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Syntheses and kinetic studies of cyclisation-based self-immolative spacers

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Abstract Kinetic analysis of disassembly of self-immolative spacers based on cyclisation processes was performed. Five compounds were synthesized belonging to two different series, and their kinetic constants were determined. Electron-donating substituents gave a slight acceleration but the main effect was steric, and the Thorpe-Ingold effect was indeed particularly effective. Comparison with the self-immolative spacers based on elimination processes showed that cyclisations gave comparable or lower rate, but the corresponding spacers are more difficult to modulate.

Introduction

Self-immolative spacers were introduced in 1981 by *Katzellenbogen et al*[1] as an original way to correlate the cleavage of two chemical bonds. In a Medicinal Chemistry context, this strategy was proposed to overcome limitations of prodrugs, classically made of two moieties: an activator, reacting with an enzyme as a substrate, and a bioactive compound, as a drug or a reporter. The introduction of the spacer core consists of adding a third moiety designed to release the effector after activation. The second bond is cleaved spontaneously after cleavage of the trigger (*scheme 1*). A variety of self-immolative spacers has been introduced over the years in the literature[2-4] and for instance used in cancer chemotherapy (Adcetris®).[5-8] In this strategy, the spontaneous release introduces a second step with its own kinetic parameters. For most applications (medicinal chemistry, analytical chemistry, materials or chemical biology), the self-immolative step needs to be fast enough in order to avoid release too far from the activation site. Our aim has been to obtain comparative data on the kinetics of the self-immolative step so that anyone can choose a spacer suited to their applications. Kinetics of some self-immolative spacers have been studied during the past, especially for elimination-based releases.[9-12] This is why we focused our present studies on the other class of spacers exploiting cyclisation in a self-immolative step. In this series of spacers, the activation step generates a nucleophilic heteroatom (like nitrogen or oxygen; less often sulfur[13]) which attacks an electrophilic centre (classically a carbonyl) on another position of the skeleton of the molecule[14] (*Scheme 1*).

To measure accurately the kinetics of the self-immolation step, a general procedure was set up based on a fast and controlled photoactivation; the fluorescence measurement of a reporter gave then access to the kinetic constants.[9-12] We have already published comparative data on kinetic constants of various elimination-based spacers (1,4 and 1,6) supported by aromatic[10-11] or heteroaromatic rings.[12] In order to complete our comprehension of rate description of self-immolative spacers, we measured the kinetics of cyclisation-based spacers. Two main questions were addressed, the effect of the nature of the spacer and the comparison between elimination and cyclisation processes.

Results and discussion

Molecular design

The structure of the targeted compounds was tripartite: a photocleavable moiety, the self–immolative spacer, and a reporter (fluorophore).

A classical 4,5-dimethoxy-2-nitrobenzyl (also called 6-nitroveratryl) group was used for the photocleavage step. This protecting group (denoted PG in Scheme 1) has been reported to be selectively cleaved by near-UV irradiation (365 nm), with excellent yield, and allows a millisecond timescale resolution.[10]

Self-immolative spacers commonly reported in literature are mainly based on phenyl cores, as aniline and phenol derivatives. The nitrogen or oxygen atom, typically involved in a bond with various protecting groups, triggers the self-immolation process. Here we

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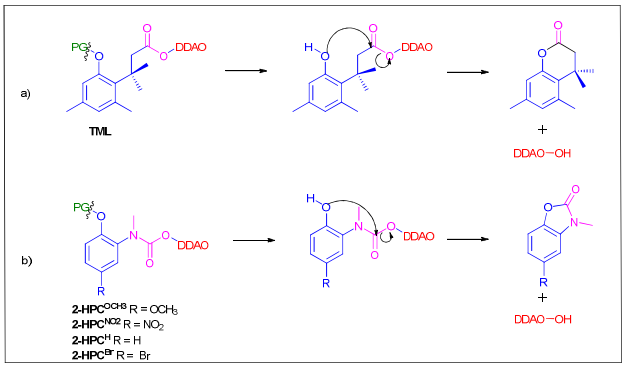
used a phenol core, acting as a nucleophile during the elimination step. Two representative series seemed particularly interesting for cyclisation studies (*scheme 1*):

- Trimethyl-lock derivatives[13-18] via 6-exo-trig-cyclisation. These are the most widely used cyclisation-based spacers

- 2-hydroxyphenyl carbamates (2-HPC) derivatives[19] via 5-exo-trig cyclisation. These derivatives are less popular but are good models to study the influence of substituents on the self-immolation rate (4 substituents were selected: methoxy, nitro, H, bromide)

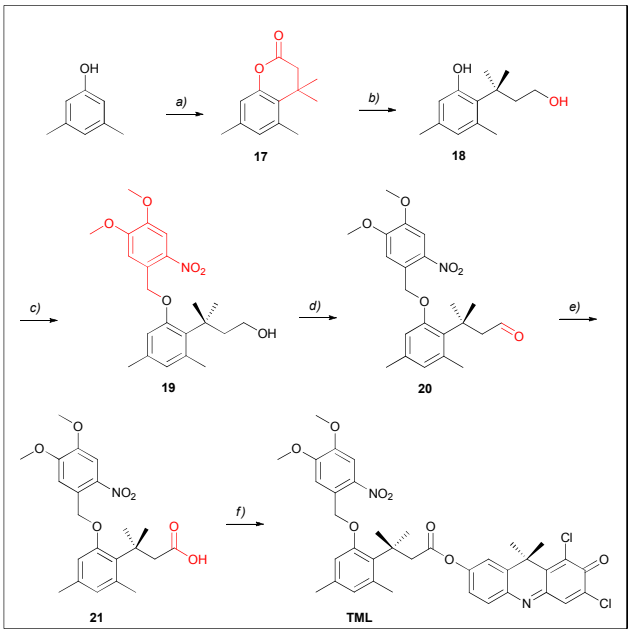


Scheme 1: Cyclisation-based spacers investigated, with a) trimethyl lock and b) 2-HPC. PG=4,5-dimethoxy-2-nitrobenzyl; DDAO=1,3-dichloro-9,9-dimethyl-9H-acridin-2(7)-one.

In order to analyse the stoichiometry and kinetics of cyclisation reactions, we used 1,3-dichloro-9,9-dimethyl-9H-acridin-2(7)-one (DDAO in Scheme 1) as a fluorescent reporter. This moiety does not emit fluorescence in caged precursors, but emits strongly in the red-wavelength region in the free phenol state.

Trimethyl-lock synthesis

The synthesis of the trimethyl-lock derivative (see *scheme 2*) began by an esterification and an aromatic electrophilic substitution of 3,5-dimethylphenol with 3,3-dimethylacrylic acid under heating and acidic conditions to form the corresponding dihydrocoumarine. The lactone was then reduced by lithium aluminium hydride, leading to the free alcohol. These two steps are common in trimethyl lock derivatives syntheses. The following steps have been adapted to produce our own compound. The phenol was engaged in an etherification reaction with 6-nitroveratryl bromide, and then the primary alcohol was successively oxidized to aldehyde and carboxylic acid by, respectively, pyridinium dichromate and a Pinnick oxidation. The acid was finally converted to acyl chloride by phosgene and esterified by DDAO to give the desired ester with an overall yield of 72%.



Scheme 2: Synthesis of the trimethyl-lock derivative. Reagents and conditions: a) 3.3-dimethylacrylic acid, methane sulfonic acid, toluene, 85°C (99%); b) $LiAlH_4$, THF, 0°C to rt (93%); c) 4,5-dimethoxy-2-nitrobenzyl bromide, Cs_2CO_3, THF, rt (93%); d) Pyridinium dichromate (PDC), CH_2Cl_2, rt (95%); e) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, acetone/tBuOH/water, rt (89%); f) 1. Phosgene, THF, rt; 2. NEt_3, 4-Dimethylaminopyridine (DMAP), DDAO, THF, rt (quant.)

Phenol-carbamate syntheses

The general synthesis of the 2-HPC (see *scheme 3*) derivatives started by the aniline protection of the corresponding commercial 2-aminophenol with a Boc group. The selective protection of the aniline function versus phenol came from a methanolysis step that led to carbonate cleavage keeping the carbamate intact. Etherification of the phenol position with 6-nitroveratryl bromide was carried out under basic conditions, and then the Boc protecting group was removed under acidic conditions using a 1:1 TFA:CH_2Cl_2 mixture. This liberated the free aniline which was then monomethylated by iodomethane. Other methods of selective monomethylation (such as reductive amination or use of a soft methylating agent like dimethyl carbonate) showed incompatibilities with our compounds. Finally, the secondary aniline was treated with phosgene, and the resulting carbamoyl chloride reacted with the phenol function of the DDAO to form the desired carbamate (overall yield, from the commercial anilines: 32 to 47%, depending on the substituents). These carbamates are constituted of two rotamers (N-C bond).

