Synthesis of novel chemiluminescent compounds.

Mu, Xiaojing

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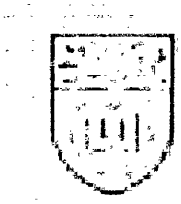

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Submitted for the Degree of Doctor of Philosophy

2002-2005

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Acknowledgements

•Special thanks should be given to my supervisor professor Keith Smith, for his encouragement, patience and excellent supervision. I would like to express thanks to all the staff in the chemistry department for their help and kindness. I am very grateful to my colleagues in G3 group for their kindly reading through an early draft of my thesis, and giving helpful proposals, warm caring and encouragement.

•Appreciation should be given to Molecular Light Technology Research Limited for its kind funding of this project and for supervision. Thanks are extended particularly to Drs. Zhaoqiang Li, Ian Weeks and Andy Rutter at MLT.

•Thanks are given to the Chinese Government for the financial support.

•Thanks are also given to the British Government for providing an O.R.S. studentship.

•I wish to thank Chongqing University (in China) for authorizing my leave to finish my Ph.D.

•Finally, many thanks with lots of love to my parents.

Summary

The thesis reports the successful synthesis of many new acridinium esters and the investigation of their chemiluminescent and stability properties. Such compounds were designed to have potential utility as biological probes.

In Chapter 1, a review on chemiluminescence, especially about acridinium derivatives, is given; and the background of the project is discussed.

In Chapter 2, synthesis of 6 acridine derivatives is reported. Each of them bears a 2,6-dibromophenoxy ring as a leaving group. They differ only in the groups bonded to the nitrogen atom of the acridine rings. **Compounds 1** and **2** have a dodecyl group and methyl group, respectively, *i.e.* the compounds are 9-(2,6-dibromophenoxycarbonyl)-10-dodecylacridinium triflate (**1**) and 9-(2,6-dibromophenoxycarbonyl)-10- methylacridinium triflate (**2**); both **compounds 3** and **4** have a 3-succinimidyloxycarbonylpropyl group at 10 position, but **3** has an iodide counter anion and **4** has a triflate counter anion. **Compounds 5** and **6** have a 5-succinimidyloxycarbonylpentyl group and a 10-succinimidyloxycarbonyldecyl group, respectively, at position 10.

In Chapter 3, synthesis of 13 acridinium derivatives is reported. They are classified into 4 groups. **Compounds 7-11** have an unsubstituted phenoxy group as leaving group and at position 10 have a methyl, dodecyl, 3-succinimidyloxycarbonylpropyl, 5-succinimidyloxycarbonylpentyl or 10-succinimidyloxycarbonyldecyl group, respectively. **Compounds 12-15** have a 2,5-dimethylphenoxy group as leaving group, and at position 10 have a methyl, 3-succinimidyloxycarbonylpropyl, 5-succinimidyloxycarbonylpentyl or 10-succinimidyloxycarbonyldecyl group, respectively. **Compounds 16-17** have a 2,6-bis(trifluoromethyl)phenoxy group as leaving group, and at position 10 have a methyl or 10-succinimidyloxycarbonyldecyl group. Finally, **compounds 18-19** have a 2,6-dinitrophenoxy group as leaving group, and at position 10 have a methyl or 10-succinimidyloxycarbonyldecyl group.

In Chapter 4, compounds with substituents on the acridinium ring, which are expected to emit chemiluminescence at different wavelengths, were synthesized. They are 2,6-dibromophenyl 1,3-dimethylacridine-9-carboxylate (**20**); 9-(2,6-dibromophenoxycarbonyl)-2-(succinimidyloxycarboxyethyl)-10-methylacridinium triflate (**21**); and 9-(4-(2-succinimidylyoxycarbonylethyl)phenoxy)-2,7-dimethoxy-10-methylacridinium trifluoromethansulfonate (**22**).

In Chapter 5, the chemiluminescence of **compounds 1-2**, **4-19**, and **21-22**, and the storage stabilities were tested.

Abbreviations

1. DGDE	Diethylene diglycol diethyl ether
2. DCM	Dichloromethane
3. DCE	1,2-Dichloroethane
4. TCE	1,1,2,2-Tetrachloroethane
5. $TsOH.H_2O$	*p*-Toluenesulfonyl monohydrate
6. TsCl	*p*-Toluenesulfonic acid chloride
7. EtOAC	Ethyl acetate
8. DMF	*N,N*-Dimethylformamide
9. DCC	Dicyclohexylcarbodiimide
10. DCU	Dicyclohexylurea
11. HMPA	Hexamethylphosphoramide
12. DHP	3,4-Dihydropyran
13. THF	Tetrahydrofuran
14. DPPF	1,1'-Bis(diphenylphosphino)ferrocene
15. NHS	*N*-Hydroxysuccinimide
16. DMAP	4-Dimethylaminopyridine
17. $(DPPF)PdCl_2$	Dichloro[1,1'-bis(diphenylphosphino)ferrocene)] palladium dichloromethane adduct
18 DPPF	1,1'-Bis(diphenylphosphino)ferrocene
19. LiAE	9-(2,6-Dibromophenoxycarbonyl)-10-(3-succinimidyloxycarbonylpropyl)acridinium iodide
20. LiAE'	9-(2,6-Dibromophenoxycarbonyl)-10-(3-Succinimidyloxycarbonylpropyl)acridinium triflate
21. CL	Chemiluminescence

Contents

Chapter 1

Introduction

1.1 What is chemiluminescence (CL)?

Luminescence is a term used to describe the emission of light, which occurs when a molecule in an excited state relaxes to the ground state. The various types of luminescence differ in the source of energy used to obtain the excited state. This energy can be supplied by electromagnetic radiation (photoluminescence; also termed as fluorescence or phosphorescence), by heat (pyroluminescence), by frictional forces (triboluminescence), by electron impact (electroluminescence) or by crystallization (crystalloluminescence). In chemiluminescence (CL), the energy is produced by a chemical reaction.[1] Chemiluminescence, which is the phenomenon observed when an excited product of an exoergic reaction relaxes to its ground state with emission of photons, can be defined in simplistic terms: chemiluminescence results when chemical reactions emit light.[1] The chemical reaction produces energy in sufficient amount (approximately 300 $kJmol^{-1}$ for blue light emission and 150 kJ mol^{-1} for red light emission) to induce the transition of an electron from its ground state to an excited electronic state. In organic molecules, transitions from a π bonding to a π* anti-bonding orbital (π→ π*) or from a non-bonding to an anti-bonding orbital (n→ π*) are most frequently encountered, but it is important to note that luminescence does not only refer to the emission of visible light, but electromagnetic radiation in general. Return of the electron to the ground state with emission of a photon is thus called luminescence.

1.2 Categories of chemiluminescence

1.2.1 Direct chemiluminescence

A direct chemiluminescent reaction takes place in two steps, which can be simplified as follows:

$$\text{Excitation reaction: } A + B \rightarrow C^*$$

$$\text{De-excitation reaction: } C^* \rightarrow C + h\nu$$

The excited molecule C^* can be either the final or an intermediate product of the reaction. The excited molecule can also relax to its ground state *via* dark routes by undergoing chemical reactions, collisional deactivation, internal conversion or inter-system crossing. These non-radiative processes are undesirable from an analytical point of view when they compete with chemiluminescence. The fraction of molecules emitting a photon on return to the ground state is the quantum yield (Φ_{cl}). It is the product of three ratios:

$$\Phi_{cl} = \Phi_c \cdot \Phi_e \cdot \Phi_f$$

where Φ_c is the fraction of reacting molecules giving a molecule of the appropriate type which relates to the yield of the chemical reaction; Φ_e is the fraction of such molecules formed in an electronically excited state and relates to the efficiency of the energy transfer process; and Φ_f is the fraction of these excited molecules that return to the ground state by emitting a photon. The total efficiency, Φ_{cl}, of a chemiluminescent reaction usually lies in the range of 1-20%, but very often can be much less than 1%, while bioluminogenic reactions may have efficiencies of up to 100%. The poor efficiency of chemiluminescent reactions, in most cases, is due to low yields of the chemical reactions or to poor energy transfer, but in some instances, the excited molecule is a poor emitter.

1.2.2 Energy transfer chemiluminescence

The addition to the system of an efficient fluorophore may result in a non-radiative energy transfer to the fluorophore which then emits intense light.

Energy transfer step: $C^* + F \rightarrow C + F^*$

De-excitation reaction: $F^* \rightarrow F + h\nu$

The molecule F is a fluorophore, which can also be excited by absorption of radiation (photoluminescence). If a molecule does not have the ability to participate in a chemiluminescent reaction, it may still be converted into another molecule, which does have this property. Alternatively, the molecule can be converted into a fluorophore by derivatization. The fluorophore can then be chemi-excited. The general requirements for energy transfer chemiluminescence are as follows.[2] (1) The molecule C should be capable of receiving the energy released from the reaction to form C^* and the efficiency of this process should be sufficiently high. (2) C^* should be capable of luminescing under the conditions of the reaction and the intensity of the radiation should be sufficiently high, and a suitable acceptor molecule, F, capable of accepting energy should be available for accepting the chemi-excitation energy and subsequently emitting the radiation. (3) The energy required for excitation must be supplied by the reaction in one step, if possible. In a multi-step reaction, the necessary energy must be released in a single step since the excitation step should occur instantaneously. (4) The energy required for luminescence in the visible region lies between 44 and 71 kcal mol^{-1}. Hence, a minimum requirement for CL is that the reaction produces 44 kcal mol^{-1} of energy.[3] A variety of organic compounds meet this requirement and, in some instances, their chemiluminescent properties have been thoroughly studied during redox reactions.[4]

The emission characteristic of the chemiluminescent process is then determined by the sensitizer and the efficiency of the transfer complies with the Förster Law [5]

$$E = d^{-6}/ (d^{-6} + R_0)$$

Where E is the efficiency, d represents the distance between the centres of the donor and acceptor molecules and R_0 is given by the equation:

$$R_0 = 9700 (J\ K^2\ \Phi_{cl}\ n^{-4})^{1/6}$$

Where J is the spectral overlap integral between donor emission and acceptor absorption spectra, K is the orientation factor for dipole-dipole interaction (2/3 for

random orientation), Φ_{cl} is the quantum yield of the chemiluminescent reaction of the energy donor in the absence of the acceptor, and n is the refractive index of the medium between the donor and the acceptor.[6]

1.3 Chemiluminescence systems

There are five classes of compounds currently used: acylhydrazides (luminol & isoluminol), acridinium derivatives, dioxetanes, coelenterazines and peroxyoxalic derivatives. Each of them has advantages well balanced by some drawbacks, with the result that none can be definitively preferred to the others in all circumstances.

1.3.1 Luminol, isoluminol and their derivatives

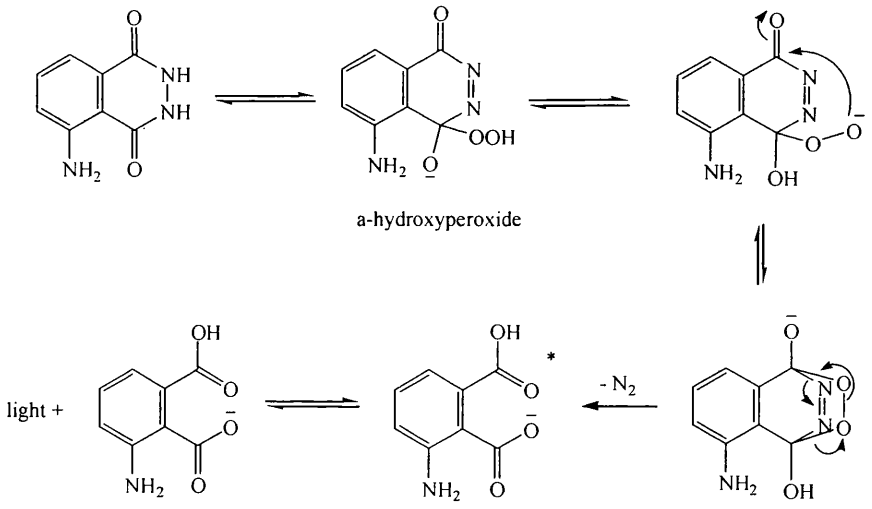


Figure 1.1, the mechanism of CL of luminol and its derivatives

The chemiluminescent mechanism of luminol derivatives follows the scheme shown in **figure 1.1**. The key intermediate is a α-hydroxyperoxide obtained by oxidation of the heterocyclic ring. The decomposition of this intermediate depends only on the pH of the system and leads to the excited state and light emission. In contrast, the first step is strongly dependent on the composition of the medium.[7] In aprotic media (DMF, DMSO), only oxygen and a strong base are required for chemiluminescence.[8] In protic media (water, alcohol) various oxygen derivatives (molecular oxygen, peroxides, superoxide anion) can oxidize luminol derivatives but catalysis by an

enzyme or a mineral catalyst is required.[9] This reaction may be used for determination of all compounds and ions that catalyse the reaction or alter the action of catalysts or produce hydrogen peroxide during a reaction.

The quantum yield of luminol does not exceed 5% in DMSO[10] and 1-1.5% in aqueous systems.[11, 12] Isoluminol is far less efficient (Φ_{cl} = 0.1%). Attempts to improve the efficiency have been made in different ways.

It has been shown that structural modifications of the heterocyclic ring lead to a complete loss of the chemiluminescent properties of both compounds.[2, 12] On the contrary, analogues with substituents in the non-heterocyclic ring are luminogenic. Electron-donating substituents are better tolerated in position 5 or 8 than in position 6 or 7, while electron-withdrawing substituents result in a loss of the chemiluminescence.[2, 12, 13] Coupling diazoluminol is also unfavourable. Alkylation of the amino group of luminol drastically decreases the efficiency, but the same modification is favourable for isoluminol (**1-2** in **figure 1.2**). Different chain lengths and coupling arms have been investigated.[1, 14]

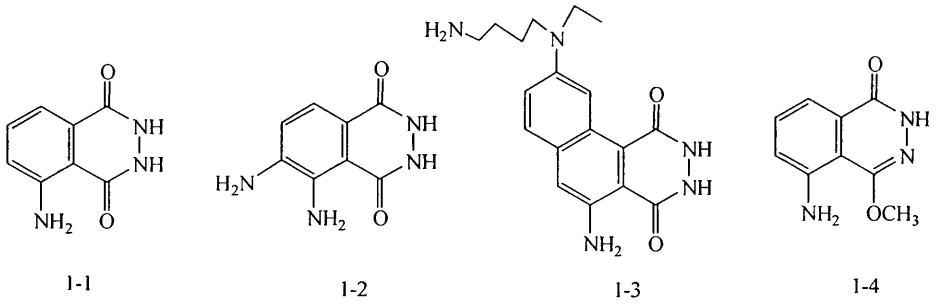


Figure 1.2, structure of luminol (**1-1**), isoluminol (**1-2**), *N*-4-aminobutyl-*N*-ethylnaphthalhydrazide (**1-3**), and *O*-methyluminol (**1-4**)

Replacement of the phenyl ring by naphthalene or benzoperylene rings leads to interesting series of molecules. *N*-4-Aminobutyl-*N*-ethylnaphthalhydrazide (**1-3** in **figure 1.2**) is about 4 times more chemiluminescent than the corresponding derivative of isoluminol and emits at longer wavelengths (515 nm rather than 420 nm) which may prevent quenching or interference from other fluorophores. However, it is easily oxidized by air oxygen at the surface of the solution.[8, 14, 15] The benzoperylene

derivative is twice as efficient as the former owing to a remarkable value of Φ_e (50%).[9, 10]

The luminol derivative **1-4**, which is an imino ether analogue, shown in **figure 1.2**, is not chemiluminescent.[16] This means that a non-chemiluminescent but photosensitive luminol derivative could release luminol on light irradiation, which would enable it to be utilized for novel CL assays. A luminol derivative that releases luminol upon light irradiation can measure light power. Luminol-*O*-2-nitrobenzylate (**1-5** in **figure 1.3**) has been reported, and its CL characteristics on light irradiation have been investigated using an ultra high-pressure mercury lamp. Furthermore, it has been applied to measure light power.[17]

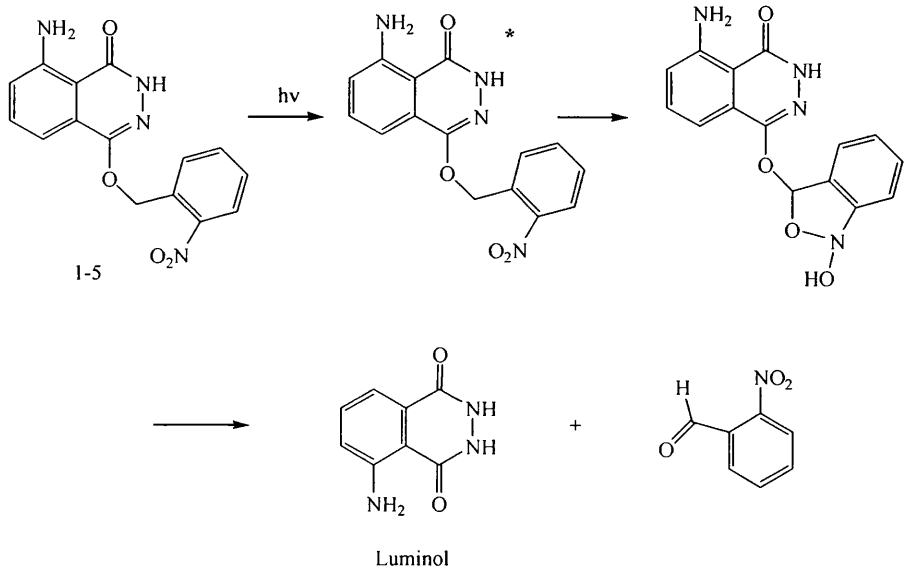


Figure 1.3, possible photosensitive luminol release mechanism.

1.3.2 Coelenterazine and synthetic derivatives

The structure of coelenterazine (R_1 = *p*-$CH_2C_6H_4OH$, $R_2 = R_3 = R_4 = R_5$ = H) is given in **figure 1.4**. Coelenterazine, which is the prosthetic group of a coelenterate protein,[18] has been synthesized by several different methods.[19-21]

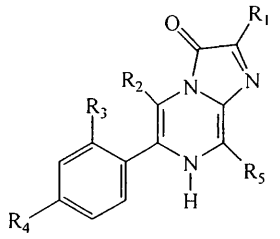


Figure 1.4, the structure of coelenterazines

The chemiluminescence of coelenterazine is triggered by superoxide anion. In contrast with luminol, the reaction is very specific and there is no need for catalytic removal of hydrogen peroxide before its determination. Consequently, coelenterazine has been proposed as a sensitive and selective chemiluminescent probe for the study of reactive oxygen metabolites released by neutrophiles.[22]

Several synthetic coelenterazine analogues have been prepared.[23, 24] CLA (2-methyl-6-phenyl-3,7-dihydroimidazo[1,2-a]pyrazin-3-one), (**figure 1.4**, R_1= CH_3, R_2 = R_3 = R_4 = R_5 = H), and especially the more efficient MCLA probe (2-methyl-6-(4-methoxyphenyl)-3,7-dihydroimidazo[1,2-a]pyrazin-3-one) (**figure 1.4**, R_1 = CH_3, R_2 = R_3 = R_5 = H, R_4 = OCH_3) have been used up to now for monitoring superoxide.[25-28] Some other analogues have been obtained recently. The old and new ones have been screened in a comparative study for their superoxide dependent chemiluminescent intensity.[29] Alkyl substitution at position 5 of the imidazopyrazinone ring results in a decrease of the luminescence intensity whereas the addition of a dimethylene bridge between position 5 and the phenyl ring bound to position 6 dramatically increases the light emission, indicating the potential usefulness of this type of compound as a probe for superoxide anion. Modification of the substituent in position 2 has little effect on luminescence. Particularly, the introduction of a propionyl or a propanamido group does not affect significantly the chemiluminescence intensity but hinders deleterious interactions with bovine serum albumin (BSA) and allows the covalent binding of the compound.

