmol) in dichloromethane (4 mL). The resulting reaction mixture was stirred at room temperature for 4 h. Saturated K_2CO_3 (20 mL) and dichloromethane (20 mL) were added to the reaction mixture. The organic layer was separated and washed with brine, dried over $MgSO_4$, and then concentrated in vacuo. The residue was purified by flash chromatography using 10% methanol in dichloromethane to give the desired product (105 mg, 58%): ^{1}H NMR (300 MHz, DMSO-d_6) $\delta = 1.32-2.02$ ppm (m, 12H) 2.88 (s, 6H) 7.51 (d, $J = 5.8$ Hz, 1H), 8.56 (s, 1H), 8.61 (d, $J = 5.8$ Hz, 1H). MS (DCI/NH_3): $m/z = 359.0$ (M + H^+). Anal. ($C_{17}H_{21}N_5O_2S \cdot 0.15H_2O$) C, H, N.

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Supporting Information Available: Elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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