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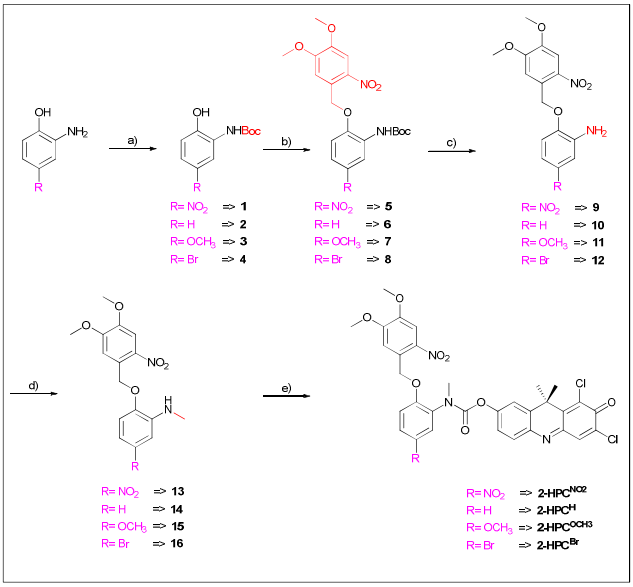
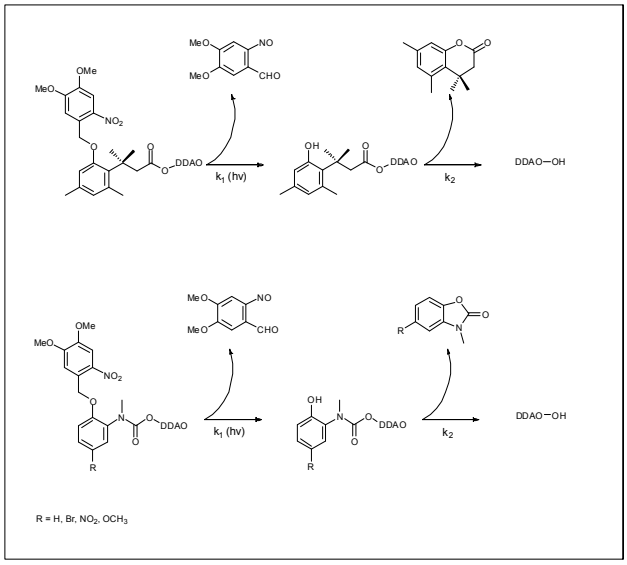


Scheme 3: General synthesis of 2-HPC derivatives, with R as a para *substituent (H, Br, NO_2, OCH_3). Reagents and conditions: a) Boc_2O, K_2CO_3, THF, H_2O, rt (96% to quant.); b) 4,5-dimethoxy-2-nitrobenzyl bromide, Cs_2CO_3, THF, rt (71% to quant.); c) Trifluoroacetic acid (TFA), CH_2Cl_2, rt (quant.); d) CH_3I, K_2CO_3, DMF, rt (42 to 50%); e) 1. Phosgene, THF, rt; 2. NEt_3, DMAP, DDAO, THF, rt (quant.)*

Cyclisation rates

For the kinetic measurements, our working hypothesis was a two-step process as depicted in *scheme 4*. The first step is a photocleavage of the caged precursor, associated to k_1, the rate constant of uncaging, and the intramolecular cyclisation, where k_2 is the rate constant for the self-immolation step.



Scheme 4: Kinetic models for disassembly from photoactivation to self-immolation of trimethyl-lock (top) and 2-HPC (bottom) derivatives.

We followed the same procedure as previously described.[11] The principle is to irradiate the compound at 365 nm and to follow the liberation of the released DDAO by fluorimetry in a quartz cuvette under temperature (293K) and pH control. The experimental data were then fitted with the kinetic model to retrieve the values of k_1 and k_2. The results are reported in table 1.

Compound	pKa of the phenol	k_2 (min^{-1})	τ_2 (min)
2-HPC^{NO_2} (pH 5)	7.6 ± 0.1	(5.2 ± 0.5)10^{-2}	19
2-HPC^{NO_2} (pH 8)	7.6 ± 0.1	(6.6 ± 0.5)10^{-2}	15
2-HPC^{Br} (pH 8)	10.0 ± 0.1	(1.1 ± 0.3)10^{-1}	9
2-HPC^{H} (pH 8)	10.7 ± 0.1	(1.9 ± 0.2)10^{-1}	5
2-HPC^{OCH_3} (pH 8)	10.8 ± 0.1	(2.9 ± 0.3)10^{-1}	3.5
TML (pH 8)	10.2 ± 0.1	(3.3 ± 0.3)10^{-1}	3

Table 1: Kinetic constants of trimethyl lock and 2-HPC derivatives. See Supporting Information for equations and methods; where $\tau = 1/k_2$, k_2 rate constant of the self-immolation step. pKa determination was carried out by following absorbance of protected aniline **1, 2, 3, 4** as a function of pH, applying the same method as in *Alouane et al.*[10-11]

Discussion

Results showed that cyclisation generally occurs in the minute range, for both trimethyl lock and 2-HPC derivatives; disassembly times were close to each other, showing only a factor of six between the largest and the smallest. For the nitro derivative of 2-HPC, which is the only derivative with a physiological pKa (7.6), we did not observe any significant dependence on pH: kinetics at pH 5 and pH 8 are close, showing only a 20% difference.

The trimethyl lock was important because of the large number of publications using it. The popularity of the trimethyl-lock is due to the Thorpe-Ingold effect, which is supposed to allow rapid intramolecular cyclisation. This steric effect is induced by the unfavourable interaction between the methyl at the *meta* position on the phenol core and the two geminal methyls on the alkyl chain (β position from ester). Because of this steric hindrance, a conformation was favoured, making the carbonyl of the ester and the phenol closer. This vicinity was already known to increase drastically the cyclisation rate[20] but the kinetics of the process was still unknown. We measured only the rate constant of the TML, in line with the huge interest to this linkage compared to other carbonyl species (amide for instance).[20] The measured lactonisation time was around 3 min for this ester derivative.

The 2-HPC series was chosen to study cyclisation rates of carbamates, which are relatively resistant to enzymes such as esterases or peptidases. The spacers have been modulated by variation of the substituents on the phenol core. The results

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enabled us to determine the effects of the substituent and of the protonation state on the kinetics of cyclisation. By lowering the pKa *via* a withdrawing group, the proportion of phenolate is increased; the phenolate anion is more nucleophilic than the phenol and so able to accelerate the cyclisation process. We focused our attention on the nitro derivative, which exhibits a pKa close to the physiological pH; in order to be *in vivo*/*in vitro* deprotonated. We observed that the kinetics of the self-immolation did not change significantly by deprotonating the nitrophenol, which led us to conclude that the gain of nucleophilicity was partially compensated by conjugation of the phenolate with the nitro group. More generally, the most electron-donor the cycle has at *para* position the faster the cyclisation occurs in line with an enhanced nucleophilicity of the phenol: Compared to the H derivative (*para*-hydrogen), we observed a relative acceleration with electron-donor groups and a moderate slow-down with electron-withdrawing ones. According to the literature, in both aliphatic[21] and aromatic[22] derivatives, nucleophilicity enhancement is a critical factor for the cyclisation rate. Indeed, in every series (including ours, phenol-based ones), electron-donating groups made the nucleophile (oxygen or nitrogen in previously cited works) more reactive. Adding this to other parameters, like decreasing pKa of the leaving group, changing heteroatom, promoting cyclisation at high pH, choosing a better electrophilic carbonyl, or increasing temperature, various cyclisation-based spacer can be generated with half-times close to the minute range, or even less.

Eventually, this work and our previous ones[11,12] show that self-immolation in elimination-based spacers is much more rapid and substituent sensitive than in cyclisation-based ones.

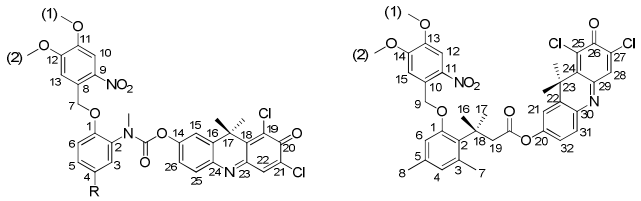
Conclusions

We investigated the self-immolation kinetics of common cyclisation-based self-immolative spacers. They exhibited release-times within the 1-10 minute range at room temperature. Steric effects were more stringent than electronic ones to modulate the self-immolation rate. The comparison between cyclisation and elimination-driven self-immolation rates (as reported by *Alouane et al)*[11,12] further suggested the later mechanism to have more potential for modulating kinetics.