Conjugates to α- or β-cyclodextrins are water-soluble and the latter is rather insensitive to matrix effects. In contrast with the other analogues, its luminescence is

not increased by hexadecyltrimethyl ammonium bromide (CTAB), probably owing to the inclusion of the chromophore in the hydrophobic cage of the cyclodextrin.

Although coelenterazine and its analogues have been widely used for superoxide monitoring, the main application of these compounds is undoubtedly their use as prosthetic groups of different photoproteins such as aequorin, obelin, mnemiopsin, beroverin and phialidin sensitive to calcium and several other inorganic ions (lanthanides, barium and strontium but not magnesium).[30] Among these photoproteins, aequorin is the best known for immunoassay applications and intracellular calcium measurements[31, 32] but these uses are beyond the scope of this review (bioluminescence).

1.3.3 Dioxetanes

In bioluminescent reactions, 1,2-dioxetanes are well known to be involved as intermediates for efficient emission of light, due to the potentially large energy of the high torsional strain of the four-membered ring system, its structure is shown in **figure 1.5**. The dioxetanes decompose thermally, chemically, or enzymatically into two carbonylic compounds, one of which is in the excited state.[33] There are two distinct modes (**figure 1.5**). The diradical mechanism mainly occurs during thermal decomposition. Very high yields of excited states are obtained but, unfortunately, often the predominant are in the T_1 state, which is rapidly quenched in aqueous solutions and, therefore, of poor utility in diagnostic applications. Enzymatic or chemical decomposition is achieved through a chemically initiated electron exchange chemiluminescence (CIEEL) mechanism: a concerted concomitant two-bond breaking process leads to an electron redistribution and the formation of the two carbonylic products. Large S_1 versus T_1 ratios are generally obtained, which makes this reaction much more efficient in aqueous solutions. Although these two mechanisms seem rather simple, several aspects are still discussed, especially those dealing with the rate determining step.[34]

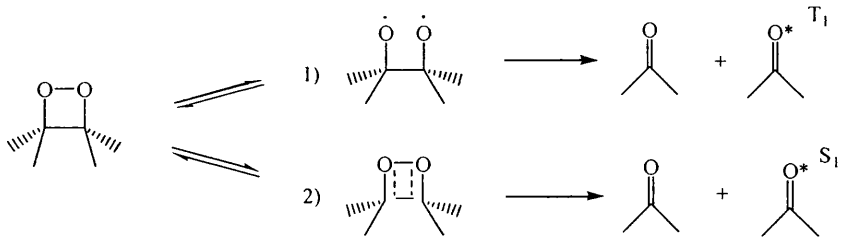


Figure 1.5, the two modes of decomposition of 1, 2-dioxetanes: 1) the diradical mechanism and 2) the chemically initiated electron exchange chemiluminescence (CIEEL). The diradical mechanism most often generates triplet excited (T_1) while CIEEL generally results in singlet states (S_1).

1.3.4 Peroxyoxalic derivatives

Several oxalate derivatives are oxidized by hydrogen peroxide giving high-energy intermediates. A gaseous intermediate has been isolated from the reaction mixture of oxalate and hydrogen peroxide and used subsequently to produce emission in the prescence of a fluorescent acceptor molecule. The proposed intermediate is dioxetanedione. In contrast with the chemiluminescent compounds cited above, the high-energy intermediate produced in this reaction is not fluorescent and, therefore, cannot emit light by itself. Light emission occurs through energy transfer to a fluorescer, which is excited in a S_1 state. As for several dioxetanes, a chemically initiated electron exchange chemiluminescence (CIEEL) mechanism is involved in the luminescent process.[1]

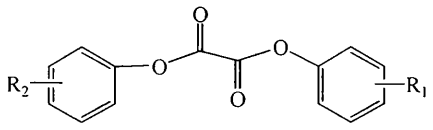


Figure 1.6, the structure of peroxyoxalic derivatives

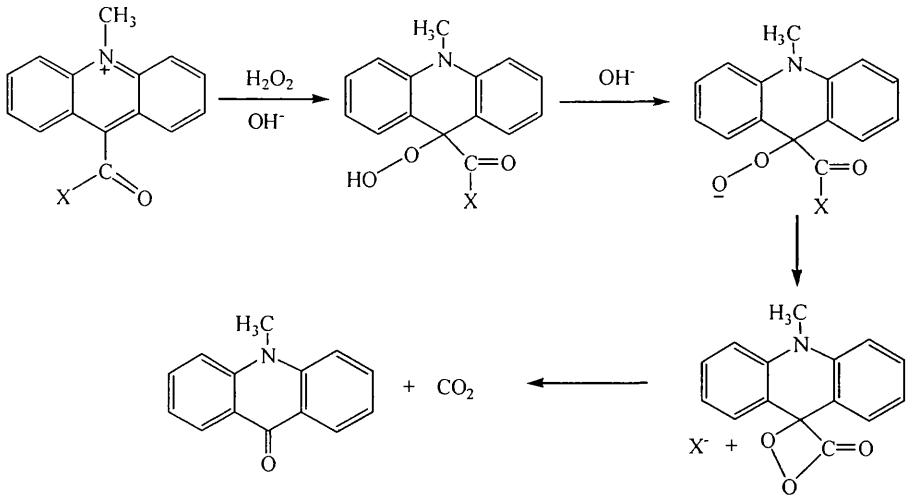
From the reaction mechanism, it is evident that the oxalate and the fluorescent sensitizer can be chosen independently. This offers flexibility because each partner of the chemiluminescent reaction can be selected either to maximize φ_c (oxalate derivatives) or to increase φ_e and φ_f (fluorescer) or to meet the requirements of the assay (aqueous or non-aqueous medium, buffer composition, pH, wavelength of the emitted light, conjugate synthesis). Energy can also be transferred to near infrared fluorescent acceptors.

By coupling the more efficient partners, overall efficiencies as high as 34% have been reported. Unfortunately, these results are obtained in non-aqueous solvents while the efficiency falls in water or water-solvent mixtures to values typical for acridinium or enhanced luminol chemiluminescence. The reaction can be carried out even in acidic media but the optimum pH is close to neutrality. Organic acids impair the reaction in contrast with weak bases, especially imidazole, which is reported to have a catalytic effect through the formation of 1,1-oxalydiimidazole.[35] γ – Cyclodextrins are proposed to preserve a minimal efficiency in water by encaging the reagents in a low polarity microenvironment.[36]

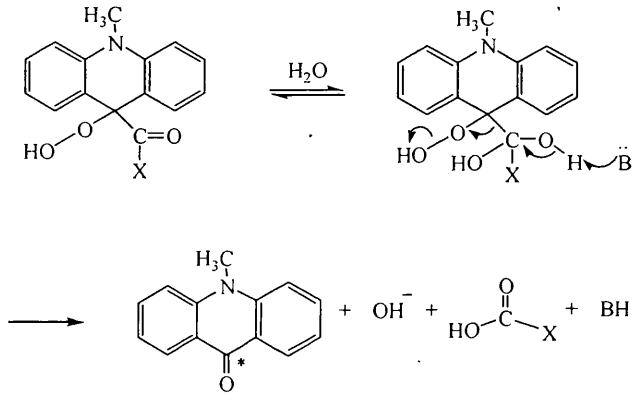
High background is frequently observed in peroxyoxalate chemiluminescence. The formation and decomposition of chemiluminescent intermediates seems to be responsible for this background. This luminescence can be distinguished kinetically from fluorophor-induced chemiluminescence and is reduced at high hydrogen peroxide–oxalate ratios. Continuous reagent addition has been proposed for suppressing background emission.

1.3.5 Acridinium derivatives

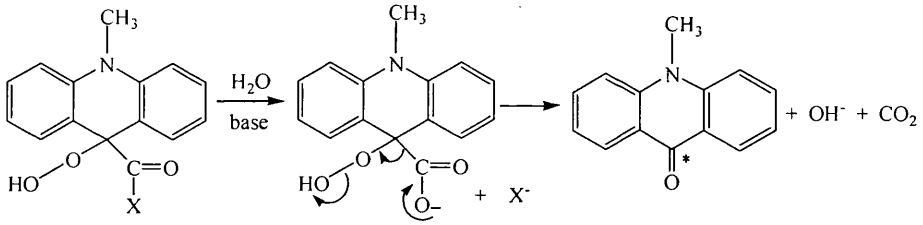
The mechanism of chemiluminescence of acridinium derivatives has been studied in detail by McCapra.[37, 38] The most widely accepted mechanism is presented at **figure 1.7**. All intermediates, except the dioxetanone, have been isolated and characterized.[39] No catalyst is involved in these reactions. According to the proposed mechanism, reversible nucleophilic addition of the hydrogen peroxide anion to the acridinium ester at the 9 position of the acridine ring is followed by cyclisation with cleavage of the leaving group, which is the rate determining step of the reaction mechanism. This intermediate then decomposes to produce carbon dioxide and *N*-methylacridone in the excited state. Only hydrogen peroxide and a strong base are needed for the chemiluminescence of acridinium derivatives.



Probable mechanism



Alternative route 1



Alternative route 2

Figure 1.7, the possible mechanism of CL of acridinium derivatives

Arylmethylene *N*-methyl dihydroacridines have epoxides and open alkylperoxides as intermediates in place of a dioxetanone.[41, 42] Lucigenin reacts with peroxide to form a dioxetane derivative.[1] Except for lucigenin and arylmethylene *N*-methyl

dihydroacridines, most chemiluminescent acridinium derivatives are constituted of two parts: the acridinium heterocycle and a leaving group X (**figure 1.7**). Each part plays a major role in the light emission. The acridinium heterocycle, after oxidation, generates the fluorescer: the excited *N*-methylacridone. Unsubstituted *N*-methylacridone emits blue light while methoxy substituted *N*-methylacridone emits green light.[43] Ring substitution as well as replacement of the *N*-methyl group by an alkyl chain terminated by a carbonylmethyl group has little effect on either the quantum yield or the chemical stability, but when the carbonylmethyl group is used for binding to an analyte, the light emitting entity will remain attached to the analyte.[44]

1.3.5.1 Effect of pH on the chemiluminescence

In the first step of the reaction mechanism, an equilibrium exists between the hydroperoxide anion and the acridinium ester and the first intermediate. Therefore a high concentration of the hydroperoxide anion will encourage the formation of the intermediate. Hydrogen peroxide and the hydroperoxide anion exist in equilibrium

$$H_2O_2 \rightleftharpoons H^+ + HOO^-$$

The pK_a of hydrogen peroxide is 12; therefore the reaction medium should have a pH higher than 12 to maximize formation of the hydroperoxide anion.

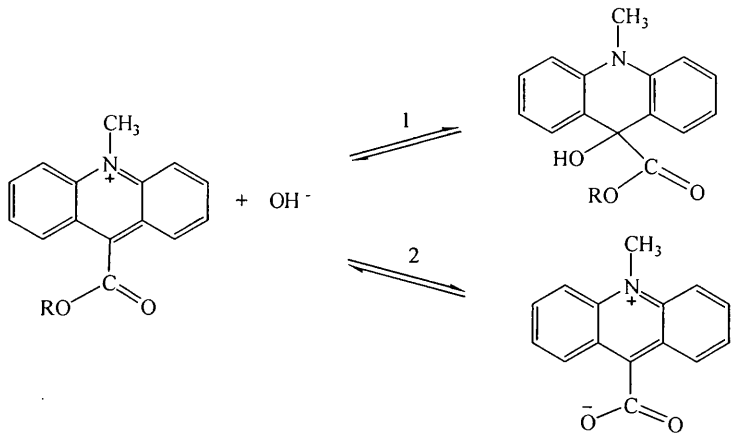
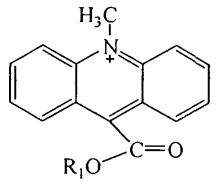


Figure 1.8, the dark reactions of 10-methylacridinium esters:
1, formation of a pseudobase; 2, base catalysed hydrolysis of the ester bond.

However, The ester bond is prone to hydrolysis at high pH. The acridinium derivatives are also in equilibrium with a non-chemiluminescent pseudobase formed by hydroxide addition at the 9 position of the heterocycle, which prevents attack form the HO_2^- (**figure 1.8**). This equilibrium is displaced toward the pseudobase in an alkaline medium. Kinetic data related to the pseudobase equilibrium are given by Littig: the half life for pseudobase formation is 26 s at pH 9, 8 s at pH 11 and only 1s at pH 13.[40] These are what are known as dark reactions, as they prevent the acridinium ester from following the chemiluminescent reaction pathway. This rapid conversion of acridinium esters to pseudobase requires that care be taken to minimize the reagent mixing time prior to observation of chemiluminescence emission. To avoid this reduction in chemiluminescent quantum yield, it is common practice to trigger the acridinium chemiluminescence by sequential addition of a solution of hydrogen peroxide in acidic medium and then a strong base, which rapidly increases the pH of the mixture, initiating the chemiluminescence.

1.3.5.2 Effect of the leaving group on the chemiluminescence

The pKa of the conjugate acid of the leaving group has a determining influence on both the chemiluminescence efficiency and the chemical stability. The leaving group must have a pKa less than that of H_2O_2 for high yields. Below that critical value, light intensity correlates with the pKa of the leaving group,[37] but very low pKa leads to faster hydrolysis.[45] Phenols, thiols, sulfonamides, fluoroalcohols, heterocyclic endocyclic amines, hydroxamic and sulfohydroxamic acids, thiolamines as well as *O*-esterfied oximes and chloroximes can be used as leaving groups. Some examples are shown in **figure 1.9**.[43, 46-50] From these compounds, those having a sulphohydroxamic acid as leaving group are probably the most chemiluminescent acridium derivatives presently known, but the poor synthesis yields (down to 0.01% overall yield) and the complex synthesis and purification process, especially for those bearing bulky groups, limit their use in practice.[46]



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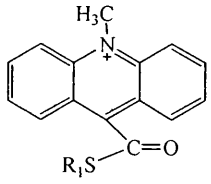

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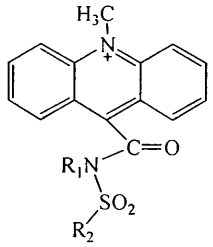

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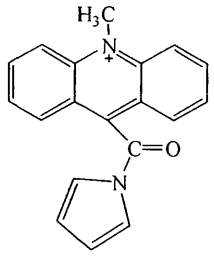

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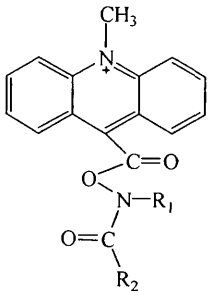

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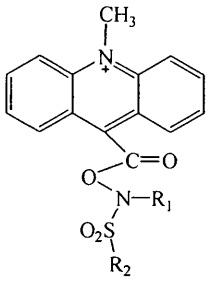

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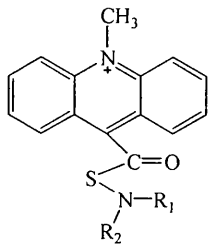

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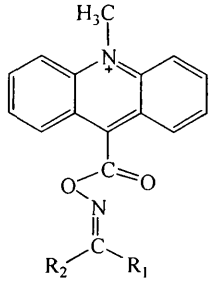

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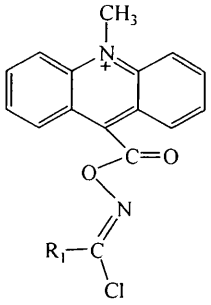

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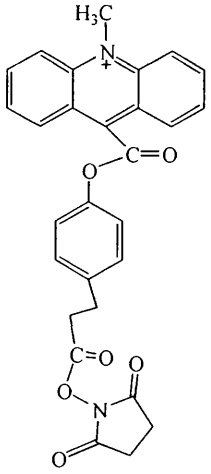

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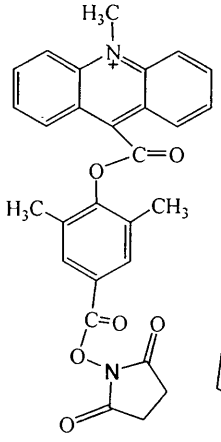

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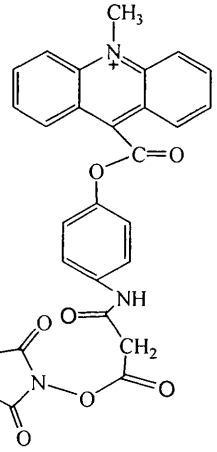

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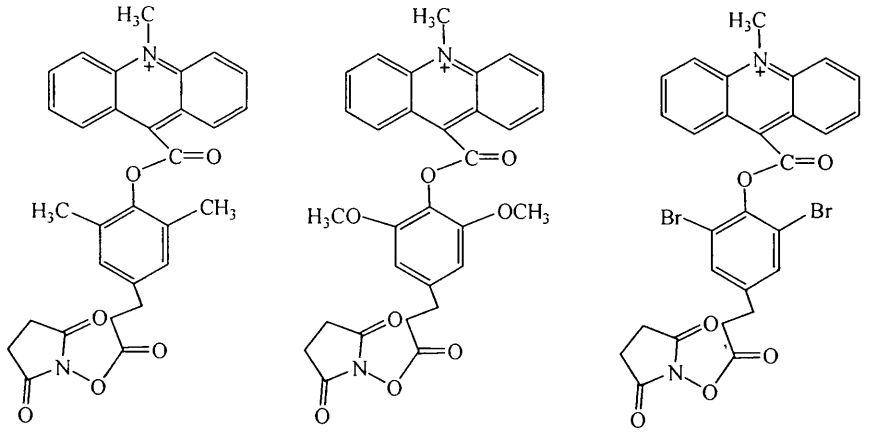


1-18 1-19 1-20

Figure 1.9, structures of acridinium derivatives bearing leaving groups based on alcohols or phenols (**1-6**), thiols (**1-7**), sulfonamides (**1-8**), heterocyclic amines (**1-9**), hydroxamic acids (**1-10**), sulfohydroxamic acids (**1-11**), thiolamines (**1-12**), oximes (**1-13**) and chloroximes (**1-14**). AE-NHS (**1-15**), DMAE-NHS (**1-16**, **1-18**), an acridinium derivative giving a long life chemiluminescence (**1-17**), DOMAE-NHS (**1-19**), DBrAE-NHS (**1-20**).

A.M. Holland has prepared a series of acridinium esters with different substituents on the leaving group, and reported the effect of the pK_a of the conjugate acid of the leaving group on the chemiluminescence in his Ph.D thesis.[51] It was shown that the quantum yield of chemiluminescence decreased, as the pK_a of the alcohol or phenol leaving group increased. **Table 1.1** shows the pK_a, chemiluminescent yield and reaction rate of various acridinium esters.

The comparison also showed that the rate of reaction increased as pK_a decreased, because the ease of forming RO^- anion resulting in the ease of formation of cyclic intermediate (**figure 1.7**) increased. The increase in pK_a also weakens the bond between RO and the carbon atom.

These arguments obviously apply to the quantum yield as well as the rate. As the product is formed more easily, a greater proportion of the reactants will form; the product and the quantum yield of reaction will be higher, which will in turn increase the overall quantum efficiency of the chemiluminescence. The quantum efficiency of emission and chemical excitation should not be affected as the excited state product is generated in the next step, and therefore would not be influenced by the leaving group.