Experimental

The commercially available chemicals were used without further purification. Anhydrous solvents were freshly distilled before use. Low actinic glassware and aluminium film were used for all experiments involving compounds bearing the nitroveratryl moiety. Column chromatography (CC): silica gel 60 (0.040-0.063mm) Merck. Analytical and thin layer chromatography (TLC): Merck silica gel 60 F-254 precoated plates; detection by UV (254 and 365 nm). ^{1}H NMR spectra were recorded at 300 MHz. ^{13}C NMR spectra were recorded at 75 MHz with complete proton decoupling; chemical shifts (δ) in ppm related to protonated solvent as internal reference (^{1}H: $CHCl_3$ in $CDCl_3$, 7.26 ppm; ^{13}C: $^{13}CDCl_3$ in $CDCl_3$, 77.0 ppm; Coupling constants *J* in Hz; current notations are used for multiplicity (s: singlet; bs: broad singlet; d: doublet; dd: double doublet; t: triplet; q: quadruplet; m: multiplet).

Scheme 5: Numeration for C^{13} and H^{1} signals



General procedure

Aniline protection (**1, 2, 3, 4**)

To a solution of 2-aminophenol derivative (1 eq) in THF/water (1:1 proportion) was added K_2CO_3 (5 eq) and di-*tert*-butyl dicarbonate (2.6 eq). After stirring for 4h at room temperature, the mixture was neutralized (Acetic acid 100%, until pH 7), the organic layer was diluted with EtOAc and washed with water and brine, then dried on $MgSO_4$, filtered and concentrated under reduced pressure. The residue was resolubilized in MeOH and we added K_2CO_3 (5 eq) and stirred at room temperature until no more evolution is observed (as seen on TLC, between 1h30 and 4h). The same work-up than previously was applied to obtain the crude product.

Nitroveratryl coupling (**5, 6, 7, 8**)

4,5-dimethoxy-2-nitrobenzyl bromide (1 eq) was introduced in a solution containing the carbamate (1 eq), and Cs_2CO_3 (1.5 eq) in THF. The mixture was stirred overnight at room temperature, and then neutralized to pH 7 by HCl 1M, washed with water then brine, dried on $MgSO_4$, filtered and evaporated. After purification on silica gel column chromatography, the corresponding ether was obtained.

Aniline deprotection (**9, 10, 11, 12**)

The protected aniline (1 eq) was solubilized in dichloromethane/trifluoroacetic acid (1:1 volume ratio) and stirred at room temperature for 30 min. After evaporation, the residue was taken up in EtOAc and washed with aqueous K_2CO_3 solution, water, and brine, then dried on $MgSO_4$, filtrated and the solvent was removed *in vacuo*. The unprotected secondary anilines were isolated without further purification.

Aniline methylation (**13, 14, 15, 16**)

The secondary anilines (1 eq), K_2CO_3 (1.5 eq), and iodomethane (1 eq) in DMF were stirred at room temperature under argon until reaction showed no more evolution on TLC. The solvent was

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removed and the residue was solubilized in EtOAc. The organic layer was washed with water several times, brine; and finally dried on $MgSO_4$, filtrated and concentrated to dryness. The secondary anilines were obtained after purification by column chromatography on silica gel.

Coupling with DDAO (**2-HPC**)

To a solution of N-methylaniline (1 eq) in anhydrous THF, was carefully injected an excess of phosgene 30% in toluene (500 µL) under argon. The mixture was stirred at room temperature for 20 min. The remaining phosgene was eliminated by an argon flux (warning, highly toxic) then the solution was added to a solution of 1,3-dichloro-7-hydroxy-9,9-dimethylacridin-2(9H)-one (0.055 mmol), 4-dimethylaminopyridine (0.055 mmol) and an excess of triethylamine (1 mL), in THF. The resulting solution was stirred under argon at room temperature overnight. The solvent was evaporated, then the residue was taken up in dichloromethane; the organic phase was washed with a solution of K_2CO_3, water and brine; dried on $MgSO_4$, filtrated and concentrated *in vacuo*. The final carbamates were purified by HPLC using 80 to 90% gradient of CH_3CN/water/0.1% TFA, Waters XBridge® Prep C18 5µm OBD™ 30 x 150mm Column.

***tert*-butyl (2-hydroxy-5-nitrophenyl)carbamate (1)**

The carbamate **1** was obtained without purification as a dark yellow powder (330 mg, 99%); rf 0.57 (40% EtOAc/cyclohexane); mp 111°C; IR (cm^{-1}) 3264, 1765, 1530, 1151; δ_H 1.54 (s, 9H, tBu), 3.81 (bs, 1H, OH), 7.9 (dd, 1H, J= 2.67/8.91 Hz, H_6), 8.06 (d, 1H, J= 2.64, H_3), 8.11 (dd, 1H, 2.70/8.94 Hz, H_5), 8.23 (bs, 1H, NH); δ_C 28.2 (tBu), 85.2 (Cq Boc), 117.2 (C_3), 121.1 (C_6), 126.2 (C_5), 126.8 (C_2), 151.8 (C_4), 153.4 (C=O), 158.6 (C_1); *m/z* 155, 199, 377 $[M+Na]^+$. HRMS, *calculated*: *m/z* 277.0793, *found*: *m/z* 277.0792 ($[M+Na]^+$), 278.0825 ($[MN^{15}+Na]^+$).

***tert*-butyl (2-((4,5-dimethoxy-2-nitrobenzyl)oxy)-5-nitrophenyl)carbamate (5)**

The crude product was purified by column chromatography on silica gel (20% EtOAc/cyclohexane) to give the ether **5** as a dark red solid (548 mg, 94%) rf 0.23 (20% EtOAc/cyclohexane); mp 122°C; IR (cm^{-1}) 2979, 1785, 1519; δ_H 1.36 (9H, s, tBu), 3.97 (3H, s, OCH_3 (1)), 4.03 (3H, s, OCH_3 (2)), 5.65 (1H, s, H_7), 7.15 (1H, d, 9.12 Hz, H_6), 7.36 (1H, s, H_{13}), 7.81 (1H, s, H_{10}), 8.13 (1H, d, 2.55 Hz, H_3), 8.26 (1H, dd, 2.58/9.06 Hz, H_5); δ_C 27.8 (CH_3 Boc), 56.4-57.2 (OCH_3 (1) & (2)), 67.8 (C_7), 83.4 (Cq Boc), 108 (C_3), 108.5 (C_{13}), 111.9 (C_5), 125.4 (C_6), 125.5 (C_2), 127.4 (C_8), 129.2 (C_{10}), 138.3 (C_9), 141.5 (C_{11}), 148.1 (C_4), 150.8 (C=O Boc), 154.7 (C_{12}), 158 (C_1); *m/z* 196, 472 $[M+Na]^+$. HRMS, *calculated*: *m/z* 472.1334, *found*: *m/z* 472.1326 ($[M+Na]^+$).

2-((4,5-dimethoxy-2-nitrobenzyl)oxy)-5-nitroaniline (9)

The aniline **9** was directly obtained pure as a deeply red solid (427 mg, quantitative yield) rf 0.18 (30% EtOAc/cyclohexane); mp 187°C; IR (cm^{-1}) 3368, 2922, 2851, 1783, 1514; δ_H 3.92 (3H, s, OCH_3 (1)), 3.97 (3H, s, OCH_3 (2)), 5.62 (1H, s, H_7), 7.15 (1H, d, 9.12 Hz, H_6), 7.36 (1H, s, H_{13}), 7.81 (1H, s, H_{10}), 8.13 (1H, d, 2.55 Hz, H_3), 8.26 (1H, dd, 2.58/9.06 Hz, H_5); δ_C 56.6 (OCH_3 (1) & (2)), 68.1 (C_7), 108.2 (C_3), 109.5 (C_5 & C_{13}), 111.1 (C_6), 114.8 (C_8), 127.4 (C_{10}), 136.9 (C_2), 139.5 (C_4), 142.5 (C_9), 148.3 (C_{11}), 150.1 (C_1), 154 (C_{12}); *m/z* 196, 350, 545 $[M+H]^+$. HRMS, *calculated*: *m/z* 350.0910, *found*: *m/z* 350.0982 ($[M+H]^+$).