Table 1.1, the relationship of pK_a with chemiluminescent yield and rate constant for reactions of various acridinium esters (from **reference 51**).

Leaving group (ROH)	pK_a of ROH	Φ_{CL} (%)	k (s^{-1})
4-acetylphenol	8.05	7.74	5.91
4-chlorophenol	9.38	5.85	6.39
2-naphthol	9.63	5.87	3.16
2-H-perfluoropropan-2-ol	9.30	5.69	2.06
4-methoxyphenol	10.21	4.29	0.17
phenol	10.00	4.82	0.66
2,2,2-trifluoroethanol	12.37	1.25	4.31×10^{-3}
2,2-dichloroethanol	12.89	0.10	3.45×10^{-3}
2-methoxyethanol	14.82	6.48×10^{-3}	3.43×10^{-3}
methanol	15.49	2.85×10^{-3}	3.56×10^{-3}

The phenoxy leaving groups had no substituents at the *ortho* positions of the ring, therefore, the attack at the 9 position in each aryl 10-methylacridinium ester had the same degree of shielding, and only the pK_a of the leaving group was changing. Substituents in the *ortho* position would not only affect the pK_a, but would also hinder attack at the 9 position, which would affect the rate and probably the quantum yield of the reaction.[51]

1.4 History and development

Luminous animals have been known since the ancient Greek civilization, but many of these are not widely understood, and progress in gaining understanding is not easy due to the problems of collecting sufficient organisms, the low amounts of material and the complexity of the molecules. Although most bioluminescence occurs in marine organisms, the best known example of bioluminescence is the firefly, which is actually a beetle. Artificial chemiluminescence was first described in 1877 by Radziszewski, who observed yellow light emission when oxygen was bubbled into an alkaline ethanolic solution of 2,4,5-triphenylimidazole (lophine).[52] Fifty years later, Albrecht reported the luminescent properties of 5-amino-2,3-

dihydrophthalazine-1,4-dione (luminol).[53] Acridinium derivatives were known as chemiluminescent molecules since Gleu and Petsch, in 1935, described the blue or green light emission of bis(*N*-methylacridinium) nitrate (lucigenin).[54] After McCapra, in 1964, proposed a mechanism based on the formation of a dioxetanone cycle for explaining the chemiluminescence of acridinium salts,[55] derivatives of dioxetane and dioxetanedione (peroxyoxalate) have been prepared and investigated.[56, 57]

1.4.1, Luminol, isoluminol and their derivatives

Since the beginning, many catalysts have been proposed for luminol oxidization: enzymes such as microperoxidase, myeloperoxidase, horseradish peroxidase, catalase and xanthine oxidase; metaloproteins such as cytochrome c, haemoglobin especially haptoglobin and deuterohemin; and mineral catalysts such as molecular ozone and halogens or persulfate anion or Fe(III), Co(III) and Cu(II) cations as well as their complexes. More recently, the bacterial peroxidase from *Arthromyces ramosus*, characterized by a very high turn-over, has been proposed and a hundred times increase in sensitivity is claimed. Moreover, many enzymes or enzyme mixtures that produce oxygen derivatives as by-products have been involved in chemiluminescent detection. Alkaline phosphatase, β-D-galactosidase and β-glucosidase in the presence of indoxyl conjugates as substrates, lactate oxidase, acylCoA synthetase and acylCoA oxidase or diamine oxidase produce hydrogen peroxide; 3-α hydroxysteroid dehydrogenase or glucose-6-phosphate dehydrogenase release NADH which reduces, in the presence of 1-methoxy-5-methylphenazinium methylsulfate, molecular oxygen to hydrogen peroxide which generates light in the luminol microperoxidase system.

Enzyme cycling is another way to increase the light emission.[58] Although the chemiluminescence efficiency of the system and the light intensity are not modified, more light is emitted after a long time because the enzyme substrates are continuously recycled. Malate dehydrogenase and alcohol dehydrogenase are cycling enzymes for NADH while hexokinase and pyruvate kinase have been proposed for ATP.

Nevertheless, the most attractive mode for increasing the sensitivity is certainly the use of chemical enhancers proposed for the improvement of luminescence catalysed by horseradish peroxidase, xanthine oxidase or Co (II).

Carbonate and bicarbonate containing media are more effective for the detection of low peroxidase concentrations.[59] The horseradish peroxidase catalysed reaction is also enhanced by several phenols, namely 6-hydroxybenzothiazole derivatives also called "synthetic luciferins" or *para*-substituted phenols (e.g. *p*-iodophenol, *p*-hydroxycinnamic acid, *p*-phenylphenol, *p*-hydroxybiphenyl).[60-62] Recently, a new class of enhancers has been proposed: for example, 4-phenylboronic acid is more effective than the basic isoenzyme of horseradish peroxidase (type VI A).[63] It will be noted that this last enhancer has been found synergistic with *p*-iodophenol.[64] The mechanism of horseradish peroxidase enhanced chemiluminescence using phenol derivatives has been studied by several authors.[65-69] Horseradish peroxidase (HRP) reacts with hydrogen peroxide to form an oxidized HRP (HRP I) that reacts with the anion of luminol to form a half reduced enzyme (HRP II) and a radical of luminol. The enzyme returns to the reduced form (HRP) by reaction with a second molecule of luminol. It is suggested that catalytic phenols preferentially form phenoxy radicals in contact with horseradish peroxidase and act as electron-transfer mediators to increase the efficiency of luminol radical formation. From chromatographic data, Jansen and van den Berg confirmed the increase in rate of reaction with the enzyme but, based on the product formation, they concluded that the mechanism of enhancement is probably different for the various enhancers.[70] Recently, Navas Diaz has given an electrochemical explanation of the phenomenon: only phenoxy radicals having a reduction potential greater than the redox potential of luminol at pH 8.5 (+ 0.8 V) can act as enhancers. They have also correlated the Hammett coefficients of substituents on the phenyl to inhibitory or enhancing effects. Such correlations are of course of predictive value for development of new enhancers.[71] Fluorescein has also been proposed but acts via a completely different mechanism (energy transfer).[72]

The xanthine oxidase reaction is enhanced by mineral or organic compounds. Sodium dithionite increases the emitted light by an unknown mechanism. With complexes of Fe (III) with ethylenediaminetetraacetic acid (EDTA) or better HEDTA (*N*-(2-hydroxyethyl)ethylenediamine-*N*,*N*',*N*'-triacetic acid), a tremendous

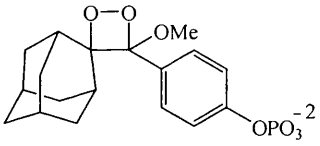
increase in light emission is observed in buffered solutions containing sodium perborate. This increase, due to the formation of hydroxyl radicals, is unfortunately matrix sensitive and consequently of little practical value, especially in homogeneous immunoassays.[73-75] Indeed, the signal is completely lost upon addition of urine or serum to a final concentration of 1%.

Penicillins have been reported to enhance the luminol–hydrogen peroxide–Co (II) system. Owing to a complexation mechanism, the β-lactam antibiotic extends the lifetime of the superoxide anion by a few orders of magnitude allowing for more efficient oxidation of luminol.[76]

Protein Cu (II) complexes have recently been found to be more efficient than Cu (II) alone for triggering the luminol peroxide luminescence. The enhancement mechanism is not yet elucidated.[77]

Surface-active agents generally impair the light emitted by enzyme mediated luminol reactions although, at selected concentrations, anionic surfactants can increase the rate of horse radish peroxidase reactions.[78, 79] Nevertheless, the non-peroxidase luminol-metal porphyrin chemiluminescence reaction is enhanced by non-ionic or negatively charged detergents.[80]

1.4.2 Dioxetanes



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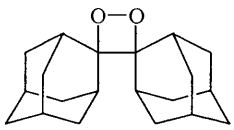
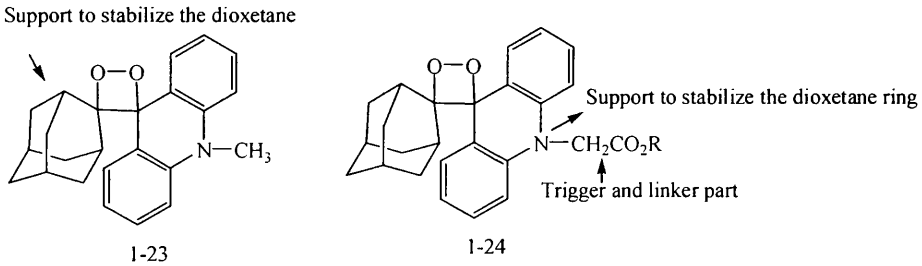


1-22

Figure 1.10 a model 1,2-dioxetanes AMPPD (**1-21**); adamantylideneadamantane-1,2-dioxetane (**1-22**).

1,2-Dioxetanes, in their earliest developments (1969), were characterized by several unpleasant properties: their thermal instability, the quenching of the luminescence in aqueous solutions and the difficulty of controlling the luminescence process make them rather unsuitable for diagnostic applications but the great dependence of the S_1

versus T_1 ratios and the half-life on the molecule substituents prompted researchers to look for more stable dioxetanes.[81-84] In 1972, Wynberg described the synthesis of the very stable adamantylideneadamantly-1,2-dioxetane (**figure 1.10**), characterized by a decomposition temperature higher than 160°C and a half-life of 21 years at 25°C.[85] It has been suggested that steric effects could explain the exceptional stability of this derivative. Nevertheless, other large substituents, such as spirobiaryl groups destabilize the dioxetane, so no conclusive theory on the role of steric effects is available yet.[81] A few years later, in 1977, McCapra prepared the first asymmetrical and sufficiently stable dioxetane: the 9-(2-adamantylidene)-*N*-methylacridan-1,2-dioxetane (**figure 1.11**) still emits light — through the excited singlet state of *N*-methylacridone — after thermal decomposition, and opens the way to a new class of asymmetrical dioxetanes whose luminescence is triggered by chemical or enzymatic removal of a protecting group of the stable state of the dioxetane.



1-23 1-24

Figure 1.11, the structure of substituted dioxetanes.

(3-(2'-Spiroadamantane)-4-methoxy-4-(3"-phosphoryloxy)phenyl-1,2-dioxetane (AMPPD, **figure 1.10**) and (3-(2'-spiroadamantane)-4-methoxy-4-(3"-β-D-galactopyrano-yloxy)phenyl-1,2-dioxetane (AMPGD) prepared by Bronstein have been widely used. These compounds are substrates of high turn-over enzymes currently used in immunoassays, alkaline phosphatase and β-D-galactosidase respectively.[81]

Another advance is the introduction of 4-methoxy-3-spiro(1,2-dioxetane-3,2'-(5'-chlorotricyclo-[3.3.1.1.3,7]decan)-4-yl)phenylphosphate disodium salt (CSPD), a derivative of AMPPD.[86, 87]

AMPPD is stable in water solution: its half-life is one year in slightly alkaline medium at room temperature. The light emission is simultaneously controlled by the kinetics of the enzymatic deprotection and the destabilized dioxetane decomposition with a finite half-life. The result of this two-step process is a delay preceding the steady-state chemiluminescence which is proportional to the alkaline phosphatase concentration. The pH influences the velocity of both reactions and the maximum light emission occurs at pH 9. The chemiluminescence-emitting moiety is the excited state of the methyl 3-hydroxybenzoate anion that emits a glow at 470 nm.[81] CSPD and AMPPD react similarly but a higher light intensity and a shorter delay to reach the steady-state are claimed using CSPD. The chlorination of the adamantane moiety of AMPPD, which minimizes the aggregation of CSPD and its dephosphorylated anion by altering their amphiphilic nature, could explain the best properties of this new molecule.[86]

AMPGD has the same behavior as AMPPD except for the deprotection that is carried out at lower pH 7.5. At this pH, the phenol is in the protonated form (pK_a of the phenol = 9) and, therefore, is a slow emitter: raising the pH above 10 produces light. It will be noted that it is possible to switch the light on and off by shifting the pH in the range 7–12.[81, 88]

As for the other chemiluminescent emitters, dioxetanes and especially emitters based on the (3-(2'-spiroadamantane)-4-methoxy-4-(3"-hydroxyphenyl)-1,2-dioxetane anion (AMPPD and AMPGD) and its chlorinated derivative (CSPD) are more efficient (3–400 times) when they are protected from proton quenching in solutions containing large proteins (BSA) or surfactants (CTAB).[81, 88]

Indirect chemiluminescence is also possible with the dioxetanes. A very hydrophobic derivative of a fluorescer (5*N*-tetradecanoylaminofluoresceine) can be included in the micelles of the surfactant CTAB (Lumi-Phos™) or the fluorescer itself can be conjugated to the AMPPD analogues by substituting the methoxy at the 4 position on the dioxetane ring.[88]

The decomposition of adamantylideneadamantyl-1,2-dioxetane has been induced by a photo-excited rare earth metal.[89]

McCapra reported that thermal decomposition of the dioxetane **1-23** produced 10-methylacridone with light emission, but dioxetane **1-23**, was too unstable to be practically handled.[90] Hoshino in 1997 [91] synthesized the compound, dioxetane **1-24**, shown in **figure 1.11**, which has an acridane structure as an excellent luminophore moiety, the adamantyl and acetate groups as stabilizer of the dioxetane ring, and the acetate as a trigger for smooth decomposition of the dioxetane by rapid generation of the acetate anion on alkaline treatment. The thermal stabilities and chemiluminescent properties of dioxetanes **1-24** having various substituents (R) were also described.

1.4.3 Acridine derivatives

The first compound that drew attention to the chemiluminescence of acridinium compounds was lucigenin, 10,10'-dimethyl-9,9'-biacridinium nitrate, which in 1935 was reported to react with alkaline peroxide to emit blue green light.[54] A mechanism was proposed for this reaction which is similar to the one for the chemiluminescent reaction of the acridinium esters, and is shown in **figure 1.12**.

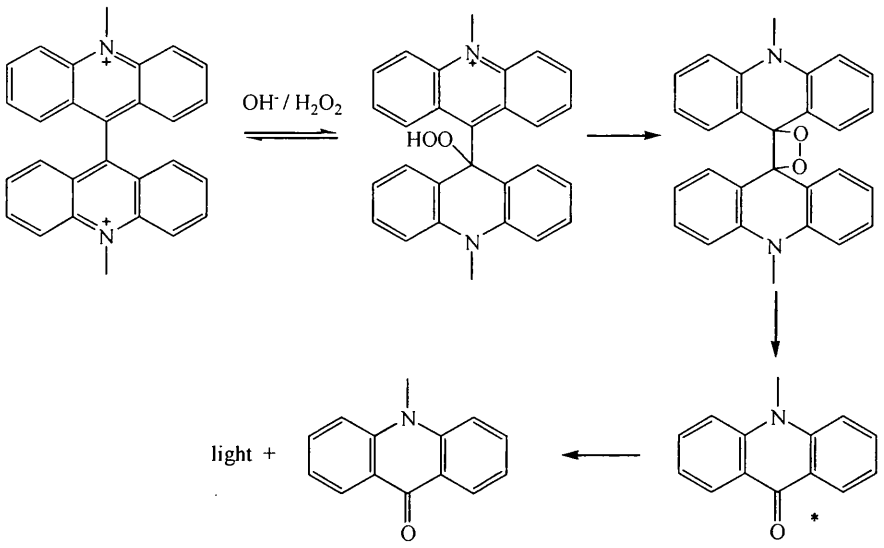


Figure 1.12, the mechanism for chemiluminescent reaction of lucigenin

In the 1960's, further attention was given to acridinium compounds, and several additional compounds were reported to be chemiluminescent, which included 9-cyano-10-methylacridinium nitrate and 9-cyano-10-methylacridan. In the following years, other chemiluminescent reactions of acridinium compounds were reported,

which included 9-chlorocarbonyl-10-methylacridinium chloride, 9-benzoyl-10-methylacridinium methanesulfonate and 9-carbonylmethoxy-10-methylacridinium iodide. All the compounds reacted to form 10-methylacridone as the emitter in the chemiluminescent reaction. In the 1970's, substituted phenyl 10-methylacridinium esters were synthesized, but little further research was carried out on this group of chemiluminescent compounds until analytical applications were developed.

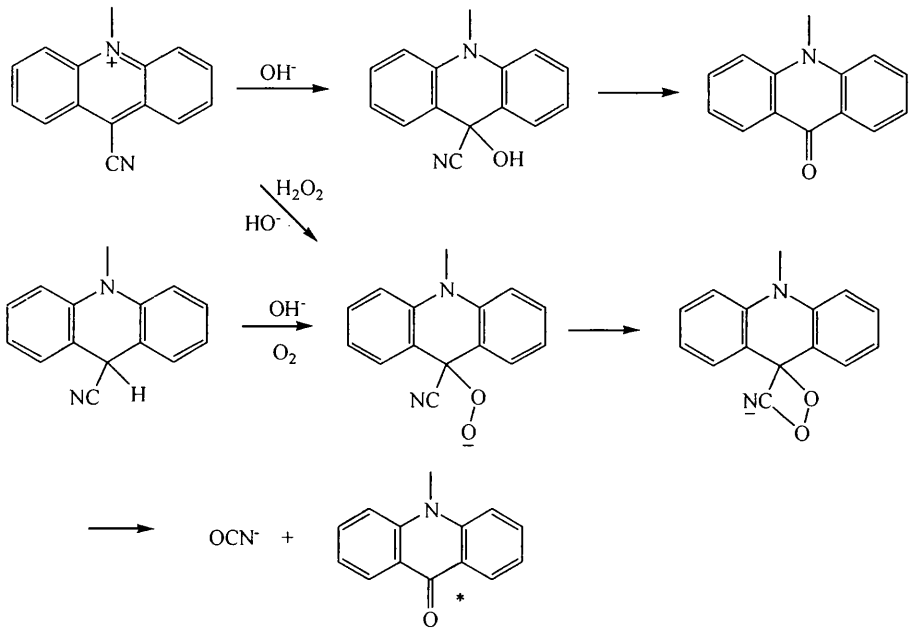
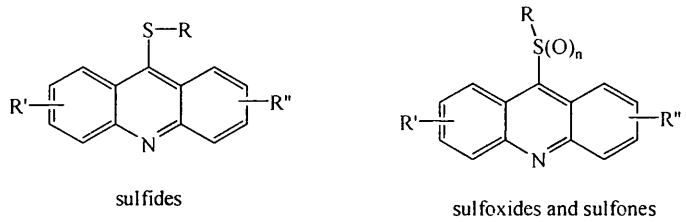


Figure 1.13, the mechanism of chemiluminescence of 9-cyano-10-methylacridinium nitrate and 9-cyano-10-methylacridan

The first acridinium ester label, which has a succinimidyl moiety as the reactive group for coupling to proteins reported was the 9-(4-(2-succinimidyl-oxycarbonylethylphenoxy)-10-methylacridinium triflate (AE-NHS, **1-15**), shown in **figure 1.9**.[92] AE-NHS does not show the best quantum yield and is not very stable, especially at room temperature, although its stability is increased after coupling to an analyte. More efficient compounds have been found in the thiol, sulfonamide, hydroxamic acid, oxime and chloroxime series.[46-47] Thiol and sulfonamide derivatives are often five times more luminogenic than AE-NHS.[93] A remarkable characteristic of sulfonamide derivatives is that the improvement in efficiency is not associated with a loss of stability: on the contrary, several compounds are much more stable than AE-NHS. No significant loss of efficiency has been observed even after one year at room temperature.[48] Compounds combining stability and efficiency were

also found in the oxime and chloroxime series. Some oxime derivatives have intrinsic stability while, up to now, chloroxime derivatives are only stable after coupling.[46, 47] Attempts to improve the stability in the phenol family have led to the development of analogues of AE-NHS with the leaving group substituted by 2,6-dimethyl, 2,6-dimethoxy, and 2,6-dibromo groups (**2-18**, **1-19**, **1-20** respectively) shown in **figure 1.9**.[50, 94] Recently, Santelli-Rouvier prepared 9-acridinyl sulfur derivatives: sulfides, sulfoxides and sulfones, 23 compounds in total and their structure are shown in **figure 1.14**.[95] And Miyashita[96] reported oxiranes having an acridane structure as a novel chemiluminescent precursor, shown in **figure 1.15**.