2-((4,5-dimethoxy-2-nitrobenzyl)oxy)-N-methyl-5-nitroaniline (13)

The aniline **13** was obtained after purification by column chromatography on silica gel (15% EtOAc/cyclohexane) as a yellow solid (170 mg, 40%); rf 0.23 (20% EtOAc/cyclohexane); mp 197°C; IR (cm^{-1}) 3424, 2921, 2851, 1271, 1219; δ_H 2.96 (s, 3H, NCH_3), 3.90 (s, 3H, OCH_3 (1)), 3.98 (s, 3H, OCH_3 (2)), 5.62 (s, 2H, H_7), 6.78 (d, 1H, J= 9 Hz, H_6), 7.03 (s, 1H, H_{13}), 7.43 (d, 1H, J= 3 Hz, H_3), 7.58 (dd, 1H, J= 3/ 9 Hz, H_5), 7.77 (s, 1H, H_{10}); δ_C 30.2 (NCH_3), 56.5 (OCH_3 (1) & (2)), 68.2 (C_7), 103.9 (C_3),108.3 (C_5), 109.7 (C_{13}), 109.9 (C_6/C_8), 113 (C_{10}), 127.2 (C_2), 139.7 (C_9), 143.1 (C_{11}), 148.4 (C_{12}), 148.9 (C_1), 153.9 (C_4); *m/z* 196, 364 $[M+H]^+$. HRMS, *calculated*: *m/z* 364.1100, *found*: *m/z* 364.1141 ($[M+H]^+$).

6,8-dichloro-9,9-dimethyl-7-oxo-7,9-dihydroacridin-2-yl(2-((4,5-dimethoxy-2-nitrobenzyl)oxy)-5-nitrophenyl)(methyl)carbamate (2-HPC^{NO2})

The crude product was purified by column chromatography on silica gel (100% dichloromethane) to give the final carbamate **2-HPC^{NO2}** (38.4 mg, quantitative yield) as a yellow solid; rf 0.14 (20% EtOAc/cyclohexane); mp 216°C; IR (cm^{-1}) 2922, 2851, 1787, 1656, 1254, 1220; δ_H 1.67 (s, 6H, CH_3 DDAO), 3.42 (s, 3H, NCH_3), 3.90 (s, 3H, OCH_3 (1)), 4 (s, 3H, OCH_3 (2)), 5.71 (s, 2H, H_7), 6.88 (d, 1H, J= 8.1 Hz, H_6), 6.9 (s, 1H, H_{13}), 7.31 (s, 1H, H_{22}), 7.35 (d, 1H, J= 4.32 Hz, H_5), 7.5 (s, 1H, H_{25}), 7.84 (d, 1H, J= 4.32 Hz, H_3), 8.34 (s, 2H, H_{15}/H_{26}), 8.37 (s, 1H, H_{10}); δ_C 26.5 (CH_3 DDAO), 30.9 (NCH_3), 56.5/56.9 (OCH_3 1 and 2), 68.3 (C_3), 108.2 (C_5), 108.3 (C_3), 108.4 (C_{13}), 112.8 (C_{15}), 119.7 (C_6), 121.2 (C_{25}), 124.8 (C_{26}), 125.7 (C_2), 131.8 (C_8), 133 (C_{10}), 135.6 (C_{19}/C_{21}), 138.4 (C_9), 136.8 (C_4/C_{24}), 138.4 (C_{16}), 139.4 (C_{11}/C_{12}), 148.5 (C_{18}), 149.8 (C_{14}), 153.5 (C_{22}), 153.6 (C_{23}), 158.5 (C_1), 173.1 (C_{20}), 207 (C=O carbamate); *m/z* 196, 289, 371, 447, 697 $[M+H]^+$. HRMS, *calculated*: *m/z* 697.1096, *found*: *m/z* 697.1105 ($[M+H]^+$), 699.1088 ($MCl^{37}+H]^+$).

***tert*-butyl (2-hydroxyphenyl)carbamate (2)**

The carbamate **2** was obtained as a dark yellow powder (919 mg, 96%); rf 0.7 (40% EtOAc/cyclohexane); mp 142°C; IR (cm^{-1}) 3284, 1690, 1147; δ_H 1.53 (s, 9H, tBu), 6.77 (bs, 1H, OH), 6.85 (d, 1H, J= 14.7 Hz, H_6), 6.94 (d, 1H, 14.6 Hz, H_4), 6.98 (d, 1H, 9.2 Hz, H_5), 7.15 (d, 1H, 7.5 Hz, H_3); δ_C 28.3 (CH_3 Boc), 82.1 (Cq Boc), 118.9 (C_6), 120.8

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(C_4), 121.4 (C_2), 125.7 ($C_{3/5}$), 147.5 (C_1), 155.1 (C=O Boc); *m/z* 176, 232 $[M+Na]^+$. HRMS, *calculated*: *m/z* 232.0917, *found*: *m/z* 232.0943 ($[M+Na]^+$).

***tert*-butyl (2-((4,5-dimethoxy-2-nitrobenzyl)oxy)phenyl)carbamate (6)**

The crude product was purified by column chromatography on silica gel (20% EtOAc/cyclohexane) to give the ether **6** as a light yellow solid (376.1 mg, quantitative yield); rf 0.3 (20% EtOAc/cyclohexane); mp 137°C; IR (cm^{-1}) 3452, 2943, 2897, 1730, 1221, 1150; δ_H 1.52 (s, 9H, tBu), 3.92 (s, 3H, OCH_3 (1)), 3.96 (s, 3H, OCH_3 (2)), 5.54 (s, 2H, H_7), 6.84 (dd, 1H, J= 1.26/14.7 Hz, H_6), 6.9 (dd, 1H, J= 1.3/14.1 Hz, H_4), 6.95 (dd, 1H, J= 1.3/14 Hz, H_5), 7.12 (bs, 1H, NH), 7.18 (s, 1H, H_{13}), 7.77 (s, 1H, H_{10}), 8.07 (d, 1H, J= 7 Hz, H_3); δ_C 28.5 (CH_3 Boc), 55.9 (OCH_3 (1) & (2)), 67.7 (C_7), 82.3 (Cq Boc), 109.5 (C_{13}), 118.7 (C_8), 119.1 (C_6), 121 (C_4), 121.6 (C_2), 122.2 (C_{10}), 125.9 ($C_{3/5}$), 136.5 (C_9), 139.1 (C_{11}), 145.7 (C_{12}), 147.7 (C_1), 155.3 (C=O Boc); *m/z* 196, 305, 349, 405 $[M+H]^+$, 427 ($[M+Na]^+$. HRMS, *calculated*: *m/z* 405.1617, *found*: *m/z* 405.1661 ($[M+H]^+$).

2-((4,5-dimethoxy-2-nitrobenzyl)oxy)aniline (10)

The aniline **10** was obtained as a yellow solid (137.7 mg, quantitative yield); rf 0.24 (30% EtOAc/cyclohexane); mp 115°C; IR (cm^{-1}) 3453, 2946, 2907, 1221, 1151; δ_H 3.90 (s, 3H, OCH_3 (1)), 3.93 (s, 3H, OCH_3 (2)), 5.49 (s, 2H, H_7), 6.64 (dd, 1H, J= 1.3/14.7 Hz, H_6), 6.69 (dd, 1H, J= 1.28/13.7 Hz, H_4), 6.76 (dd, 1H, J= 1.3/14 Hz, H_5), 6.8 (dd, 1H, J= 1.7/14.2 Hz, H_3), 7.25 (s, 1H, H_{13}), 7.72 (s, 1H, H_{10}); δ_C 56.1 (OCH_3 (1) and (2)), 68.1 (C_7), 109.7 (C_{13}), 118.9 (C_8), 119.5 (C_6), 121.5 (C_4), 122.1 (C_2), 122.3 (C_{10}), 126.4 ($C_{3/5}$), 136.9 (C_9), 139.3 (C_{11}), 145.9 (C_{12}), 148.1 (C_1); *m/z* 196, 305 $[M+H]^+$. HRMS, *calculated*: *m/z* 305.1093, *found*: *m/z* 305.1131 ($[M+H]^+$).

2-((4,5-dimethoxy-2-nitrobenzyl)oxy)-N-methylaniline (14)

The aniline **14** was obtained after purification by column chromatography on silica gel (15% EtOAc/cyclohexane) as an orange solid (60 mg, 48%); rf 0.36 (20% EtOAc/cyclohexane); mp 130°C; IR (cm^{-1}) 2976, 2999, 1215; δ_H 2.9 (s, 3H, NCH_3), 3.90 (s, 3H, OCH_3 (1)), 3.95 (s, 3H, OCH_3 (2)), 4.32 (bs, 1H, NH), 5.51 (s, 2H, H_7), 6.58 (d, 1H, J= 1.32 Hz, H_5), 6.61 (d, 1H, J= 1.32 Hz, H_4), 6.65 (d, 1H, J= 7.62 Hz, H_6), 6.93 (d, 1H, J= 7.89 Hz, H_3), 7.21 (s, 1H, H_{13}), 7.44 (s, 1H, H_{10}); δ_C 30.5 (NCH_3), 56.4 (OCH_3 (1) & (2)), 68.1 (C_7), 109.7 (C_{13}), 118.9 (C_8), 119.5 (C_6), 121.5 (C_4), 122.1 (C_2), 122.3 (C_{11}), 126.4 ($C_{3/5}$), 136.9 (C_9), 139.3 (C_{11}), 145.9 (C_{12}), 148.1 (C_1); *m/z* 196, 319 $[M+H]^+$. HRMS, *calculated*: *m/z* 319.1249, *found*: *m/z* 319.1288 ($[M+H]^+$).

6,8-dichloro-9,9-dimethyl-7-oxo-7,9-dihydroacridin-2-yl(2-((4,5-dimethoxy-2-nitrobenzyl) oxy)phenyl)(methyl)carbamate (2-HPC^H)

The crude product was purified by column chromatography on silica gel (100% dichloromethane) to give the carbamate **2-HPC^H** (110.4 mg, quantitative yield) as a yellow solid; rf 0.27 (20% EtOAc/cyclohexane); mp 160°C; IR (cm^{-1}) 3382, 2915, 2850, 2813, 1710, 1502; δ_H 1.58 (s, 6H, CH_3 DDAO), 3.32 (s, 3H, NCH_3), 3.82 (s, 3H, OCH_3 (1)), 3.95 (s, 3H, OCH_3 (2)), 5.52 (s, 2H, H_7), 6.58 (d, 1H, J= 1.32 Hz, H_5), 6.61 (d, 1H, J= 1.32 Hz, H_4), 6.75 (d, 1H, J= 7.62 Hz, H_6), 6.87 (s, 1H, H_{22}), 6.93 (d, 1H, J= 7.89 Hz, H_3), 7.11 (s, 1H, H_{26}), 7.21 (s, 1H, H_{13}), 7.22 (s, 1H, H_{25}), 7.38 (s, 1H, H_{15}), 7.44 (s, 1H, H_{10}); δ_C 27.8 (CH_3 DDAO), 30.5 (NCH_3), 32.8 (C_{17}), 56.4 (OCH_3 (1) & (2)), 68.1 (C_7), 109.7 (C_{13}), 118.8 (C_{15}), 118.9 (C_8), 119.5 (C_6), 120.1 (C_{26}), 120.4 (C_{25}), 121.5 (C_4), 122.1 (C_2), 122.3 (C_{10}), 126.4 ($C_{3/5}$), 129.4 (C_{21}), 129.9 (C_{19}), 136.9 (C_9), 139.3 (C_{11}), 140.8 (C_{24}), 144.6 (C_{16}), 145.9 (C_{12}), 147.2 (C_{18}), 148.1 (C_1), 151.2 (C_{22}), 158.8 (C_{14}), 164.6 (C_{23}), 170.2 (C_{20}); *m/z* 196, 345, 652 $[M+H]^+$. HRMS, *calculated*: *m/z* 652.1146, *found*: *m/z* 652.1252 ($[M+H]^+$), 654.1235 ($MCl^{37}+H]^+$).

tert-butyl (2-hydroxy-5-methoxyphenyl)carbamate (3)

The carbamate **3** was obtained as a dark red oil (555.1 mg, quantitative); rf 0.77 (40% EtOAc/cyclohexane); IR (cm^{-1}) 3225, 2857, 1726, 1150; δ_H 3.66 (s, 3H, methoxy), 6.14 (dd, 1H, J= 2.94/8.94 Hz, H_5), 6.36 (d, 1H, J= 2.91 Hz, H_3), 6.58 (d, 1H, J= 8.58 Hz, H_6); δ_C 55.6 (OCH_3 phenol), 106.3 (C_3), 110 (C_6), 118.2 (C_5), 126.3 (C_2), 140.3 (C_1), 153.5 (C_4); *m/z* 140 $[M+H]^+$, 206, 262 $[M+Na]^+$. HRMS, *calculated*: *m/z* 262.1091, *found*: *m/z* 262.1050 ($[M+Na]^+$).

tert-butyl (2-((4,5-dimethoxy-2-nitrobenzyl)oxy)-5-methoxyphenyl)carbamate (7)

The crude product was purified by column chromatography on silica gel (50% dichloromethane/cyclohexane) to give the ether **7** as a red solid (485.5 mg, 71%); rf 0.33 (20% EtOAc/cyclohexane); mp 143°C; IR (cm^{-1}) 3451, 2975, 2904, 2863, 1712, 1271; δ_H 1.49 (s, 9H, tBu), 3.68 (s, 3H, methoxy), 6.47 (dd, 1H, J= 2.88/8.70 Hz, H_5), 6.78 (d, 1H, J= 8,5 Hz, H_6), 7.12 (d, 1H, J= 2.6 Hz, H_3); δ_C 28.3 (CH_3 Boc), 55.6 (methoxy), 81.8 (Cq Boc), 106.5 (C_3), 110.2 (C_6), 118.4 (C_5), 126.5 (C_2), 140.5 (C_1), 152.5 (C=O Boc), 153.7 (C_4); *m/z* 140, 196, 335, 379 $[M+H]^+$, 457 $[M+Na]^+$. HRMS, *calculated*: *m/z* 435.1789, *found*: *m/z* 435.1763 ($[M+H]^+$).

2-((4,5-dimethoxy-2-nitrobenzyl)oxy)-5-methoxyaniline (11)

The aniline **11** was obtained as a red solid (m= 639 mg, quantitative yield); rf 0.35 (30% EtOAc/cyclohexane); mp 117°C; IR (cm^{-1}) 3193, 2918, 2863, 1208; δ_H 3.67 (s, 1H, methoxy), 3.82 (s, 1H, OCH_3 (1)), 3.91 (s, 1H, OCH_3 (2)), 5.4 (s, 2H, H_7), 6.78 (dd, 1H, J= 2.3/8.9 Hz, H_5), 6.85 (d, 1H, 8.9 Hz, H_6), 6.99 (d, 1H, 2.3 Hz, H_3), 7.12 (s, 1H, H_{13}), 7.65 (s, 1H, H_{10}), 11.08 (bs, 2H, NH_2); δ_C 55.5 (methoxy), 56.5 (OCH_3 (1) and (2)), 68.7 (C_7), 102.3 (C_3), 102.5 (C_5), 108 (C_6), 109.5 (C_8), 114.3 (C_{10}/C_{13}), 129.9 (C_2), 139.2 (C_4), 140.2 (C_1), 148.8 (C_9), 153.9 (C_{12}), 155.2 (C_{11}); *m/z* 154, 182, 196, 335, 357 $[M+H]^+$. HRMS, *calculated*: *m/z* 335.1165, *found*: *m/z* 335.1239 ($[M+H]^+$).

2-((4,5-dimethoxy-2-nitrobenzyl)oxy)-5-methoxy-N-methylaniline (15)

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The aniline **15** was obtained after purification by column chromatography on silica gel (15% EtOAc/cyclohexane) as a yellow solid (148.9 mg, 45%); rf 0.51 (20% EtOAc/cyclohexane); mp 153°C; IR (cm^{-1}) 3406, 2929, 1516; δ_H 3.87 (s, 3H, NCH_3), 3.76 (s, 3H, methoxy), 3.92 (s, 3H, OCH_3 (1)), 3.96 (s, 3H, OCH_3 (2)), 5.46 (s, 2H, H_7), 6.1 (dd, 1H, J= 3.00/9.00 Hz, H_5), 6.24 (d, 1H, J= 2.8 Hz, H_3), 6.67 (d, 1H, J= 8.9 Hz, H_6), 7.24 (s, 1H, H_{13}), 7.75 (s, 1H, H_{10}); δ_C 30.3 (NCH_3), 56.1 (methoxy), 56.4 (OCH_3 (1) & (2)), 70.1 (C_7), 107 (C_3), 110.7 (C_6), 113.4 (C_{13}), 118.9 (C_5), 127 (C_2), 127.8 (C_8) 129.9 (C_{10}), 140.4 (C_9), 140.9 (C_1), 142.4 (C_{11}), 154.2 (C_4), 156.6 (C_{12}); *m/z* 152, 349 $[M+H]^+$. HRMS, *calculated*: *m/z* 349.1355, *found*: *m/z* 349.1393 ($[M+H]^+$).