sulfides sulfoxides and sulfones

Figure 1.14 the structures of 9-acridinyl sulfer derivatives

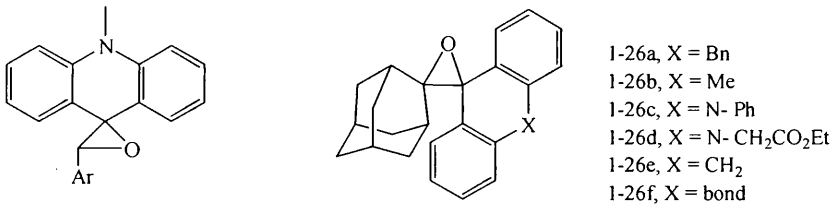


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Figure 1.15, a chemiluminescent precursor

Generally, acridinium derivatives emit light as a short flash within 5 seconds or less after triggering the chemical reaction. Nevertheless, slower or faster emission rates have been observed after modification of the acridinium ring as well as after substitution of the leaving group. In the phenol series, methylation of the acridinium ring slows the kinetics of light emission. Electron-withdrawing groups introduced into the phenyl ring increase both efficiencies and reaction rates while electron donating groups have the opposite effect. Hydrolysis rates are also affected by substituents of both the phenyl and acridinium rings. A parallelism is often observed between the effects on hydrolysis and chemiluminescent reactions because both involve nucleophilic attack, the sites of which are only one carbon apart. Nevertheless, the relative magnitude of the effects can differ greatly from one

compound to another and, in some cases, increased stabilities can be associated with fast emission properties. Steric hindrance could explain these characteristics since most of the compounds exhibiting this stability are substituted in the *ortho* position of the phenol by bulky groups (methyl) or atoms (Br) while the same substitution in another position is not efficient from this point of view.[97] Recently, substitution of the phenyl ring by alkylcarboxamido groups has led to unexpected results: the luminescence efficiency is unaffected but the kinetics of the emission and the rate of hydrolysis are strongly dependent on the position of the substituent. To our knowledge, the *para* substituted alkylcarboxamido derivatives are the slowest emitters described up to now in the acridinium series (duration $\approx$ 60 s).[98]

Large excesses of various nucleophilic reagents (hydroxide, thiolate and sulfite ions) attack acridinium carboxylate derivatives at the carbon bearing the carboxy group. The adducts formed with thiols or sulfite are more stable than the corresponding native compound but the chemiluminescent properties are lost. The chemiluminescence is slowly recovered after dilution with water (sulfite) or after reagent removal by 2,2'-dipyridyldisulfide (thiols). Formation of adducts has been proposed for long term storage of unstable acridinium conjugates.[99] By reaction with hydroxide ion, a non-luminogenic pseudobase is reversibly obtained. Before triggering the chemiluminescence, the equilibrium has to be displaced to the acridinium by reaction with an acid (HNO_3, HCl).[99, 100]

Epinephrine in cationic surfactant micelles containing periodate has been reported to increase the luminescent signal of lucigenin.[101] A patent has described methods for enhancing the chemiluminescent signal of an acridinium ester, in a chemiluminescent reaction in the presence of an enhancer selected from the group consisting of: (a) a cationic surfactant; (b) a nonionic surfactant; and (c) a sulfated primary alcohol (d) zwitterionic surfactants, and (e) anionic surfactants.[102]

1.5 Applications

In the last few years, several papers dealing with new chemiluminescent compounds and more than 1500 per year dealing with applications in immunoassay and

biomedical research have been published. Although significant improvements of noise and sensitivity, new developments in multi-analyte analysis and homogeneous immunoassays, advances in selectivity of coupling and triggers are expected in the near future, chemiluminescence has already become an essential tool in routine analysis.

Acylhydrazides like (iso)luminol are still the most frequently used chemiluminescent compounds in immunoassays and in oxygen metabolism studies partly because they can be used for different kinds of assays, but they need a catalyst for light emission and an enhancer to be competitive in terms of sensitivity. This can result in higher background signals.

Acridinium derivatives have high quantum yields even after easy coupling to proteins. As they do not need catalysts, background signals are low and high sensitivities are frequently obtained. The instantaneous light emission, which has been considered in the past as a disadvantage (measuring problems), allows high rates in automated analyzers.

The dioxetanes used for diagnostic applications are enzyme triggered dioxetanes. As for acridinium derivatives, low background signals are observed. Moreover, dioxetanes exhibit a prolonged light emission but they need a rather long period of time before reaching a constant signal. This last feature represents an unwelcome added incubation time in immunoassays.

Coelenterazine and its analogues are essentially used in association with catalytic proteins such as apoaequorin. Used alone, it is a specific luminogenic reagent for superoxide anion.

In the presence of a fluorescer, oxalate derivatives are the most efficient non-biological emitters. Fluorescers and oxalates are chosen independently. Efficiency and flexibility are therefore the main advantages of this system. Non-resolved problems of water solubility and stability added to a loss of efficiency in water certainly explain the little success of peroxyoxalate chemiluminescence in immunoassays and biomedical applications.

1.5.1 Applications in immunoassay

Immunoassay takes advantage of the properties of a group of molecules called antibodies that are involved in the acquired immune system of mammals. In response to the invasion of a substance that is alien to the system, the *B*-lymphocytes produce antibodies. This alien material is termed the antigen, as its presence initiates the production of antibodies. An antibody will specifically bind to one antigen. A labelled antigen (Ag*) is introduced into the sample, which competes with the antigen in the sample for the antibody, and the amount of antigen-antibody complex is related directly to the amount present in the blood. Or, the antibody is labelled, and the complex, which is now labelled, can be separated and assayed. This is an area in which chemiluminescent compounds provide many advantages over the existing radioactive compounds that are currently used.

Immunoassay has a wide range of applications, as an antigen can be a range of substances, such as hormones, therapeutic drugs, illegal drugs (*e.g.* heroin or an anabolic steroid) and pathogens (*e.g.* salmonella, HIV). Therefore it can provide information of direct value to doctors, such as the identification and location of tumors or the identification of infections, and it can also be used to follow drug therapy. It can be used in veterinary medicine, agriculture, forensic medicine and the food industry.

There are two classes of assays that use this principle: immunoassay and immunometric assay. The first immunoassay used radioactive iotopes, such as ^{125}I, so the classes were referred to as radioimmunoassay and immunoradiometric assay, but fluorescent and chemiluminescent assays are also used, which are naturally called fluorescent immunoassay and chemiluminescent immunoassay respectively.

The first class, radioimmunoassay (RIA), conventionally uses a labelled antigen and unlabelled antibody, usually at a concentration that will produce about 50% binding of the labelled antigen. Unlabelled antigen will also bind to the antibody, and will compete, as there is a limited amount of antibody. As the amount of unlabelled antigen increases, the amount of free unlabelled antigen will increase. The bound

antigen is separated from the free antigen, and a standard curve can then be produced, plotting counts against concentration.

After reaching the equilibrium in the immunoassay, it is necessary to separate the free antigen from the antigen-antibody complex. This can be achieved by attaching the antibody to a surface, such as the surface of the tube the reaction takes place in, or glass or plastic beads, and a variety of other complexes. After the reaction, the free antigen can be removed by washing.

The second class, immunoradiometric assay (IMRI), always uses labelled antibodies, and is a non-competitive assay. Large amounts of the labelled antibodies are used to push the equilibrium over to the right, then the excess free antibody is removed, and the remaining bound antibody is measured.

Addition of a solid phase antigen can be used to separate the free antibody from the complex, and either the activity of the complex or the free antibody can be measured. IMRA is a lot more sensitive than RIA, and can used to cover a wide range of analyte concentrations (μM of marker proteins and drugs to pM of free hormones), but RIA is still used.

Luminol, isoluminol and their analogues have been extensively applied in immunoassays. Because the oxidation of luminol derivatives has to be catalysed, antigen or antibody labelling with either the catalyst or the luminogenic substrate has been investigated and heterogeneous immunoassays in various formats (direct or indirect detection in competitive or non competitive mode) as well as homogeneous immunoassays have been proposed. In an excellent review, Rongen have listed more than 60 immunoassays from different classes.[103] Recently Kricka has given a review of chemiluminescence in clinical application.[104] In recent years, enzyme labelled immunoassays using enhanced luminol detection have been proposed for the detection or the determination of fentanyl,[105] DDT (1,1,1-trichloro-2,2-bis(*p*-chlorphenyl)ethane, one of the most widely used organochlorine pesticides) and related compounds,[106] human serum albumin,[107] immunoglobulin E serum levels,[108] 2,4-dichlorophenoxyacetic acid,[109] ferrocene and eleven of its derivatives,[110]

prostaglandin E2 (*p*-iodophenol enhancer),[111] endothelin,[112] antibodies [113], proinsulin [114], human chorionic gonadotrophine β-subunit in broad range using microsampling,[115] and free thyroxine.[116] A sensitive non-enhanced chemiluminescent assay based on glucose oxidase for human granulocyte colony stimulating factor (G-CSF) is also described.[117]

Acridinium esters and their analogues are widely used in immunoassay as well as luminol and its derivatives. Recently, detections of vitellogenin have been described by Fukada and Soh[118-120] in five salmonid species and in fish meal. Silvaieh has developed an enantioselective sequential-injection chemiluminescence immunoassay for α-amino acids, 3,3',5-triiodothyronine (T_3) and thyroxine (T_4).[121- 123] Leidy described the detection of somatostatin in unextracted rat plasma,[124] and rat growth hormone-releasing hormone.[125, 126] In Schmidt-Gayk' s group, competitive immunoassay has been used for immunoglobulin A[127] and lysozyme in faeces.[128] Other immunoassays have been reported for detection of human cardiac troponin I,[129] for glucose oxidase activity and alkaline phosphatase amplification cascade,[130] for estradiol based on hapten heterology,[131] for intact human proinsulin and its conversion intermediates,[132] for studying the kinetics of production and consumption or degradation of human interferon-γ,[133] for the sensitive measurement of apolipoprotein B100,[134] and so on.

In the acridinium series, only lucigenin has been used for non-immunoassay applications, namely for specific quantification of cellular superoxide anion response after oxidative stress.[135-137] Although chemiluminescent isomers of acridinium and related heterocycles (phenanthridinium and isoquinolinium) have been claimed more than 10 years ago, no applications have been found in the medical literature.[138]

1.5.2 Applications in nucleic acid assay

Acridinium compounds reveal their best capabilities in labelling DNA strands to produce chemiluminescent DNA probes. Indeed, after inclusion into the groove of a DNA double helix, acridinium labels show increased stability toward hydrolysis and no more reactivity with nucleophilic thiols and sulfites. Various non-separative

determinations are based on these properties,[97] which have also been exploited for the very accurate determination of hybridization rate constants and thermodynamic affinities of oligonucleotide probes binding to simple synthetic targets as well as to complex biological targets.[139] Multianalyte determinations based on different decay kinetics of several acridinium derivatives have been made using DNA probes, although these methods could probably be applied for labelled antibody based assays.[140] In 1989, Arnold developed several hybridization assay formats involving acridinium ester labelled DNA probes,[141] in which target sequences in the 10^{-16} to 10^{-17} mol L^{-1} range can be detected; when rRNA is the target, this translates to 3000 to 300 bacterial organisms. The entire assay can be carried out in < 30 min. This is the first homogeneous DNA probe to be of practical use in the clinical laboratory, and it represents a major simplification of hybridization formats. This enhances assay sensitivity about 10-fold, to a range of 10^{-17} to 10^{-18} mol L^{-1} of target. Highly sensitive hepatitis C virus RNA detection methods have been described by Sarrazin, in which RNA amplicons are detected by a hybridization protection assay with amplicon-specific acridinium ester labelled DNA probes.[142] The acridinium ester labelled oligonucleotides hybridize to the RNA amplicons. As the RNA amplicons are not immobilized within the reaction tube, unhybridized acridinium ester labelled probes cannot be easily washed out but have to be deactivated. This is performed by addition of alkaline reagent. The unhybridized acridinium ester labelled probes are hydrolyzed at high pH, while the hybridized probes are protected from degradation and thereby retain the chemiluminescent label.

1.6 Advantages of acridinium esters as chemiluminescent labels

There are various problems associated with radioactive isotopes, and therefore chemiluminescent compounds were developed as an alternative. There are several important features of this system.

1 When a sample containing a typical acridinium ester bound to an antibody is reacted with hydrogen peroxide and base, the acridinium ester detaches itself from the antibody and forms excited *N*-methylacridone. Therefore the chemiluminescence should not be affected. If the acridinium compound were bound to the antibody *via* another route, for example *via* the nitrogen of the acridinium ring, the antibody

would remain attached during chemiluminescence and this might affect the chemiluminescence. However, with the Woodhead-Weeks label, the nature of the biological molecule does not interfere with the chemiluminescence, since attachment is *via* the phenolic part of the phenolic ester.

2 With the modifications that have been made to the phenoxy component of the label, the stability of some labels is greater than that of labels that use radioactive isotopes. ^{125}I is often used as the radioactive isotope and it releases high-energy particles that can damage the antibodies, and therefore reduce the shelf life of the label. The chemiluminescent labels however can be stored for a long time without a loss in sensitivity.

3 There are also health hazards presented by using radioactive isotopes. Iodine accumulates in the thyroid gland, and a high concentration of radioactive isotopes increases the risk of cancer. Although the acridinium esters are suspected to be carcinogenic, they pose a much lower risk than radioactive isotopes.

4 The acridinium ester chemiluminescent labels are very sensitive (detection limit of 1 - 10 amol – 10^{-17} – 10^{-18} mol L^{-1}), being more sensitive than ^{3}H isotope and as sensitive as ^{125}I isotope.[143]

5 Radioactive isotopes are expensive to use. The health hazards dictate that expensive safety standards have to be met, and the isotopes are costly to buy. Acridinium esters, in the small quantities that are required, are very cheap.

1.7 Improvements to the model label

Generally, a typical model acridinium ester label is composed of three parts, emitter, spacer and linker, illustrated in **figure 1.16**. There are several problems that this model label presents, and there are areas in which improvements could be made.

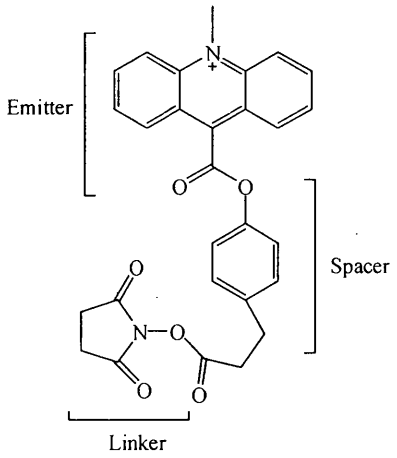

Figure 1.16, a model acridinium ester label [92]

1.7.1 Changes to the phenol ring[50, 51, 94]

The simple model acridinium ester, shown in **figure 1.16**, is susceptible to hydrolysis, reducing the stability of the label in solution. This makes the label unsuitable for some commercial applications, but there have been modifications made to the label that have improved its stability. The introduction of substituents at the *ortho* position on the phenyl ring provides shielding from hydrolysis.[50, 51] In addition to labels derived from 2,6-dimethyl phenol, which are sufficiently stable for more commercial uses, a variety of other *ortho* substituted phenol derivatives have been produced.

The chemiluminescence reaction is carried out at high pH, which can present a problem when using some biological molecules, as they are not stable at high pH. By substitution of electron-withdrawing groups to decrease the pK_a of the leaving group, the reaction rate can be improved so that the reaction can be carried out at lower pH.[51]

1.7.2 Changes to the acridinium ring [94, 144, 145, 146]

The emitter in the chemiluminescent reaction is 10-methylacridone, and the only changes that will affect the wavelength of the emitted light are changes to the acridinium ring. If acridinium ester based systems could be developed which emitted light at different frequencies, multi-analyte systems could be developed. However,

substituents on the acridinium ring can also affect the kinetics and stability of the acridinium ester, in a similar way to modifications to the phenoxy ring.

2,7-Dimethoxy acridium derivative was prepared by Li, and also 1,8-dichloro-2,7-dimethoxy acridinium compound was produced.[94] They have reduced efficiency, possibly due to the heavy atom effect. Additionally, fluorophores could be linked to the acridinium ring *via* the nitrogen or at other parts of the ring. This would allow the energy to be transferred to the fluorophore, producing light of different wavelengths.

1.7.3 Changes to the spacer and the positions of the linkers

Patel described the use of energy transfer for detecting antibody and antigen.[147]An antigen was labelled with a chemiluminescent compound (a luminol analogue), and its corresponding antibody with a fluorescent molecule (a fluorescin analogue). In the absence of binding to antibody, the emission detected in a luminometer corresponds to a wavelength characteristic of the chemiluminescent molecule. Upon immune complex formation between the antigen and the antibody, the doner/acceptor pair is sufficiently close for energy transfer, and the emission characteristics of the fluorescent acceptor were seen.

The energy transfer occurs between chemiluminescent emitter (labels) and energy acceptor (quencher) labels as means of monitoring complementary nucleic acid binding reactions. Typically, efficient quenching occurs when the distance between the emitter and the quencher is of the order of 10 nm or less, so the spacer may have some effect on the quenching possibly.

In this thesis, a series of AEs possessing a linker and spacer bonded to the N atom (position 10) of the acridinium ring, instead of to the leaving group, have been reported. Their stabilities and chemiluminescent kinetics have been investigated.

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Chapter 2

Synthesis of 9-(dibromophenyl) 10-methyl, 10-dodecyl and 10-(ω-succinimidyloxycarbonylalkyl)-acridinium carboxylates

2.1 Introduction

9-(2,6-Dibromophenoxycarbonyl)-10-(3-succinimidyloxycarbonylpropyl)acridinium iodide (**figure 2.1**, n = 3, Y = I) was synthesized successfully by Li in Smith's group, and has been called LiAE for short, in honour of Li's excellent work. LiAE has shown interestingly properties with potentially useful applications. When it attaches to an oligonucleotide fragment *via* a linker group bonded to the acridinium nitrogen atom, and then takes part in a chemiluminescent reaction, the luminescent moiety remains attached to the oligonucleotide and doesn't drift into solution. Consequently, energy transfer from the luminescent moiety to a quencher that was itself associated with the oligonucleotide should be more reliable than with a similar label linked *via* the phenolic moiety. This could result in both a higher quantum yield of energy transfer and a lower background luminescence from the doner *N*-alkylacridinium moiety.

However, as reported by Li, the synthesis of LiAE is both tedious and low yielding, giving a yield of less than 3% overall. Furthermore, it is possible that the relatively short spacer group between the linker moiety and the luminescent moiety might result in oligonucleotide-bound products having diminishing luminescent performance. For these reasons, it was of interest to synthesize the range of novel labels with different lengths of spacers (**figure 2.1**, n = 3, 5, 10).