6,8-dichloro-9,9-dimethyl-7-oxo-7,9-dihydroacridin-2-yl(2-((4,5-dimethoxy-2-nitrobenzyl) oxy)-5-methoxyphenyl)(methyl)carbamate (2-HPC^{OCH3})

The crude product was purified by column chromatography on silica gel (100% dichloromethane) to give the final carbamate **2-HPC^{OCH3}** (44.4 mg, quantitative yield) as a brown solid; rf 0.27 (20% EtOAc/cyclohexane); mp 103°C; IR (cm^{-1}) 2924, 2801, 1726, 1657, 1235; δ_H 1.58 (s, 6H, CH_3 DDAO), 3.31 (s, 3H, NCH_3), 3.77/3.81 (s, 6H, OCH_3 (1) & (2)), 3.91 (s, 3H, methoxy), 5.46 (s, 2H, H_7), 6.87 (m, 3H, H_3/H_5/H_6), 6.97 (d, 1H, J= 3Hz, H_{15}), 7.03 (s, 1H, H_{25}), 7.38 (s, 1H, H_{13}), 7.44 (d, 1H, J= 9Hz, H_{26}), 7.51 (s, 1H, H_{22}), 7.74 (s, 1H, H_{10}); δ_C 29.7 (CH_3 DDAO), 39.1 (NCH_3), 56.9/56.5/55.8 (OCH_3 (1)&(2), methoxy), 67.7 (C_7), 106.6 (C_3/C_5), 108 (C_{13}), 108.6 (C_6), 113.4 (C_{15}), 113.9 (C_{25}), 114.9 (C_{26}), 120 (C_2), 121.5 (C_8), 121.5 (C_9), 129 (C_{21}), 132.1 (C_{19}), 132.9 (C_{10}), 135.4 (C_{16}), 137.2 (C_9), 138.2 (C_{16}), 138.6 (C_{11}), 139.2 (C_{12}), 139.4 (C_1), 140.3 (C_{18}), 147.4 (C_{14}), 148 (C_{22}), 149.6 (C_{23}), 154.1 (C_4), 154.4 (C=O carbamate), 173.1 (C=O DDAO); *m/z* 149, 448, 682 $[M+H]^+$. HRMS, *calculated*: *m/z* 682.1251, *found*: *m/z* 682.1356 ($[M+H]^+$), 684.1341 ($MCl^{37}+H]^+$).

tert-butyl (5-bromo-2-hydroxyphenyl)carbamate (4)

The carbamate **4** was obtained as a red solid (750 mg, quantitative yield); rf 0.74 (40% EtOAc/cyclohexane); mp 135°C; IR (cm^{-1}) 3262, 2978, 1691, 1150; δ_H 1.52 (s, 9H, tBu), 6.65 (s, 1H, H_3), 6.82 (d, 1H, J= 8.58 Hz, H_6), 7.11 (dd, 1H, J= 3.87/8.49 Hz, H_5), 7.33 (s, 1H, OH), 7.87 (bs, 1H, NH); δ_C 28.2 (CH_3 Boc), 82.6 (Cq Boc), 112.4 (C_4), 120 (C_6), 123.8 (C_3), 127.1 (C_2), 128.1 (C_5), 146.4 (C_1), 154.6 (C=O Boc); *m/z* 187, 231 $[M+H]^+$, 310 $[M+Na]^+$. HRMS, *calculated*: *m/z* 310.0037, *found*: *m/z* 310.0048 ($[M+Na]^+$), 312.0029 ($MBr^{81}+Na]^+$).

tert-butyl (5-bromo-2-((4,5-dimethoxy-2-nitrobenzyl)oxy)phenyl)carbamate (8)

The crude product was purified by column chromatography on silica gel (20% EtOAc/cyclohexane) to give the ether as a brown solid (1.23 g, 94%); rf 0.34 (20% EtOAc/cyclohexane); mp 148°C; IR (cm^{-1}) 3452, 2976, 2927, 1726; δ_H 1.52 (s, 9H, tBu), 3.92 (s, 3H, OCH_3 (1)), 3.97 (s, 3H, OCH_3 (2)), 5.53 (s, 2H, H_7), 6.69 (d, 1H, J= 8.70 Hz, H_6), 7.03 (dd, 1H, J= 2.31/8.61 Hz, H_5), 7.11 (s, 2H, H_3/H_{13}), 7.77 (s, 1H, H_{10}), 8.3 (bs, 1H, NH); δ_C 28.3 (tBu), 56.4 (OCH_3 (1) & (2)), 68.3 (C_7), 81.1 (Cq Boc), 108.2 (C_{13}), 109 (C_4), 113.3 (C_6), 114.7 (C_3), 121.4 (C_2), 125.1 (C_8), 129.6 (C_{10}), 139.1 (C_9), 145.1 (C_{11}), 148.2 (C_{11}), 152.3 (C=O Boc), 154 (C_{12}); *m/z* 196, 385, 427, 483 $[M+H]^+$. HRMS, *calculated*: *m/z* 483.0668, *found*: *m/z* 483.0766 ($[M+H]^+$), 485.0738 ($MBr^{81}+H]^+$).

5-bromo-2-((4,5-dimethoxy-2-nitrobenzyl)oxy)aniline (12)

The aniline **18** was obtained as a dark red solid (955.1 mg, quantitative yield); rf 0.23 (30% EtOAc/cyclohexane); mp 142°C; IR (cm^{-1}) 3366, 2917, 2884, 1271, 1192; δ_H 3.64 (bs, 2H, NH_2) ; 3.92 (s, 3H, OCH_3 (1)) ; 3.95 (s, 3H, OCH_3 (2)) ; 5,49 (s, 2H, H_7) ; 6,62 (d, 1H, J= 8.58 Hz, H_6) ; 6.75 (1H, dd, 2.19/8.52 Hz, H_5), 6.86 (1H, d, 2.22 Hz, H_3), 7.17 (1H, s, H_{13}), 7.74 (1H, s, H_{10}); δ_C 56.4 (OCH_3 (1) & (2)), 68.0 (C_7), 108.1 (C_{13}), 109.4 (C_4), 114.1 (C_3), 114.4 (C_6), 118.0 (C_5), 121.0 (C_8), 128.9 (C_{10}), 138.0 (C_2), 139.3 (C_9), 144.7 (C_{11}), 148.0 (C_1), 153.9 (C_{12}); *m/z* 196, 383 $[M+H]^+$. HRMS, *calculated*: *m/z* 383.0144, *found*: *m/z* 383.0234 ($[M+H]^+$), 385.0217 ($MBr^{81}+H]^+$).

5-bromo-2-((4,5-dimethoxy-2-nitrobenzyl)oxy)-N-methylaniline (16)

The aniline **16** was obtained after purification by column chromatography on silica gel (15% EtOAc/cyclohexane) as a yellow solid (496 mg, 50%); rf 0.26 (20% EtOAc/cyclohexane); mp 170°C; IR (cm^{-1}) 2922, 28.47, 1209, 1155; δ_H:2.88 (3H, s, NCH_3), 3.91 (3H, s, OCH_3 (1)), 3.96 (3H, s, OCH_3 (2)), 5.50 (2H, s, H_7), 6.60 (1H, d, J= 8.46 Hz, H_6), 6.76 (1H, d, J= 8.31 Hz, H_5), 6.82 (1H, s, H_3), 7.15 (1H, s, H_{13}), 7.75 (1H, s, H_{10}).; δ_C 30.4 (NCH_3), 56.5 (OCH_3 (1) & (2)), 68.3 (C_7), 108.0 (C_{13}), 109.5 (C_3), 113.0 (C_4), 115.4 (C_6), 119.1 (C_5), 129.0 (C_8), 139.4 (C_{10}), 140.6 (C_9), 144.5 (C_2), 148.3 (C_{11}/C_1), 154.1 (C_{12}); *m/z* 196, 397 $[M+H]^+$. HRMS, *calculated*: *m/z* 397.0300, *found*: *m/z* 397.0392 ($[M+H]^+$), 399.0373 ($MBr^{81}+H]^+$).

6,8-dichloro-9,9-dimethyl-7-oxo-7,9-dihydroacridin-2-yl(5-bromo-2-((4,5-dimethoxy-2-nitrobenzyl)oxy) phenyl)(methyl)carbamate (2-HPC^{Br})

The crude product was purified by column chromatography on silica gel (100% dichloromethane) to give the carbamate **2-HPC^{Br}** (24.1 mg, quantitative yield) as a dark green solid; rf 0.31 (20% EtOAc/cyclohexane); mp 146°C; IR (cm^{-1}) 2923, 1728, 1658, 1222; δ_H 1.67 (s, 6H, CH_3 DDAO), 3.38 (s, 3H, NCH_3), 3.88 (s, 3H, OCH_3 (1)), 3.99 (s, 3H, OCH_3 (2)), 5.57 (s, 2H, H_7), 6.93 (d, 1H, J = 9Hz, H_5), 7.03 (s, 1H, H_{13}), 7.09 (d, 1H, J= 6 Hz, H_3), 7.39 (s, 1H, H_6), 7.52 (m, 3H, H_{25}/H_{26}/H_{15}), 7.60 (s, 1H, H_{10}), 7.82 (s, 1H, H_{22}); δ_C 25.6/26.4 (CH_3 DDAO), 29.7 (NCH_3), 56.5 (OCH_3 (1)), 56.9 (OCH_3 (2)), 67.6 (C_{17}), 68 (C_7), 108.2 (C_{13}), 108.4 (C_4), 113.5 (C_6), 114.7 (C_{15}), 119.8 (C_{26}), 121.4 (C_{25}), 128 (C_3), 131.6 (C_8), 132.3 (C_5), 132.7 (C_2), 135.6 (C_{21}), 137.3 (C_{19}), 138.2 (C_{24}), 138.7 (C_{16}), 139.3 (C_{11}), 140.2 (C_{12}/C_{23}), 148.2 (C_{18}), 149.6 (C_{14}/C_{22}), 152.6 (C_1), 153.9 (C_9), 154.4 (C=O carbamate), 173.1

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(C_{20}); *m/z* 397, 730 $[M+H]^+$. HRMS, *calculated*: *m/z* 730.0260, *found*: *m/z* 730.0358 ($[M+H]^+$), 732.0333 ($MBr^{81}+H]^+$), 734.0319 ($MCl^{37}Br^{81}+H]^+$).

4,4,5,7-Tetramethyl-2-chromanone (17)

3,5-dimethylphenol (500 mg, 4.09 mmol) and 3,3-dimethylacrylic acid (409,5 mg, 4.09 mmol) were dissolved in toluene (15 mL). Then methyl sulfonic acid was added (2.92 mL, 20.45 mmol). The mixture was stirred for 4 h under argon at 85°C. After solvent evaporation, the reaction mixture was diluted with EtOAc and washed with K_2CO_3 (1M) solution, water and brine; and finally dried over anhydrous magnesium sulphate. The solvent was removed under vacuum affording a yellow oil (827 mg, 99%); rf 0.72 (30% EtOAc/cyclohexane); IR (cm^{-1}) 2966, 1766; δ_H 1.42 (6H, s, H_{10}/H_{11}), 2.25 (3H, s, H_7), 2.45 (3H, s, H_8), 2.56 (2H, s, H_{12}), 6.72 (2H, d, J= 4.86 Hz, H_4/H_6); δ_C 20.9 (C_7), 23.9 (C_8), 28.2 (C_{10}/C_{11}), 35.5 (C_9), 46.0 (C_{12}), 117.0 (C_6), 126.9 (C_4), 130.1 (C_3), 136.6 (C_2), 138.2 (C_5), 152.0 (C_1), 169.3 (C_{13}); *m/z* 175, 205 $[M+H]^+$. HRMS, *calculated*: *m/z* 205.1184, *found*: *m/z* 205.1222 ($[M+H]^+$).

2-(4-hydroxy-2-methylbutan-2-yl)-3,5-dimethylphenol (18)

To a solution of 4,4,5,7-Tetramethyl-2-chromanone (500 mg, 2.45 mmol) in anhydrous THF (10 mL) in an ice bath was added lithium aluminium hydride (766.6 mg, 12.12 mmol) portion wise. The heterogeneous mixture was allowed to warm at room temperature and stirred 1h30 under argon. The excess of $LiAlH_4$ was neutralized with NH_4Cl at 0°C, then the suspension was filtrated on Celite 535; the resulting filtrate was diluted by EtOAc and treated by HCl 1M, water and brine; and finally dried on $MgSO_4$, filtrated and concentrated *in vacuo*. The crude product was purified on silica gel column chromatography, giving the alcohol **18** as an off-white powder (479 mg, 94%); rf 0.35 (30% EtOAc/cyclohexane); mp 78°C; IR (cm^{-1}) 3508; δ_H (CD_3OD) 1.55 (6H, s, H_{10}/H_{11}), 2.17 (3H, s, H_7), 2.27 (2H, t, J= 6.24 Hz, H_{12}), 2.48 (3H, s, H_8), 3.62 (2H, dd, J= 7.24/14.61 Hz, H_{13}), 6.34 (1H, s, H_4), 6.45 (1H, bs, OH), 6.49 (1H, s, H_6); δ_C (CD_3OD) 20.6 (C_7), 26.0 (C_8), 32.1 (C_{10}/C_{11}), 45.2 (C_{12}), 61.9 (C_{13}), 113.6 (C_6), 127.2 (C_4), 136.7 (C_2), 138.4 (C_3), 139.9 (C_5), 156.1 (C_1); *m/z* 123, 189, 209 $[M+H]^+$, 231 $[M+Na]^+$. HRMS, *calculated*: *m/z* 209.1497, *found*: *m/z* 209.1531 ($[M+H]^+$).

3-(2-((4,5-dimethoxy-2-nitrobenzyl)oxy)-4,6-dimethylphenyl)-3-methylbutan-1-ol (19)

K_2CO_3 (559.8 mg, 4.05 mmol, 1.5 eq) was added to a solution of 2-(4-hydroxy-2-methylbutan-2-yl)-3,5-dimethylphenol (563 mg, 2.7mmol, 1 eq) in DMF (12 mL). After 2 min, 4,5-dimethoxy-2-nitrobenzyl bromide (740mg, 2.7mmol, 1 eq) was added. The mixture was stirred under argon overnight, then treated by an aqueous solution of K_2CO_3, water and brine; dried on $MgSO_4$, filtrated and evaporated. The crude product was purified on silica gel column chromatography (40% EtOAc/cyclohexane) to give the ether as an orange solid (879 mg, 81%); rf 0.17 (30% EtOAc/cyclohexane); mp 86°C; IR (cm^{-1}) 3326, 2969, 1274; δ_H 1.58 (6H, s, H_{10}/H_{11}), 2.21 (3H, s, H_7), 2.24 (2H, t, J= 7.24 Hz, H_{12}), 2.51 (3H, s, H_8, 3.57 (2H, t, J= 7.23 Hz, H_{13}), 3.94 (3H, s, OCH_3 (1)), 3.97 (3H, s, OCH_3 (2)), 5.50 (2H, s, H_{14}), 6.58 (2H, s, H_4/H_6), 7.37 (1H, s, H_{20}), 7.78 (1H, s, H_{17}); δ_C 21.0 (C_7), 26.2 (C_8), 32.7 (C_{10}/C_{11}), 40.5 (C_9), 46.0 (C_{12}), 56.5 (OCH_3 (1) & (2)), 61.5 (C_{13}), 69.4 (C_{14}), 108.5 (C_{20}), 110.6 (C_6), 114.0 (C_4), 129.0 (C_{15}), 130.6 (C_2), 131.4 (C_{17}), 137.1 (C_3), 138.3 (C_5), 139.3 (C_{16}), 148.5 (C_{18}), 154.3 (C_1), 158.8 (C_{19}); *m/z* 196, 318, 404, 421 $[M+H]^+$. HRMS, *calculated*: *m/z* 404.2028, *found*: *m/z* 404.2069 ($[M+H]^+$).