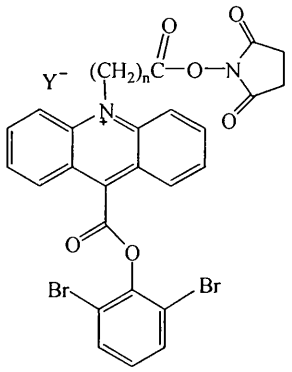


Figure 2.1, the structure of LiAE and desirable novel labels with a NHS linker group

A new compound (**figure 2.1**, n = 3, Y = CF_3SO_3) was designed, which differs from LiAE only in the nature of the counter anion Y^-. This is not expected to have any significant effect on the properties of the luminescence. However, it would be derived from an alkyl triflate rather than the corresponding iodide, and the triflate is expected to be a more reactive electrophile. Therefore it may be easier to obtain 9-(2,6-dibromophenoxycarbonyl)-10-(3-succinimidyloxycarbonylpropyl)acridinium trifluoromethanesulfonate in a better yield than had proved to be the case for synthesis of the corresponding iodide derivative. Another 2 compounds (**figure 2.1,** n = 5 or 10 respectively, Y = CF_3SO_3) were designed so that the effects of different lengths of spacer groups can be investigated.

It was clear that the synthesis of novel labels with a NHS linker group, would require the expenditure of considerable time and effort. However, it was possible that the expected benefits might not be realizable, either because the alkyl triflates did not react as expected with AE or because the presence of a long chain alkyl group on the nitrogen might adversely affect the luminescent properties of the product. Therefore before attempting the syntheses of derivatives with a linker and a spacer (**figure 2.1**), it was decided to investigate the synthesis of a simple model compound without a linker (**figure 2.2**), which would show up any difficulties of the types listed. The dodecyl derivative (9-(2,6-dibromophenoxycarbonyl)-10-dodecylacridinium triflate was chosen because it has only one functional group, without the distractions from other functional groups, and it has a relative molecular mass similar to the succinimidyloxycarbonylpropyl group (**figure 2.1**, n = 3), and a length of carbon chain similar to succinimidyloxycarbonyldecyl group (**figure 2.1**, n = 10). The availability of dodecyl derivative (9-(2,6-dibromophenoxycarbonyl)-10-dodecylacridinium triflate, would also allow a comparison between it and the simple methyl compound, 10-methyl AE (**section 2.2.2**). Therefore, the first compound to be synthesized would be dodecylated AE, and the experience gained was intended to be applied in the synthesis of other derivatives shown in **figure 2.1** (n = 3, 5, 10, Y = CF_3SO_3).

2.2 Results and discussion

2.2.1 The scheme for synthesis of 9-(2,6-dibromophenoxycarbonyl)-10-dodecylacridinium trifluoromethanesulfonate (1)

There are 4 steps involved in the synthesis of 9-(2,6-dibormophenoxycarbonyl)-10-dodecylacridinium trifluoromethanesulfonate (**1**): (i) substitution of dodecyl iodide with silver triflate to form dodecyl triflate (**1a**); (ii) conversion of acridine-9-carboxylic acid to the corresponding acid chloride (**1b**) with $SOCl_2$; (iii) coupling of **1b** with 2,6-dibromophenol to give 2,6-dibromophenyl acridine-9-carboxylate (**1c**); (iv) coupling of **1a** with **1c** to give **compound 1**.

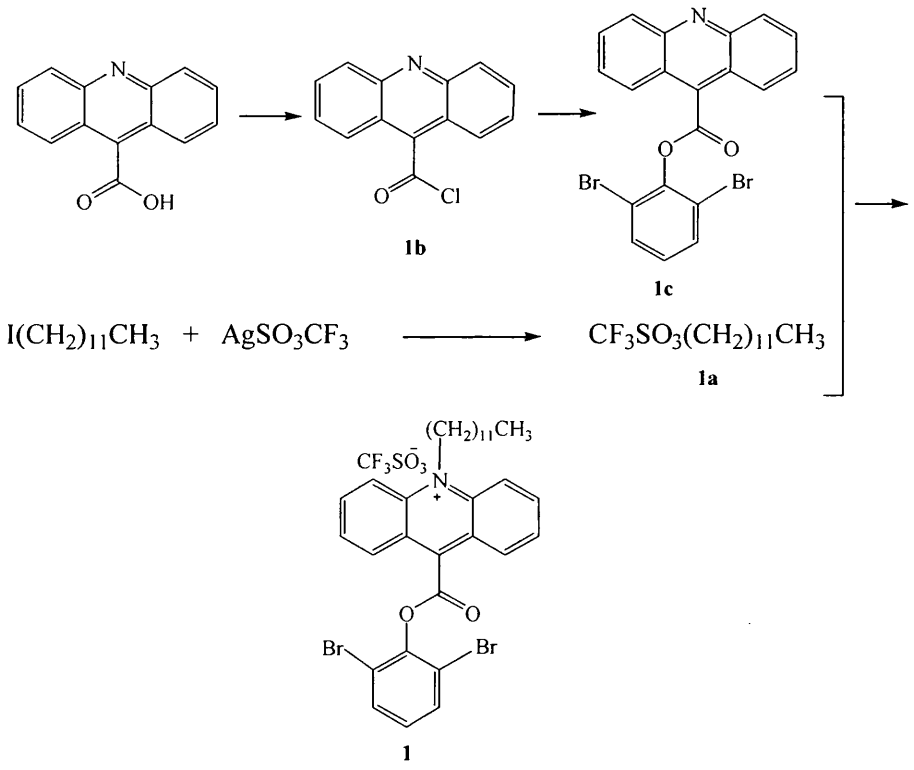


Figure 2.2, the synthetic pathway for **compound 1**

2.2.1.1 Synthesis of dodecyl triflate[1] (1a)

The proposed synthesis of dodecyl triflate is illustrated in **figure 2.3**. Silver triflate

does not dissolve in DCM or other common solvents except for benzene and toluene. According to the literature, the reaction of dodecyl iodide with silver triflate was accomplished by stirring in benzene for 23 h.[1] Therefore the reaction was carried out under similar conditions, and its progress was checked by TLC. A by-product, a yellow precipitate of AgI, was produced, and its precipitation may have helped drive the reaction to completion. The resulting mixture was extracted with DCM and then chromatographed on a silica column to remove the triflic acid produced by hydrolysis. The purified product obtained showed a triplet peak at 4.57 ppm in the ^{1}HNMR spectrum, which was attributed to the CH_2 protons next to the trifluoromethanesufonyloxy group. If the dodecyl triflate was hydrolyzed, the typical peak at 4.57 ppm disappeared, and in its place, a new triplet at 3.42 ppm appeared. By monitoring these two peaks, the percentage of decomposition could be calculated. The percentage of decomposition during the reaction and chromatography are shown in **table 2.1** (**note**: Five parallel reactions were conducted under the same conditions and then chromatographed on different solid phases and with different eluents separately). Due to the toxicity of benzene, the reaction was tried in toluene instead and a similar result and yield was obtained, so toluene could be an alternative solvent.

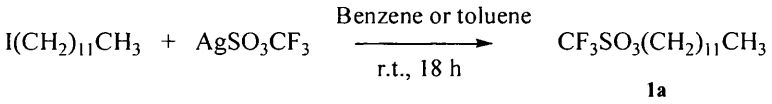


Figure 2.3, the synthesis of dodecyl triflate (1a)

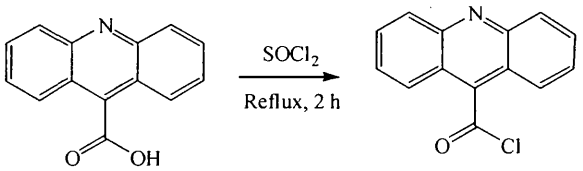
From the results in **table 2.1**, it is clear that chromatography on silica, eluted with DCM provided material of reasonable purity. Therefore this method was used to produce a sample of **compound 1a** for use in the later stages of the synthesis of **compound 1**.

Table 2.1, the percentage of decomposed triflate on the column chromatography.

Solid phase / mobile phase	Hexane	Hexane+ether (2:1)	ether	DCM
Neutral alumina	100%	60.8%	62.0%	15.6%
Silica gel				Less than 10%

2.2.1.2 Synthesis of acridine-9-carboxylic acid chloride (1b)

The acridine-9-carboxylic acid chloride (**1b**) was synthesized according to a procedure (**Figure 2.4**) in the literature.[2-4] Acridine-9-carboxylic acid hydrate was suspended in excess thionyl chloride and refluxed. About 1 h later, the mixture turned clear, after which, the reflux was maintained for a further 1 h. The mixture was stripped of excess thionyl chloride to provide a bright yellow solid. IR revealed the absence of a band at 3500 cm^{-1}. The yield should be more than 90% according to the literature, but the amount obtained was not recorded. Instead the compound **1b** was used in the next step without further purification.[2]



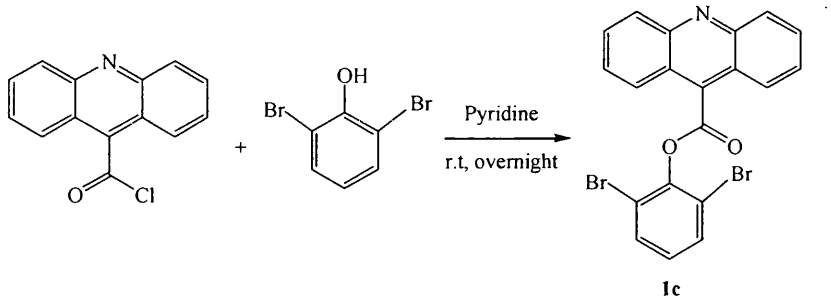
1b

Figure 2.4, the synthesis of acridine-9-carboxylic acid chloride (**1b**)

2.2.1.3 The synthesis of 2,6-dibromophenyl acridine-9-carboxylate (1c)

The 2,6-dibromophenyl acridine-9-carboxylate was synthesized by using acridine-9-carboxylic acid chloride and 2,6-dibromophenol suspended in pyridine, according to a literature procedure.[2-7] The temperature was raised to 70 °C to dissolve the starting materials. And then the mixture was stirred vigorously at room temperature for 20 h (**figure 2.5**). The resulting mixture was chromatographed on

silica, eluted with toluene (Tol) + EtOAC = 4:1. The fractions with Rf = 0.49 were combined, and the solvents were evaporated under vacuum to give a pale yellow solid which was re-dissolved in DCM and washed with aqueous NaOH (0.1mol L^{-1}) solution to remove the 2,6-dibromophenol (Rf value 0.43). The pure product was obtained in 54% yield. Its ^{1}HNMR spectrum showed 3 doublets and 3 triplets in the aromatic region, integrating in a ratio 2:2:2:2:2:1. In the ^{13}CNMR spectrum, there were 12 peaks, while the starting material (**1b**) has only 8 different carbon atoms. A peak in the IR spectrum at 1747 cm^{-1}, which was typical for such an ester, and in the mass spectrum, a molecular ion for the product at m/z 457 (^{79}Br ^{81}Br isotope peak) corroborated the product structure.



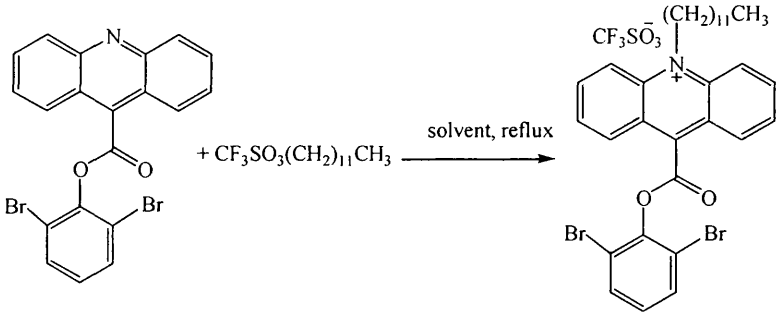
1c

Figure 2.5, the synthesis of 2,6-dibromophenyl acridine-9-carboxylate (**1c**)

2.2.1.4 Synthesis of 9-(2,6-dibromophenoxycarbonyl)-10-dodecylacridinium trifluoromethanesulfonate[8] (1)

In order to investigate the best conditions for synthesis of 9-(2,6-dibromophenoxycarbonyl)-10-dodecylacridinium trifluoromethanesulfonate (**1**), the reaction shown in **figure 2.6** was carried out separately in toluene and 1,2-dichloroethane (DCE) at different temperatures for different lengths of reaction time. Then volatiles were evaporated under vacuum and the resulting mixtures were separately extracted with chloroform-d for recording a ^{1}HNMR spectrum to calculate the crude yield in each case. The crude yield was obtained by using the ratio of peak integrals in the ^{1}HNMR spectrum at 5.58 ppm and $\delta >=$ 7.40 ppm, then multiplying by 11/2 and 100 to give the result as a percentage. The crude product was purified by

the method developed by Dr. Li for synthesis of LiAE,[4] involving removal of the solvent, dissolving the product in DCM, precipitating with diethyl ether, removing the solvent with a pipette, and then repeating the re-dissolving and re-precipitating procedure again and again until a pure product was obtained. The results are shown in **table 2.2**. Reflux in DCE for 30 h afforded the best yield. Although the crude yield calculated by the ^{1}HNMR spectrum, was as high as 31%, the pure product obtained was far less, just 19%.



1

Figure 2.6, the synthesis of 9-(2,6-dibromophenoxycarbonyl)-10-dodecylacridinium trifluoromethanesulfonate (**1**)

Table 2.2 the results of synthesis of **compound 1** in different conditions

Solvent	Reaction time	The amount of reactant 1	The amount of dodecyl triflate	Yield, / crude
Toluene	3 h	52.5mg, 0.115mmol	50mg, 0.157mmol	8.0%
DCE	10.5 h	83.0mg, 0.182mmol	100mg, 0.314mmol	12.0%
DCE	31 h	119mg, 0.260mmol	70mg, 0.220mmol	31.0%
DCE	31.5 h	74.8mg, 0.164mmol	80mg, 0.252mmol	30.6%

In the ^{1}HNMR spectrum of the product, the 3 doublets and 3 triplets in the aromatic region integrating for 11 protons were at lower field compared with those in the ^{1}HNMR spectrum of 2,6-dibromophenyl acridine-9-carboxylate (**1c**). There was a typical peak at 5.58 ppm attributed to the 2 protons of the CH_2 group next to the N atom and 25 protons in the aliphatic region in total were found. The mass spectrum showed one cation at m/z 626 ($[M\text{-}CF_3SO_3]^+$ for $^{79}Br^{81}Br$ isotope peak), another at

m/z 392 (**figure 2.7**), and an anion at m/z 149 ($[CF_3SO_3]^-$). In the ^{13}CNMR spectrum, a peak at 53.0 ppm attributed to the CH_2 group close to the acridinium ring further corroborated the structure of the product.

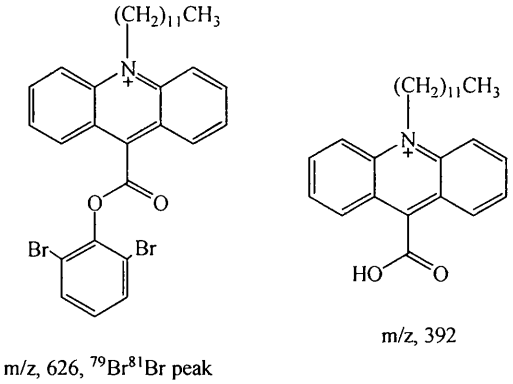


Figure 2.7, MS fragments for **compound 1**

2.2.2 Synthesis of 9-(2,6-dibromophenoxycarbonyl)-10-methylacridinium trifluoromethanesulfonate (2)

The synthesis of **compound 2** was performed by stirring 2,6-dibromophenyl acridine-9-carboxylate (**1c**) and methyl triflate in dried DCM at room temperature for 3 h, as illustrated in **figure 2.8**.[2-7] Because the product has a poor solubility in DCM, while the solubility of the starting material is good, the product could be isolated by filtration and followed by washing with diethyl ether. In the ^{1}HNMR spectrum, the 3 doublets and 3 triplets in the aromatic region integrating for 11 protons and a singlet at 6.05 ppm integrating to 3 protons attributed to the methyl group bonded on the N atom were found. In the ^{13}CNMR spectrum, a peak at 162.8 ppm for the carbonyl group and 40.9 ppm for the carbon atom of the methyl group were found. In the ^{19}FNMR spectrum, a peak at –79.3 ppm showed the presence of $CF_3SO_3^-$ group. The IR spectrum showed a strong absorption at 1760 cm^{-1}. The mass spectrum showed cations at m/z, 472 ($[M\text{-}CF_3SO_3]^+$ for $^{79}Br^{81}Br$ isotope peak) and 193 (**figure 2.9**), and an anion at m/z 149 ($[CF_3SO_3]^-$).

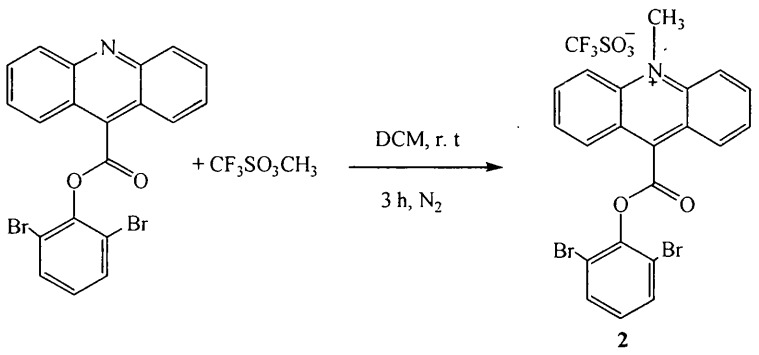


Figure 2.8, synthesis of 9-(2,6-dibromophenoxycarbonyl)-10-methylacridinium trifluoromethanesulfonate (**2**)

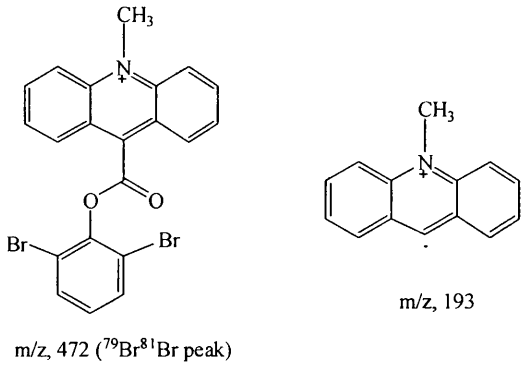


Figure 2.9, MS fragments for **compound 2**

2.2.3 The scheme for synthesis of 9-(2,6-dibromophenoxycarbonyl)-10-(3-succinimidyloxycarbonylpropyl)acridinium iodide (LiAE, 3)

Compound 3 (**LiAE**) was synthesized successfully by Li.[4] In order to improve the yield, the reaction was conducted by using 2,6-dibromophenyl acridine-9-carboxylate (**1c**) and succinimidyl 4-iodobutanoate (**compound 3a**) under different conditions. **Compound 3a** was in turn synthesized by condensing 4-iodobutyric acid and *N*-hydroxysuccinimide using the dehydrating agent dicyclohexylcarbodiimide (DCC).

2.2.3.1 Synthesis of succinimidyl 4-iodobutanoate (3a)

The succinimidyl 4-iodobutanoate (**3a**) was synthesized by using *N*-hydroxysuccinimide and 4-iodobutyric acid together with dicyclohexylcarbodiimide (DCC), as illustrated in **figure 2.10**.[2-4] The reaction mechanism was thought to be as shown in **Figure 2.11**.[3] The reaction was performed in dry THF at 0 °C for 3 h, followed by continued stirring at room temperature overnight. Some white precipitate, dicyclohexylurea (DCU), was produced. The precipitate was removed by filtration, and the filtrate was evaporated and then chromatographed on a silica column, eluted with Tol + EtOAC. The fractions with Rf = 0.48 were combined and evaporated to give a yellow solid in 84% yield.

The ^{1}HNMR spectrum showed 2 triplets, 1 quintet and 1 singlet integrating to a ratio of 2:2:2:4. In the ^{13}CNMR spectrum, two peaks at 169.4 and 168.0 ppm were found for the 3 carbons of the ester and amide carbonyl groups. The IR spectrum showed absorptions at 1739 (ester) and 1657 cm^{-1} (amide). The mass spectrum showed the molecular ion peak at m/z 311 and another ion at m/z 197.

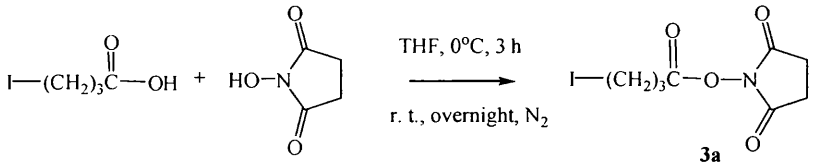


Figure 2.10, synthesis of succinimidyl 4-iodobutanoate (**3a**)

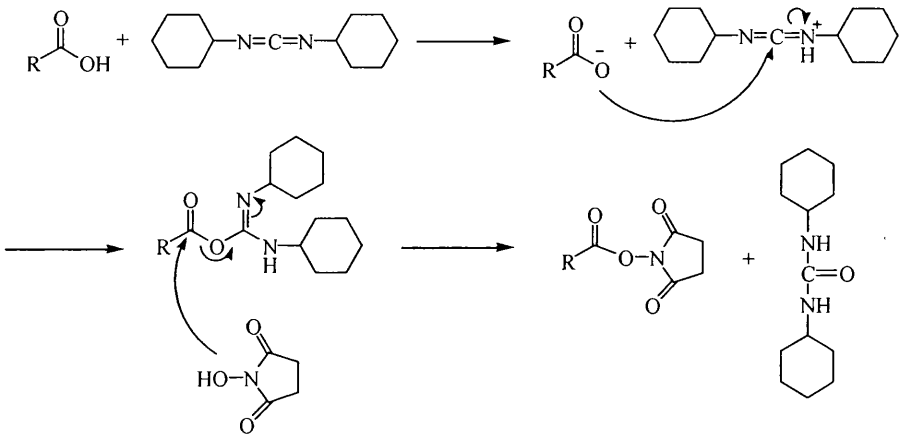


Figure 2.11, the mechanism of esterification by DCC dehydration

2.2.3.2 Synthesis of 9-(2,6-dibromophenoxycarbonyl)-10-(3-succinimidyloxycarbonylpropyl)acridinium iodide (LiAE, 3)

Different attempts at the synthesis of LiAE were performed by heating **compounds 3a** and **1c** in different solvents, at different temperatures and for different lengths of time, separately. Firstly, the starting materials were heated at 120 °C in nitrobenzene for 2 h under N_2. TLC showed a spot different from the starting materials, and a chemiluminescent test of the mixture gave out a quick and strong greenish blue flash, which proved the presence of the acridinium salt (desired product), because the 2,6-dibromophenyl acridine-9-carboxylate itself emits chemiluminescence too slowly to be seen by the naked eye. To the resulting mixture, diethyl ether and petroleum ether were added to precipitate the product. Some dark brown oil precipitated, but unfortunately, TLC showed that it was a mixture. Because of the small scale of the reaction and low yield, no pure product was obtained. Nitrobenzene maybe is a suitable solvent for the reaction, but its removal was difficult because of its high boiling point (210-212 °C), thus, limiting some ways for purification. The reaction was also tried in DMF and in diethylene glycol diethyl ether (DGDE), however, no pure product was obtained in either case, for the same reasons. Under Dr. Li's guidance, the reaction was tried with the two starting materials without solvent at 150 °C for 0.5 h under nitrogen. The pathway is illustrated in **figure 2.12**. The mixture turned dark brown after heating. The product was extracted from the resulting mixture with acetonitrile, and then precipitated with diethyl ether. The precipitate was washed with ethyl acetate again and again, until TLC showed absence of starting materials, to give a brown-red product. The whole synthesis and purification procedure is illustrated in **figure 2.13**.

The residue after separation of the LiAE was evaporated under vacuum. It could be reused for the same reaction. The synthesis of LiAE using the recycled starting materials gave a result similar to the original reaction. After this recycling, a yield (3%) in total was obtained. The ^{1}HNMR spectrum of the LiAE looked good, but the

product had a deeper dark colour than the previous sample, and its solubility in acetonitrile decreased. Possibly, other side reactions occurred under the prolonged high temperature conditions, and the side product had a bad solubility and a dark colour. It was tedious to purify LiAE by washing with solvent many times and part of the product was washed away in the process. Therefore, column chromatography was used to purify LiAE. The extract with acetonitrile was loaded onto a mini silica gel column for chromatography. A gradient mobile phase was assumed. First, Tol + EtOAc (4:1) was used to wash through the starting materials, 2,6-dibromophenyl acridine-9-carboxylate (Rf = 0.49) and succinimidyl 4-iodobutanoate (Rf = 0.48); and then EtOAc + CH_3CN (2:1) was employed to wash out a bright yellow band. The ^{1}HNMR spectrum showed that it was not the desired LiAE, but its isomer, as reported by Dr. Chen.[2] There is an equilibrium between LiAE and its isomer, shown in **figure 2.14**. Due to the loss of the positive charge, the acridine ring protons resonated at high field in the ^{1}HNMR spectrum.

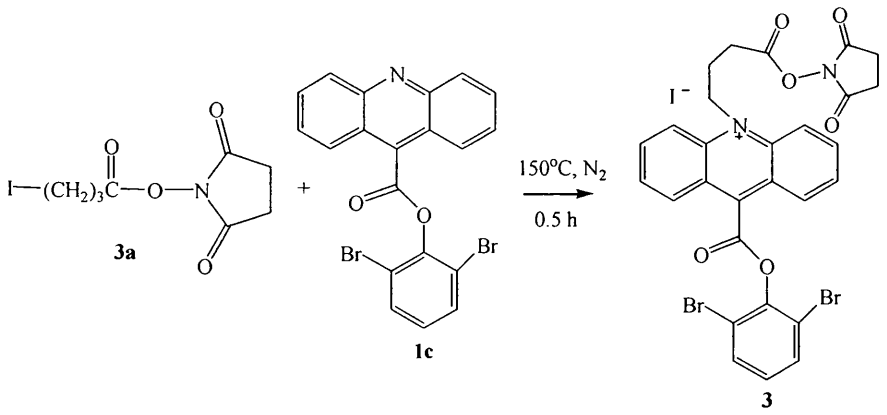


Figure 2.12, synthesis of LiAE (**3**)

In the ^{1}HNMR spectrum, 3 doublets and 3 triplets in the aromatic region and 4 peaks in the aliphatic region integrating in a ratio of 2:2:2:2:2:1:2:2:2:4 were found. A typical triplet at 5.33 ppm was attributed to the CH_2 group bonded to the N atom and a singlet at 2.69 ppm was attributed to the 4 protons of the succinimidyloxy moiety. Due to the low yield and small scale, the amount of product was not big enough for a ^{13}CNMR spectrum; and no IR spectrum or mass spectrum were

recorded either.

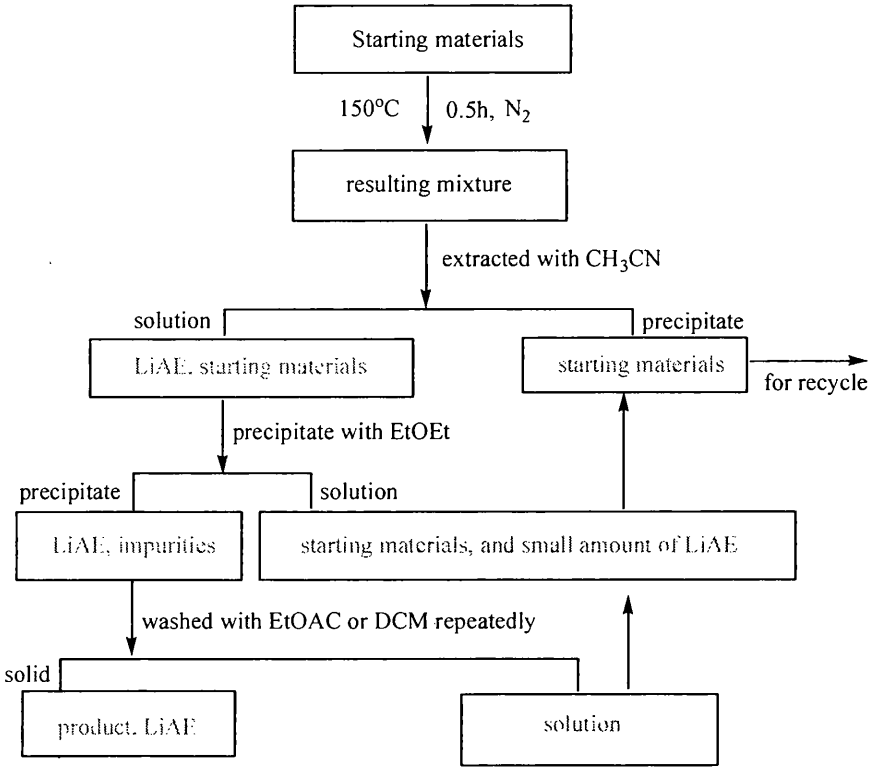


Figure 2.13, outline for synthesis and purification of LiAE (**3**)

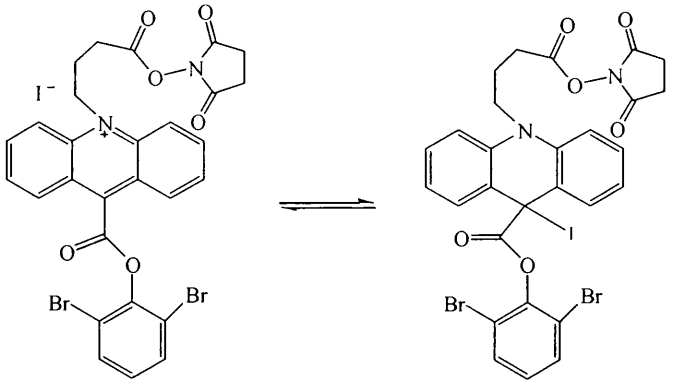


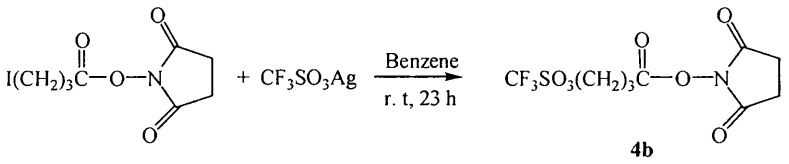
Figure 2.14, the suggested equilibrium between LiAE and its isomer

2.2.4 The scheme for synthesis of 9-(2,6-dibromophenoxycarbonyl)-10-(3-succinimidyloxycarbonylpropyl)acridinium trifluoromethanesulfonate (4)

Alkyl triflates are expected to be more reactive than the corresponding iodides for attacking the N atom of the acridine ring. Therefore, **compound 4 (figure 2.1**, n = 3, Y = CF_3SO_3) was designed. The steps involved in the synthesis of **compound 4** included substitution of succinimidyl 4-iodobutanoate (**3a**) to form the succinimidyl 4-(trifluoromethanesulfonyloxy)butanoate (**4b**). **Compound 4b** was coupled with **1c** to give the product, **compound 4**.

2.2.4.1 Synthesis of succinimidyl 4-(trifluoromethanesulfonyloxy)butanoate[1] (4b)

The succinimidyl 4-(trifluoromethanesulfonyloxy)butanoate (**4b**) was synthesized by stirring silver triflate and succinimidyl 4-iodobutanoate (**3a**) in benzene under nitrogen for 23 h, as illustrated in **figure 2.15**. Upon the contact of the starting materials at room temperature, a yellow precipitate appeared immediately. Succinimidyl 4-(trifluoromethanesulfonyloxy)butanoate was purified according to the method used for purifying dodecyl triflate (**1a**) (**section 2.2.1.1**). The resulting mixture was stripped of solvent, taken up in DCM, and chromatographed to remove the precipitate (AgI) and triflic acid produced by hydrolysis of silver triflate. The fractions with Rf = 0.25 were combined and evaporated under vacuum to leave a white solid in 47% yield. In the ^{1}HNMR spectrum, a typical triplet at 4.57 ppm was attributed to the protons next to the trifluoromethanesulfonyloxy group, while the corresponding protons next to iodide for **compound 3a** resonated at 3.22 ppm. In the ^{13}CNMR spectrum, the peak at 75.3 ppm was attributed to the carbon next to the trifluoromethanesulfonyloxy group, while the corresponding carbon of **compound 3a** gave a peak at 42.4 ppm. Although the mass spectrum did not show any desired peak in a good abundance, the successful use of the product in the next step proved it was the desired compound.



4b

Figure 2.15, the synthesis of succinimidyl 4-(trifluoromethanesulfonyloxy)butanoate (**4b**)

2.2.4.2 Synthesis of 9-(2,6-dibromophenoxycarbonyl)-10-(3-succinimidyloxycarbonylpropyl)acridinium trifluoromethanesulfonate (LiAE', 4)

LiAE' (**4**) was synthesized by using 2,6-dibromophenyl acridine-9-carboxylate and succinimidyl 4-(trifluoromethanesulfonyloxy)butanoate under different conditions, as illustrated in **figure 2.16**. The product was isolated by the same method used for isolating LiAE. Firstly the conditions for synthesis of **compound 1** (*N*-dodecyl derivative) were applied to synthesis of LiAE'; the starting materials were refluxed in 1,2-dichloroethane (DCE) for 23 h under N_2. However, the product was obtained in less than 1% yield. **Compound 4b** is not so reactive as **compound 1a** as expected, the reaction temperature should therefore be raised, and the solvent should be changed. The reaction was therefore conducted in nitrobenzene and DGDE separately. Unfortunately, due to the high boiling points and high viscosity of these solvents, the purification method used for LiAE did not work very well in these cases. When the reaction was performed in *p*-xylene, however, a good yield was obtained. Although the ^{1}HNMR spectrum showed the proton integrals were in a good ratio, the product had a black color and bad solubility in acetonitrile, possibly because the product was not pure and the impurity had a black colour and did not dissolve in acetonitrile. The solvents used for syntheses of **compounds 1** and **2** were both chloro substituted, so the reaction was carried out in 1,1,2,2-tetrachloroethane (TCE), which has a boiling point as high as 147 °C, but not so high that it can not easily be removed under vacuum. The starting materials were heated in TCE at 140 °C for 4 h under N_2. Then the product was isolated in a way similar to that used to purify LiAE.

LiAE' was obtained in 7% yield with an expected yellow color and a better solubility in acetonitrile. The results are listed in **Table 2.3**. **Compound 4** was characterized by ^{1}HNMR, IR and mass spectrometry, but not by ^{13}CNMR because of the limited amount. Because the product differs from LiAE only in the counter anion, it gave a same ^{1}HNMR spectrum as LiAE (**3**).

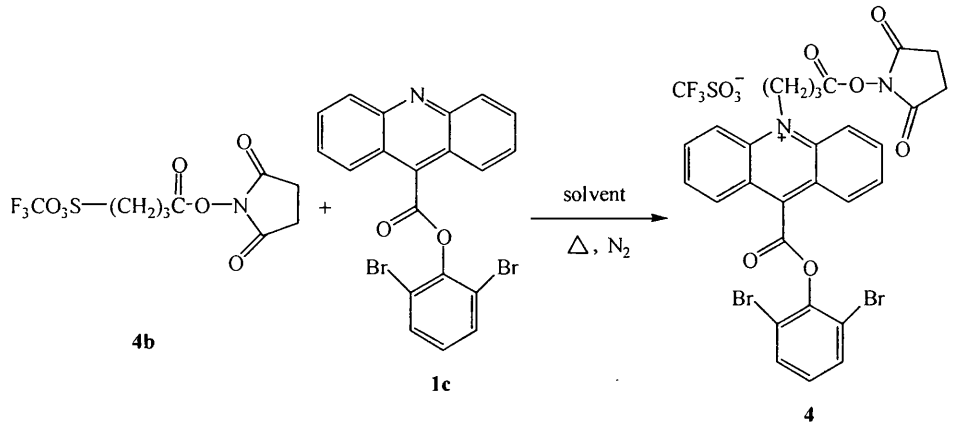


Figure 2.16, the pathway of synthesis of LiAE' (**4**)

Table 2.3, the results of synthesis of LiAE' (**4**) under different conditions.

Solvents	Reaction time & temperature	The amount of compound **1c**	The amount of compound **4b**	Yield (isolated)
p-xylene	4 h, reflux	126 mg, 0.28 mmol	140 mg, 0.42 mmol	6.0%
p-xylene	6.75 h, reflux	91 mg, 0.20 mmol	81 mg, 0.24 mmol	5.0%,
DCE	23 h, reflux	101 mg, 0.22 mmol	106 mg, 0.32 mmol	0.9%,
nitrobenzene	3.5 h, 150 °C	75 mg, 0.25 mmol	84 mg, 0.25 mmol	2.4%
DGDE	4 h, 150 °C	56 mg, 0.12 mmol	41 mg, 0.12 mmol	Nearly 0
TCE	2 h, 140 °C	62 mg, 0.14 mmol	57 mg, 0.17 mmol	7%

2.2.5 The scheme of synthesis of 9-(2,6-dibromophenoxycarbonyl)-10-(5-succinimidyloxycarbonylpentyl)acridinium trifluoromethanesulfonate (5)

The synthesis of 9-(2,6-dibromophenoxycarbonyl)-10-

(5-succinimidyloxycarbonylpentyl)acridinium trifluoromethanesulfonate (**5**) was carried out in a synthetic pathway similar to that used for **compound 1** by using **compound 1c** and succinimidyl 6-(trifluoromethanesulfonyloxy)hexanoate (**5c**). The synthetic pathway is illustrated in **figure 2.17**. However, unlike in the analogous synthetic pathway to **compound 4**, the iodo compound, 6-iodohexanoic acid (**5a**), was not commercially available, so a step for synthesis of 6-iodohexanoic acid (**5a**) was added. Condensing **compound 5a** with *N*-hydroxysuccinimide gave succinimidyl 6-iodohexanoate (**5b**). **Compound 5b** was converted into the corresponding triflate, **compound 5c**, which would couple with **1c**, affording the final product.