3-(2-((4,5-dimethoxy-2-nitrobenzyl)oxy)-4,6-dimethylphenyl)-3-methylbutanal (20)

To a solution of 3-(2-((4,5-dimethoxy-2-nitrobenzyl)oxy)-4,6-dimethylphenyl)-3-methylbutan-1-ol (714.9 mg, 1.77 mmol, 1 eq) in 10 mL of dichloromethane was added pyridinium dichromate (2.66 g, 7.08 mmol, 4 eq). The mixture was stirred under argon for 24h, then treated by NH_4Cl, water and brine; dried over $MgSO_4$, filtrated and concentrated *in vacuo*. The crude product was purified by column chromatography (20% EtOAc/cyclohexane) to give the aldehyde as an orange solid (675 mg, 95%); rf 0.51 (30% EtOAc/cyclohexane); mp 106 °C; IR (cm^{-1}) 2919, 1721, 1275; δ_H 1.62 (6H, s, H_{10}/H_{11}), 2.16 (3H, s, H_7), 2.51 (3H, s, H_8), 2.97 (2H, s, H_{12}), 3.90 (3H, s, OCH_3 (1)), 3.94 (3H, s, OCH_3 (2)), 5.47 (2H, s, H_{14}), 6.52 (1H, s, H_4), 6.58 (1H, s, H_6), 7.20 (1H, s, H_{20}), 7.74 (1H, s, H_{17}), 9.54 (1H, s, H_{13}); δ_C 21.0 (C_7), 26.2 (C_8), 32.3 (C_{10}/C_{11}), 39.2 (C_9), 57.1 (OCH_3 (1) & (2)), 69.2 (C_{14}), 108.5 (C_{20}), 110.2 (C_6), 113.9 (C_4), 128.9 (C_{15}), 129.9 (C_2), 130.2 (C_{17}), 137.4 (C_3), 138.0 (C_5), 139.3 (C_{12}), 148.4 (C_{18}), 154.4 (C_1), 157.8 (C_{19}), 204.1 (C_{13}); *m/z* 196, 338, 384, 402, 419 $[M+H]^+$. HRMS, *calculated*: *m/z* 402.1872, *found*: *m/z* 402.1911 ($[M+H]^+$).

3-(2-((4,5-dimethoxy-2-nitrobenzyl)oxy)-4,6-dimethylphenyl)-3-methylbutanoic acid (21)

3-(2-((4,5-dimethoxy-2-nitrobenzyl)oxy)-4,6-dimethylphenyl)-3-methylbutanal (116 mg, 0.29 mmol) was dissolved in 10 mL of acetone/*tert*-butanol/water (17:12:3) mixture, then 2-methyl-2-butene (208 µL, 1.96 mmol, 6.75 eq), sodium chlorite (137.7 mg, 1.52 mmol, 5.25 eq), and sodium dihydrogenophosphate (53 mg, 0.44 mmol, 1.5 eq) were added. The mixture was stirred overnight, and then neutralized by NH_4Cl. The organic phase was diluted by EtOAc, treated by water and dried on $MgSO_4$. After filtration, evaporation and purification on silica gel (40% EtOAc/cyclohexane), the acid was obtained as a light yellow powder (107.6 mg, 89%); rf 0.40 (40%EtOAc/cyclohexane); mp 120 °C; IR (cm^{-1}) 2927, 1711, 1275; δ_H 1.79 (6H, s, $H_{10}H/_{11}$), 2.26 (3H, s, H_8), 2.37 (3H, s, H_7), 3.64 (2H, s, H_{12}), 3.76 (3H, s, OCH_3 (1)), 3.90 (3H, s, OCH_3 (2)), 5.61 (2H, s, H_{14}), 6.65 (1H, s, H_4), 6.68 (1H, s, H_6), 7.40 (1H, s, H_{20}), 7.68 (1H, s, H_{17}); δ_C 19.2 (C_7), 21.4 (C_8), 26.0 (C_9), 32.3 C_{10}/C_{11}), 40.1 (C_{12}), 57.1 (OCH_3 (1) & (2)), 69.3 (C_{14}), 108.4 (C_{20}), 110.0 (C_6), 114.0 (C_4), 128.8 (C_{15}), 130.4 (C_2), 130.7 (C_{17}), 136.6 (C_3), 138.2 (C_5), 139.2 (C_{16}), 148.3 (C_{18}), 154.3 (C_1), 158.1 (C_{19}), 177.7 (C_{13}); *m/z* 196, 318, 418 $[M+H]^+$, 435 $[M+NH_4]^+$. HRMS, *calculated*: *m/z* 418.1821, *found*: *m/z* 418.1860 ($[M+H]^+$).

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6,8-dichloro-9,9-dimethyl-7-oxo-7,9-dihydroacridin-2-yl-3-(2-((4,5-dimethoxy-2-nitrobenzyl)oxy)-4,6-dimethylphenyl)-3-methylbutanoate (TML)

To a solution of 3-(2-((4,5-dimethoxy-2-nitrobenzyl)oxy)-4,6-dimethylphenyl)-3-methylbutanoic acid (15 mg, 0.036 mmol, 1 eq) in anhydrous THF (5 mL), was injected an excess of phosgene 30% in toluene (300 µL) under inert atmosphere. The mixture was stirred at room temperature for 45 min. The remaining phosgene was eliminated by an argon flux; this solution was then added to a solution of 1,3-dichloro-7-hydroxy-9,9-dimethylacridin-2(9H)-one (11.1 mg, 0.036 mmol), 4-dimethylaminopyridine (4.4 mg, 0,036 mmol) and an excess of triethylamine (1 mL) in THF (5mL). The resulting solution was stirred under argon at room temperature overnight. The solvent was evaporated, then the residue was taken up in dichloromethane; the organic phase was washed water and brine; dried on $MgSO_4$, filtrated and concentrated *in vacuo*. The ester was purified by HPLC using 80 to 90% gradient of CH_3CN/water/0.1% TFA, Waters XBridge® Prep C18 5µm OBD™ 30 x 150mm Column, to give the pure product as a deep dark green solid (25 mg, quantitative yield); rf 0.40 (40%EtOAc/cyclohexane); mp 220°C; IR (cm^{-1}) 2918, 1754, 1621; δ_H 1.71 (s, 6H, CH_3 DDAO), 2.21 (s, 2H, H_7), 2.57 (s, 2H, H_8), 3.09 (s, 2H, H_{19}), 4.01 (s, 6H, OCH_3 (1) & (2)), 5.56 (s, 2H, H_9), 6.55-7.82 (m, 7H, ArH); δ_C 11 (C_7), 14.1 (C_8), 20.7 (C_{16}), 23 (C_{17}), 23.7/25.8 (CH_3 DDAO), 38.9 (C_{18}), 56.4 (OCH_3 1 and 2), 68.2 (C_9), 70.1 (C_{19}), 108.1 (C_2), 109.7 (C_3/C_5), 128.5 (C_4), 128.8 (C_{15}/C_{23}), 129.8 (C_{10}), 130.9 (C_{14}/C_{13}), 132.4 (C_6), 147.9 (C_1/C_{11}), 153.7 (C_{25}/C_{27}), 167.8 (C_{26}), 187.8(C=O ester); *m/z* 350, 447, 647, 707 $[M+H]^+$. HRMS, *calculated*: *m/z* 707.1819, *found*: *m/z* 707.1922 ($[M+H]^+$), 709.1911 ($MCl^{37}+H]^+$).

Analytical Solutions

All kinetic experiments have been performed in CH_3CN/0.1 M Britton-Robinson buffer[23] 1:1 (v:v). All solutions were prepared using water purified through a Direct-Q 5 (Millipore, Billerica, MA).
UV-Visible Absorption: UV/Vis absorption spectra were recorded in 1 cm × 1 cm quartz cuvettes (Hellma) on a diode array UV/Vis spectrophotometer (Cary 300, Agilent, Thermo Scientific) at 298 K.

Steady-state fluorescence emission

Corrected fluorescence spectra upon one-photon excitation were recorded with a Photon Technology International QuantaMaster QM-1 spectrofluorimeter (PTI, Monmouth Junction, NJ) equipped with a Peltier cell holder (TLC50, Quantum Northwest, Shoreline, WA). Solutions for fluorescence measurements were adjusted to 10 µM.

Irradiation experiments

One-photon irradiation experiments were carried out on the spectrofluorimeter. Irradiations were performed using a filtered 75 W xenon lamp at several slit widths on 400 µL samples in 0.2 × 1 cm^2 quartz fluorescence cuvettes (Hellma) under constant stirring.

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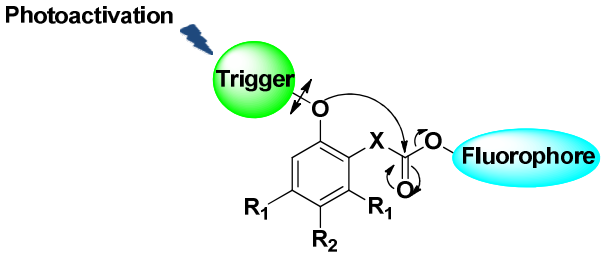

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