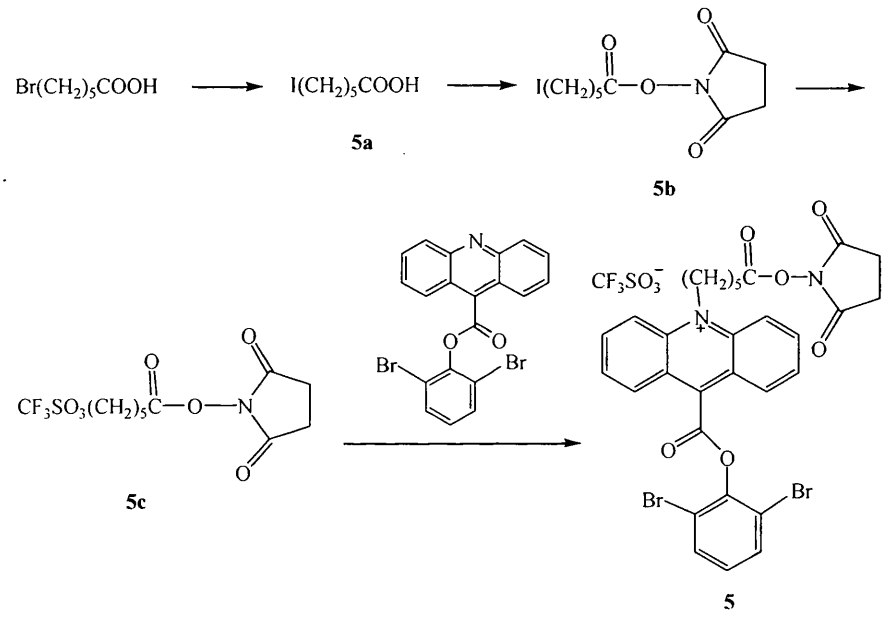


Figure 2.17, the synthetic pathway for 9-(2,6-dibromophenoxycarbonyl)-10-(5-succinimidyloxycarbonylpentyl)acridinium trifluoromethanesulfonate (**5**)

2.2.5.1 Synthesis of 6-iodohexanoic acid[9] (5a)

The synthesis of 6-iodohexanoic acid (**5a**) was achieved by stirring 6-bromohexanoic acid and sodium iodide in dry acetone under N_2 overnight, as illustrated in **figure 2.18**. The starting material NaI is soluble in acetone, but the by-product NaBr is not, so the reaction would be driven to completion. The resulting mixture (brown solution with white precipitate) was poured into water, and extracted with diethyl ether. The combined extracts were washed with saturated $Na_2S_2O_3$ to remove the yellow colour,

dried over $MgSO_4$, filtered and evaporated. The crude product was chromatographed, eluted with ether + hexane + formic acid (1:1:0.01) according to a method in the literature.[8] The fractions with Rf = 0.38 were pooled and evaporated to give snow-white flakes, the product could be purified further by recrystallization in hexane: ether (9:1). When the reaction was not protected by N_2, the saturated $Na_2S_2O_3$ couldn't remove the yellow colour completely. However, although the product obtained had a yellow colour, the ^{1}HNMR spectrum did not give any peaks of the yellow impurities. In the ^{1}HNMR spectrum, the typical triplet at 3.12 ppm attributed to the protons next to the iodide was found, while for the corresponding protons of the starting material, 6-bromohexanoic acid, the chemical shift was 3.45 ppm. No peak for the carbonyl group was found in the ^{13}CNMR spectrum because the signal was too weak, but in the IR spectrum, a strong absorption at 1703 cm^{-1}, attributed to the carbonyl group, was found. In the negative ion mass spectrum (ES^-), an anion at m/z 483 for the dimer of the product having lost 1 proton, and another at m/z 241 for the molecular anion having lost 1 proton were found.

$$Br\,(CH_2)_5COOH + NaI \xrightarrow[\text{overnight, r.t.}]{\text{Acetone, } N_2} \underset{\textbf{5a}}{I\,(CH_2)_5COOH} + NaBr$$

Figure 2.18, the synthesis of 6-iodohexanoic acid (**5a**)

2.2.5.2 Synthesis of succinimidyl 6-iodohexanoate[2-7] (5b)

The synthesis of succinimidyl 6-iodohexanoate was performed by using DCC as dehydration initiator, as shown in **figure 2.19**; the mechanism is shown in **figure 2.11** in **section 2.2.3.1**. The procedure was carried out in a way similar to that used for synthesis of **compound 3a**. **Compound 3a** (**section 2.2.3.1**) could be purified by chromatography, but this was not the case for succinimidyl 6-iodohexanoate (**5b**). The ^{1}HNMR spectrum of the product after chromatography showed that there were extra peaks in the high field region, attributed to DCU. The product was therefore further purified by recrystallisation. In its ^{1}HNMR spectrum, there were 2 triplets, 1 singlet and 3 multiplets integrating in a ratio 2:4:2:2:2:2. A triplet at 3.13 ppm was

attributed to the CH_2 group next to the iodide, another one at 2.57 ppm was attributed to the CH_2 group next to the ester bond; a singlet at 2.78 ppm was attributed to the succinimidyloxy moiety, where there was not such a peak in the ^{1}HNMR spectrum of **compound 5a**. The ^{13}CNMR spectrum showed peaks at 169.5 and 168.8 ppm attributed to the 3 carbons of the carbonyl groups, 26.0 ppm for the carbons of the 2 CH_2 groups of the succinimidyloxy moiety and another 5 peaks for the other 5 carbons of the 5 different CH_2 groups. The IR spectrum showed an absorption at 1738 cm^{-1}. The CI mass spectrum showed a desired peak at m/z 357 for the cation resulting from the whole molecule plus ammonium.

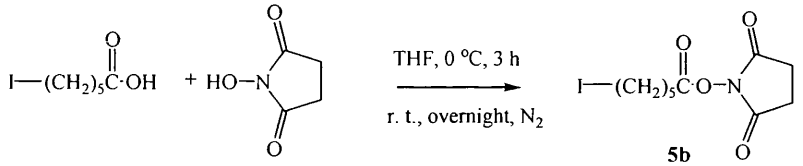


Figure 2.19, the synthesis of succinimidyl 6-iodohexanoate (**5b**)

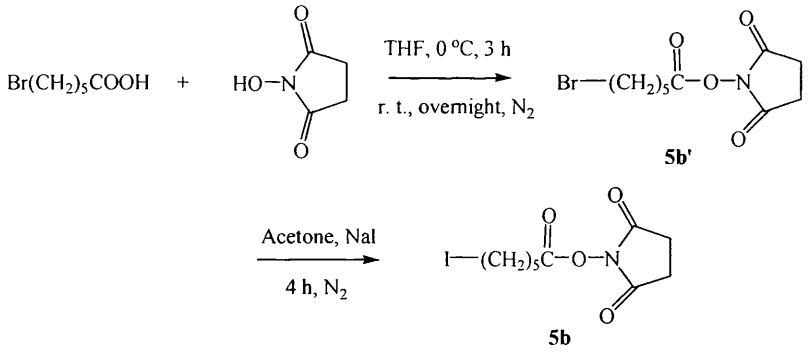


Figure 2.20, an alternative synthetic pathway for succinimidyl 6-iodohexanoate (**5b**).

Compound 5b could be synthesized *via* an alternative route, as shown in **figure 2.20**. Succinimidyl 6-bromohexanoate (**5b'**) was synthesized by condensing 6-bromohexanoic acid and *N*-hydroxysuccinimide, and then the bromo atom was substituted with iodide, which was accomplished by stirring in acetone with NaI. Reasonable proton positions and perfect proton integrals in the ^{1}HNMR spectrum

verified the structures of succinimidyl 6-bromohexanoate and succinimidyl 6-iodohexanoate.

2.2.5.3 Synthesis of succinimidyl 6-(trifluoromethanesulfonyloxy)hexanoate[1] (5c)

The synthesis of succinimidyl 6-(trifluoromethanesulfonyloxy)hexanoate (**5c**) was firstly tried by using succinimidyl 6-bromohexanoate (**5b'**) and silver triflate, as illustrated in **figure 2.21**, because if the Br atom of **5b'** could be substituted with the CF_3SO_3 group, 1 step (**section 2.2.5.1**) for synthesis of **5c** would be saved. The reaction was carried out under similar conditions as for synthesis of **compound 4b**. The resulting mixture was passed through a silica column to remove the triflic acid. The ^{1}HNMR spectrum showed that the desired triplet at 4.57 ppm attributed to the protons next to the CF_3SO_3 group was very small, but showed a strong triplet at 3.45 ppm which was attributed to the protons next to the bromo atom of the starting material. Based on the proton integrals at 4.57 and 3.45 ppm in the ^{1}HNMR spectrum, the crude yield was calculated. Only 1% of the starting material was converted to the desired product. The bromo substituted compound is not so reactive for this type of reaction as the corresponding iodo compound, because the iodide is a better leaving group than bromide during the substitution.

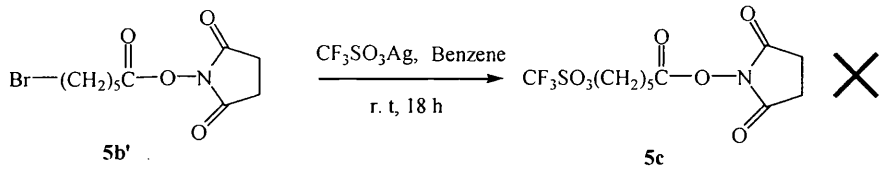


Figure 2.21, an attempt to convert **compound 5b'** to **5c**

Succinimidyl 6-iodohexanoate (**5b**) was used for the synthesis of **5c**. **Compound 5b** and silver triflate were stirred in benzene under nitrogen for 18 h,[2] under similar conditions to those for synthesis of **1a** and **4b** (**section 2.2.4.2**), as illustrated in **figure 2.22**. The resulting mixture was chromatographed (silica, eluted with DCM).

The fractions with Rf = 0.28 were combined and evaporated under vacuum to give a white gel. The presence of a small amount of impurities with Rf = 0.38 changed the colour of the product to brown, and made the gel a liquid. So when the product became liquid on standing, or became brown in colour, it was passed through a silica column again; otherwise, the impurity would affect the synthesis at the next stage. The freshly prepared triflate (**5c**) was more reactive than after standing for 1 or 2 days. In the ^{1}HNMR spectrum, the triplet for the protons next to the CF_3SO_3 group was found at 4.45 ppm, and the integrations for all protons were in good ratio, 2:4:2:2:2:2. In the ^{13}CNMR spectrum, 2 peaks at 169.5 ppm and 168.6 ppm were attributed to the 3 carbons of the 2 different carbonyl groups and a peak at 77.4 ppm for the carbon atom next to the CF_3SO_3 group was found. The IR spectrum showed the strong absorption of the carbonyl group at 1736 cm^{-1}. However the mass spectrum did not show the expected molecular ion. Nevertheless, the successful use of the compound in the next step proved it was the desired compound.

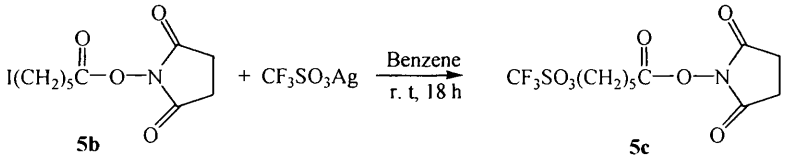


Figure 2.22, synthesis of succinimidyl 6-(trifluoromethanesulfonyloxy)hexanoate (**5c**)

2.2.5.4 Synthesis of 9-(2,6-dibromophenoxycarbonyl)-10-(5-succinimidyl-oxycarbonylpentyl)acridinium trifluoromethanesulfonate[8] (5)

Compound 5 was synthesized under conditions similar to those used for **compound 1 (section 2.2.1.4)**, by refluxing 2,6-dibromophenyl acridine-9-carboxylate (**1c**) and succinimidyl 6-(trifluoromethanesulfonyloxy)hexanoate (**5c**) in redistilled DCE for 21.5 h, as illustrated in **figure 2.23**. The product was then separated by the same method used for purifying LiAE.

In the ^{1}HNMR spectrum, 3 doublets and 3 triplets were found in the aromatic region and 5 peaks in the aliphatic region, integrating in a ratio of 2:2:2:2:2:1:2:4:2:2:4. A

triplet at 5.70 ppm was attributed to the protons of the CH_2 group linked to the N atom, while a singlet at 2.88 ppm was attributed to the succinimidyloxy moiety. The peaks for the acridinium ring appeared from 9.07 ppm to 8.13 ppm, while the chemical shifts for the acridine ring of **compound 1c** were at a relatively high field, from 8.73 to 7.61 ppm. In the ^{13}CNMR spectrum, 3 peaks at 169.8, 168.9 and 161.5 ppm were attributed to the 4 carbons of the 3 different carbonyl groups; there were peaks in the aromatic region, and a typical peak at 52.6 ppm for the carbon of the CH_2 group linked to the N atom, and a peak at 26.0 ppm for the succinimidyloxy moiety. The IR spectrum showed a strong absorption at 1734 cm^{-1}, which was lower than typical signals for comparable ester carbonyl groups (for **compound 1c**, the IR signal of the ester carbonyl group was at 1754 cm^{-1}); and higher than typical signals for comparable amide carbonyl groups. It seemed that the IR signals of the 3 carbonyl groups partly overlapped and showed only one strong absorption. The mass spectrum showed cations at m/z 669 ($[M\text{-}CF_3SO_3]^+$ for the $^{79}Br^{81}Br$ isotope peak) (**figure 2.24**), and an anion at m/z 149 ($[CF_3SO_3]^-$), corroborating the structure of the product.

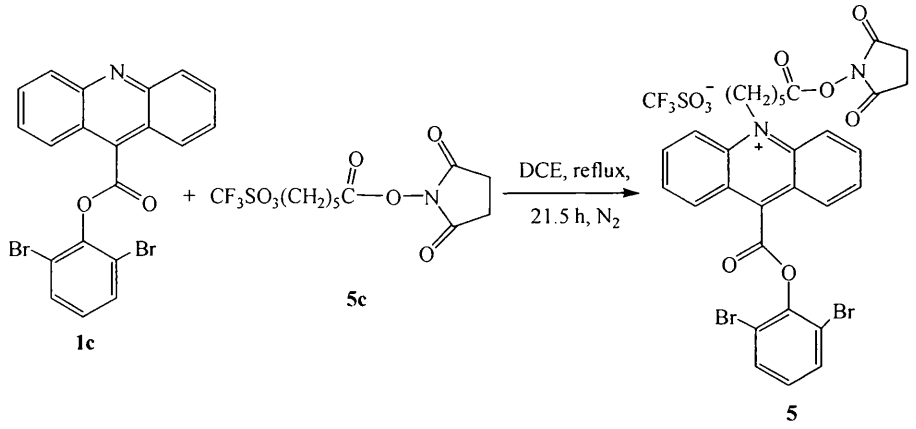
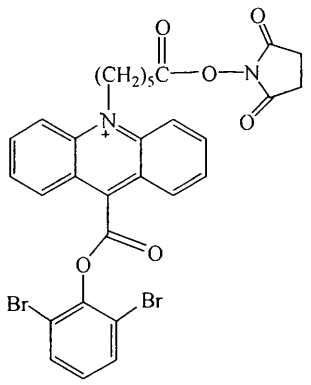


Figure 2.23 the synthesis of 9-(2,6-dibromophenoxycarbonyl)-10-(5-succinimidyloxycarbonylpentyl)acridinium trifluoromethanesulfonate (**5**).



m/z, 669 ($^{79}Br^{81}Br$ peak)

Figure 2.24, MS fragment for **compound 5**.

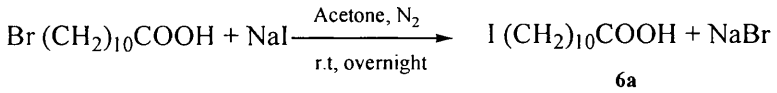
2.2.6 The scheme for synthesis of 9-(2,6-dibromophenoxycarbonyl)-10-(10-succinimidyloxycarbonyldecyl)acridinium trifluoromethanesulfonate (6)

The synthesis of **compound 6** was carried out in a synthetic pathway similar to that used for **compound 5** (**section 2.2.5**). 11-Bromoundecanoic acid was converted to 11-iodoundecanoic acid (**6a**), which was esterified with *N*-hydroxysuccinimide to form succinimidyl 11-iodoundecanoate (**6b**). The iodide group of **compound 6b** was then substituted with a trifluoromethanesulfonyloxy group to provide the corresponding triflate, **compound 4c**, which was coupled with **compound 1c** to give the final product (**6**).

2.2.6.1 Synthesis of 11-iodoundecanoic acid (6a)

The synthesis of 11-iodoundecanoic acid was carried out by stirring 11-bromoundecanoic acid and sodium iodide in acetone under N_2 overnight, as illustrated in **figure 2.25**, under similar conditions to those used to synthesize 6-iodohexanoic acid (**5a**).[9] The resulting mixture was poured into water and extracted with diethyl ether, the combined extracts were washed with saturated $Na_2S_2O_3$ to remove the yellow colour, dried over $MgSO_4$, filtered and evaporated. The crude product was recrystallized in hexane + ether = 9:1. In the ^{1}HNMR spectrum, a typical

triplet at 3.12 ppm attributed to the CH_2 protons next to the iodide was found, while for the corresponding protons of the starting material, its chemical shift was 3.45 ppm. Unlike **compound 5a**, the signal for the carbon of the carbonyl group at 179.9 ppm was strong enough to be seen in the ^{13}CNMR spectrum. The IR spectrum showed a strong absorption at 1693 cm^{-1}. The CI mass spectrum showed the desired pseudo molecular ions at m/z 313 ($[M+H]^+$) and 330 ($[M+NH_4]^+$).

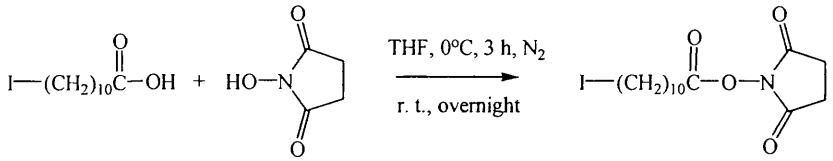


6a

Figure 2.25, the synthesis of 11-iodoundecanoic acid (**6a**)

2.2.6.2 Synthesis of succinimidyl 11-iodoundecanoate (6b)

Succinimidyl 11-iodoundecanoate (**6b**) was synthesized by stirring 11-iodoundecanoic acid and *N*-hydroxysuccinimide in dry THF at 0 °C for 3 hours, then at room temperature overnight, as illustrated in **figure 2.26**.[2-7] The resulting mixture was filtered to remove DCU, and then followed by chromatography and recrystallization in a similar way as used for **compound 5b** in **section 2.2.5.2**. White flakes were obtained in 65% yield. In the ^{1}HNMR spectrum, a singlet at 2.81 ppm for the succinimidyloxy group and another 6 peaks integrating in the ratio 4:20 were found. In the ^{13}CNMR spectrum, peaks at 169.5 and 169.0 ppm were attributed to the 3 carbons of 2 different carbonyl groups. The IR spectrum gave a signal at 1725 cm^{-1}. The mass spectrum (CI) showed a peak at m/z 427 ($[M+NH_4]^+$).



6b

Figure 2.26, synthesis of succinimidyl 11-iodoundecanoate (**6b**)

2.2.6.3 Synthesis of succinimidyl 11-(trifluoromethanesulfonyloxy)undecanoate[1] (6c)

Succinimidyl 11-(trifluoromethanesulfonyloxy)undecanoate (**6c**) was synthesized by stirring silver triflate and succinimidyl 11-iodoundecanoate (**6b**) in benzene under nitrogen for 18 h, as illustrated in **figure 2.27**.[1] the product was purified in a way similar to those used for **compound 4b** in **section 2.2.4.2** and **compound 5c (section 2.2.5.3)**. In the ^{1}HNMR spectrum, a peak at 4.44 ppm for the protons next to the triflate group was found, and there were 7 peaks in a proton-integrating ratio of 2:4:2:2:2:2:10. In the ^{13}CNMR spectrum, peaks at 169.6 ppm and 169.0 ppm were attributed to the 3 carbons of the two different carbonyl groups. A signal at 78.1 ppm for the carbon atom next to the CF_3SO_3 group was found, while for the corresponding iodide compound (**6b**), the signal for the corresponding carbon was found at 7.6 ppm. The appearance of only one peak at –74.8 ppm in the ^{19}FNMR spectrum corroborated the structure of the product. The IR spectrum showed the absorption of carbonyl groups at 1736 cm^{-1}, but the mass spectrum did not show the desired molecular ion peak as with other triflates. The instability of the triflate group was possibly responsible for this. The successful use of the product in the next step proved it was the desired compound.

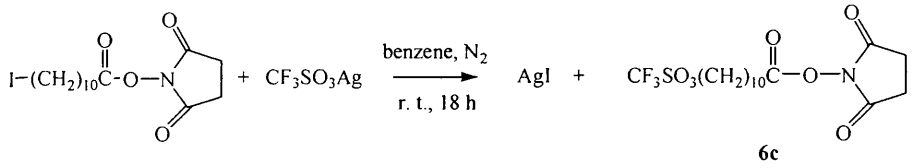


Figure 2.27, the synthesis of succinimidyl 11-(trifluoromethanesulfonyloxy)undecanoate (**6c**)

2.2.6.4 Synthesis of 9-(2,6-dibromophenoxycarbonyl)-10-(10-succinimidyloxycarbonyldecyl)acridinium trifluoromethanesulfonate[8] (6)

Compound 6 was synthesized by a pathway similar to that used for **compound 5 (section 2.2.5.4)**, by refluxing 2,6-dibromophenyl acridine-9-carboxylate (**1c**) and succinimidyl 10-(trifluoromethanesulfonyloxy)undecanoate (**6c**) in DCE for 21 h under N_2. The reaction was also carried out separately in TCE at 140 °C for 4 h for comparison reasons. The synthesis is illustrated in **figure 2.28**. The product (**6**) was

separated according to the method developed by Li, washing the crude product with DCM and ether. The reaction conducted in TCE at higher temperature gave a slightly higher yield. However, the product showed a brown colour, which deepened on standing.

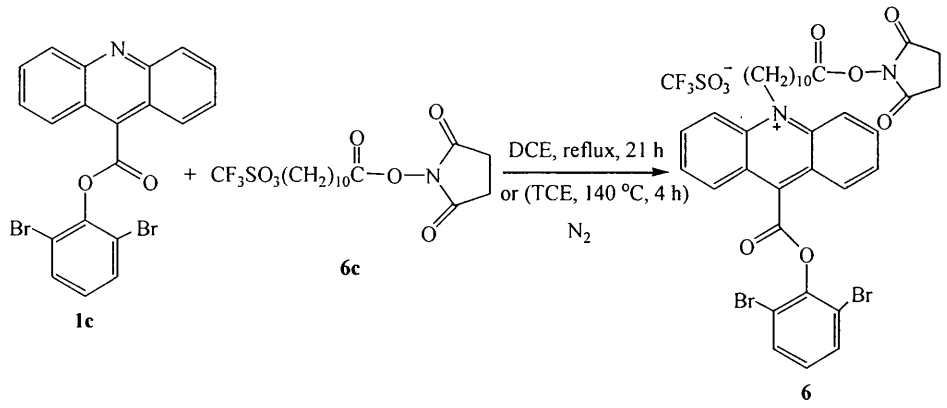
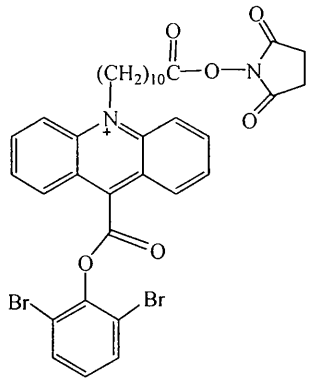


Figure 2.28, the synthesis of 9-(2,6-dibromophenoxycarbonyl)-10-(10-succinimidyloxycarbonyldecyl)acridinium trifluoromethanesulfonate (**6**)

In the ^{1}HNMR spectrum, 3 doublets and 3 triplets in the aromatic region were found and 7 peaks in the aliphatic region integrating in a ratio of 2:2:2:2:2:1:2:4:2:2:2:2:10. A triplet at 5.47 ppm was attributed to the protons of the CH_2 group linked to the N atom, while a singlet at 2.65 ppm was attributed to the succinimidyloxy moiety. The peaks for the acridinium ring appeared from 8.84 ppm to 7.58 ppm, while the chemical shifts for the acridine ring of **compound 1c** were at a relatively high field, from 8.73 to 7.61 ppm. In the ^{13}CNMR spectrum, 3 peaks at 169.7, 169.1 and 161.5 ppm were attributed to the 4 carbons of the 3 different carbonyl groups; there were 11 peaks in total in the aromatic region, and a typical peak at 52.9 ppm for the carbon of the CH_2 group linked to the N atom, and a peak at 26.0 ppm for the succinimidyloxy moiety. The IR spectrum showed a strong absorption at 1728 cm^{-1}, which was lower than signals from the ester carbonyl groups and higher than signals from the amide carbonyl groups. It seemed that the IR signals of the 3 carbonyl groups partly overlapped and showed only one strong absorption. The mass spectrum showed cations at m/z 793 and 739 ($[M\text{-}CF_3SO_3]^+$) (**figure 2.29**), and an anion at m/z 149 ($[CF_3SO_3]^-$), corroborating the structure of the product. ^{19}FNMR gave only

one peak at –78.2 ppm.



m/z, 739 ($^{79}Br^{81}Br$ peak)

Figure 2.29, MS fragment for **compound 6**

2.3 Experimental section

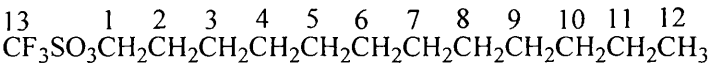
Melting points (mp) were measured on a Griffin capillary melting point apparatus and are not corrected. ^{1}HNMR and ^{13}CNMR (400MHz) spectra were obtained on a Bruker AV 400 spectrometer. Chemical shifts are reported in δ units downfield from internal tetramethylsilane (Me_4Si). Abbreviations used are: s = singlet; d = doublet; dd = double doublet; ddd = double double doublet; dt = double triplet; t = triplet; m = multiplet. Coupling constants (J) are expressed in Hz. IR spectra were recorded on a Perkin Elmer spectrometer 1 with Universal ATR Sampling accessory. Only strong absorptions (*e.g.* C=O) are reported. Low resolution electron ionisation (EI), chemical ionisation (CI) and electrospray (ESI) MS spectra were determined on a Micromass Quattro II low resolution triple quadrupole mass spectrometer. The data are presented as m/z ratios for the molecular ion and other most abundant ions with their percentage relative intensity given in brackets. The dibromo compounds give multiple isotop peaks, but only the $^{79}Br^{81}Br$ are reported.

TLC was performed on Whatman aluminium silica gel plates and visualised by UV 254 nm; and columns for chromatography were packed with Fisher Chemicals

Matrex * Silica 60 (35-70) micron).

2.3.1.1 Synthesis of dodecyl triflate (1a)

To a dry round bottom flask (5 ml) equipped with a magnetic stirrer bar, silver triflate (278 mg, 2.08 mmol) and 12-iodododecane (300 mg, 1.01 mmol) were added. Then the flask was flushed with N_2 for 5 minutes. Afterwards, dry benzene (1 ml) was introduced with a syringe. The mixture was stirred for 18 h at room temperature. The resulting mixture was stripped of volatiles over a rotary evaporator, and then extracted with DCM (0.6 ml) for loading on a silica column for chromatography, and eluted with DCM. The fractions with Rf = 0.75 were pooled and evaporated under vacuum to give a colourless oil (219 mg, yield, 68%).



^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 4.57 (2H, H_1, t, J = 7 Hz), 1.85 (2H, H_2, quintet, J = 7 Hz), 1.45 (2H, H_{11}, quintet, J = 7 Hz), 1.39-1.21 (16H, H_3, H_4, H_5, H_6, H_7, H_8, H_9, H_{10}, m), 0.91 (3H, H_{12}, t, J = 7 Hz).

^{13}CNMR (400 Hz, chloroform-d, δ, ppm), 119.0 (C_{13}, splitting into 4 peaks 123.8, 120.6, 117.5, 114. 3), 78.1 (C_1), 33.0, 30.1-29.2, 26.6 (C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}), 25.4 (C_{11}), 14.5 (C_{12}).

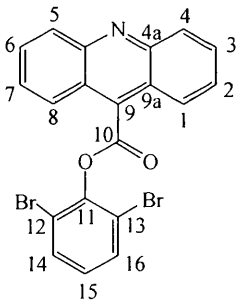
2.3.1.2 Synthesis of acridine-9-carboxylic acid chloride (1b)

In a round bottom flask (50 ml) equipped with a condenser, a stirrer bar and a $CaCl_2$ drying tube, acridine-9-carboxylic acid (1.88 g, 8.42 mmol) was refluxed in thionyl chloride (10 ml). About 1 h later, the suspension turned clear. The heating was maintained for a further hour, and the excess of thionyl chloride was evaporated under reduced pressure. The yellow solid obtained was pumped under vacuum

overnight to provide the material for the next step without further purification.

2.3.1.3 Synthesis of 2,6-dibromophenyl acridine-9-carboxylate (1c)

In a round bottom flask (50 ml) equipped with a $CaCl_2$ drying tube and a magnetic stirrer bar, acridine-9-carboxylic acid chloride (2.036g, 8.42 mmol) in pyridine was heated to 70 °C. After the solid had dissolved completely, the solution was cooled to room temperature, 2,6-dibromophenol (2.325 g, 9.23 mmol) was added and the mixture was stirred vigorously overnight. The resulting mixture was stripped of pyridine to leave a brown solid. DCM (10 ml) was used to extract the product and the extract was subjected to column chromatography, eluted with Tol + EtOAc (4:1). The fractions with Rf 0.49 were collected and evaporated to give a pale yellow solid. The solid was re-dissolved in DCM (40 ml), washed with dilute NaOH aqueous solution (0.1 mol L^{-1}, 5 × 40 ml), dried over $MgSO_4$, filtered and evaporated to give a pale yellow powder (2.080 g, 54% yield), m.p. 159-160 °C.



^{1}HNMR (400 MHz, chloroform-d_3, δ, ppm), 8.73 (2H, H_1, H_8, d, J = 8 Hz), 8.27 (2H, H_4, H_5, d, J = 8 Hz), 7.79 (2H, H_3, H_6, t, J = 8 Hz), 7.66 (2H, H_{14}, H_{16}, d, J = 8 Hz), 7.61 (2H, H_2, H_7, t, J = 8 Hz), 7.09 (1H, H_{15}, t, J = 8 Hz).

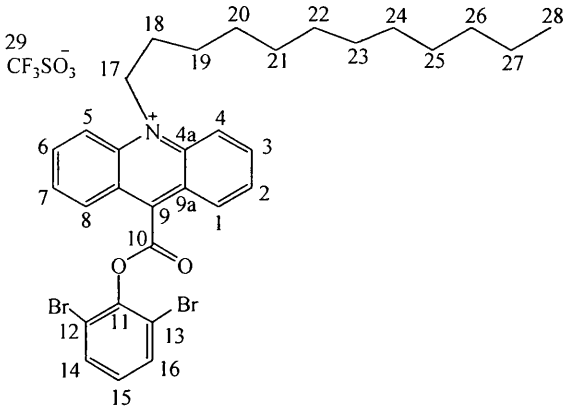
^{13}CNMR (400 MHz, chloroform-d, δ, ppm), 163.0 (C_{10}), 147.7 (C_{11}), 145.5 (C_{4a}/$C_{4a'}$), 132.8 (C_9), 132.0 (C_{14}/C_{16}), 129.3 (C_4/C_5), 129.1 (C_3/C_6), 127.9 (C_{15}), 126.5 (C_2/C_7), 124.9 (C_1/ C_8), 122.0 (C_{9a}/$C_{9a'}$), 116.8 (C_{12}/C_{13}).

IR (υ, cm^{-1}), 1754 (C=O)

MS (m/z), EI: 457 ([M, $^{79}Br^{81}Br$ isotope peak]$^+$, 2%), 206 ([M-Br_2PhO]$^+$, 100%), 178 (72%); CI: 458 ([M+H, $^{79}Br^{81}Br$ isotope peak]$^+$, 21%).

2.3.1.4 Synthesis of 9-(2,6-dibromophenoxycarbonyl)-10-dodecylacridinium trifluoromethanesulfonate (1)

To a round bottom flask (5 ml) equipped with a stirrer bar and a condenser (25 ml), 2,6-dibromophenyl acridine-9-carboxylate (74.8 mg, 0.16 mmol) was added. The flask was flushed with N_2 for 5 min. Then dodecyl triflate (70 mg, 0.22 mmol) and DCE were introduced by syringes respectively. The mixture was refluxed for 31 h. The solvent was evaporated under vacuum. The product was extracted with DCM (0.6 ml). The extract was precipitated with diethyl ether (3 ml), and the upper solution was removed with a pipette carefully. The yellow precipitate was re-dissolved in DCM (0.6 ml), and re-precipitated with diethyl ether repeatedly (x 6) to give a greenish yellow solid (11.5 mg, 19.0% yield), m.p. 92-96 °C.



^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 8.79 (2H, H_4, H_5, d, J = 8 Hz), 8.62 (2H, H_1, H_8, d, J = 9 Hz), 8.34 (2H, H_3, H_6, dd, J = 8, 7 Hz), 7.83 (2H, H_2, H_7, dd, J = 9, 7 Hz), 7.53 (2H, d, H_{14}, H_{16}, J = 8 Hz), 6.99 (1H, H_{15}, t, J = 8 Hz), 5.43 (2H, H_{17}, t, J = 8

Hz), 1.98 (2H, H_{27}, m), 1.62-1.48, 1.26-1.16, 1.15-0.95, (4H, 2H, 12H, m , m, m, H_{18}, H_{19}, H_{20}, H_{21}, H_{22}, H_{23}, H_{24}, H_{25}, H_{26}), 0.64 (3H, H_{28}, t, J = 7 Hz).

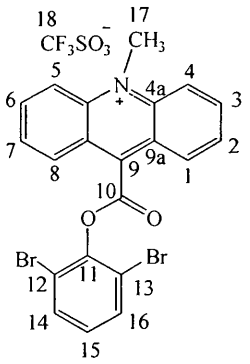
^{13}CNMR (400 Hz, chloroform-d, δ, ppm), 161.5 (C_{10}), 147.1 (C_{11}), 145.9 (C_9), 141.7 (C_{4a}), 140.7 (C_{14}/C_{16}), 130.3 (C_{15}), 133.8, 129.8, 129.3, 119.9 (C_1/C_8, C_2/C_7, C_3/C_6, C_4/C_5), 123.9 (C_{9a}), 117.7 (C_{12}/C_{13}), 53.0 (C_{17}), 32.3 (C_{26}), 30.2, 30.1, 30.0 (2 lines), 29.9, 29.8, 29.7 (C_{19}, C_{20}, C_{21}, C_{22}, C_{23}, C_{24}, C_{25}), 27.1 (C_{18}), 23.1 (C_{27}), 14.5 (C_{28}). C_{29} has not been found.

IR (υ, cm^{-1}), 1764 (C=O).

MS (m/z), ES^+: 626 ([M-CF_3SO_3, $^{79}Br^{81}Br$ isotope peak]$^+$, 84%), 414 (25%), 406 (47%), 392 (100%); ES^-: 149 ([$CF_3SO_3^-$], 100%). Acc-MS: calculated mass: 624.1107 ([M-CF_3SO_3, $^{79}Br^{79}Br$ isotope peak]$^+$); measured mass: 624.1108.

2.3.2 Synthesis of 9-(2,6-dibromophenoxycarbonyl)-10-methylacridinium trifluoromethanesulfonate (2)

To a round bottom flask (5 ml), equipped with a stirrer bar, 2,6-dibromophenyl acridine-9-carboxylate (89 mg, 0.19 mmol) was added. The flask was flushed with nitrogen *via* a septum for 5min. Afterwards dry dichloromethane (2 ml) and methyl triflate (200 ul, 1.75 mmol) were introduced with syringes respectively. The mixture was stirred at room temperature for 3 h. The resulting mixture was filtered, the precipitate was washed with DCM (1 ml), followed by EtOAC (1 ml) and EtOEt (1 ml), and a yellow solid (83 mg, 68% yield,) was collected, m.p. 249- 250 °C.



^{1}HNMR (400 Hz, acetonitrile-d_3, δ, ppm), 8.87 (2H, H_4, H_5, d, J = 9 Hz,), 8.53 (2H, H_1, H_8, d, J = 9 Hz), 8.34 (2H, H_3, H_6, dd, J = 9, 7 HZ), 7.98 (2H, H_2, H_7, dd, J = 9, 7 Hz), 7.68 (2H, H_{14}, H_{16}, d, J = 8 Hz), 7.16 (1H, H_{15}, t, J = 8 Hz), 4.72 (3H, H_{17}, s).

^{13}CNMR (400 Hz, acetonitrile-d_3, δ, ppm), 162.8 (C_{10}), 147.4 (C_{11}), 146.3 (C_9), 143.3 (C_{4a}), 140.6 (C_{14}/C_{16}), 131.5(C_{15}), 134.6, 130.6, 129.3, 120.3 (C_1/C_8, C_2/C_7, C_3/C_6, C_4/C_5), 124.5 (C_{9a}), 118.0 (C_{12}/C_{13}), 40.9 (C_{17}), C_{18} has not been seen.

^{19}FNMR (400 Hz, acetonitrile-d_3, δ, ppm), -79.3.

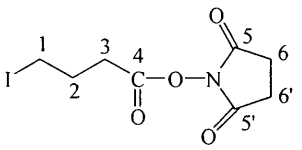
IR (υ, cm^{-1}), 1760 (C=O).

MS (m/z), ES^+: 526 (11%), 472 ([M-CF_3SO_3, $^{79}Br^{81}Br$ isotope peak]$^+$, 31%), 252 (49%), 193 (100%); ES^-: 149 ([CF_3SO_3] $^-$, 100%). Acc-MS: calculated mass: 469.9386 ([M-CF_3SO_3, $^{79}Br^{79}Br$ isotope peak]$^+$); measured mass: 469.9385.

2.3.3.1 Synthesis of succinimidyl 4-iodobutanoate (3a)

In a flask (25 ml), equipped with a stirrer bar, 4-iodobutyric acid (480 mg, 2.24 mmol) in dry THF (10 ml), was cooled to 0 °C in an ice bath under N_2. *N*-Hydroxysuccinimide (247 mg, 2.14 mmol) in THF (2 ml) and DCC (523 mg, 2.53 mmol) were then added respectively. The mixture was stirred at 0 °C for 3 h, and then at room temperature overnight. The resulting mixture was filtered, the filtrate was

condensed on a rotary evaporator and loaded onto a silica column for chromatography, eluted with Tol + EtOAC (4: 1). The fractions with Rf = 0.48 were combined and evaporated to give a yellow solid (567 mg, 84% yield), m.p. 86-87 °C.



^{1}HNMR (400 MHz, chloroform-d, δ, ppm), 3.22 (2H, H_1, t, J = 7 Hz), 2.78 (4H, H_6, $H_{6'}$, s), 2.71 (2H, H_3, t, J = 7 Hz), 2.18 (2H, H_2, quintet, J = 7 Hz).

^{13}CNMR (400 MHz, chloroform-d, δ, ppm), 169.4 ($C_5/C_{5'}$), 168.0 (C_4), 32.2 (C_3), 28.5 (C_2), 26.0 ($C_6/C_{6'}$), 4.24 (C_1).

IR (υ, cm^{-1}), 1739 (C=O), 1657 (C=O).

MS (m/z), EI: 311 (M^+, 1%), 197 ($[M-C_4H_4NO_3]^+$, 100%), 184 ($[M-I]^+$, 29%) 169 ($[M- C_4H_4NO_2-COO]^+$, 52%); CI: 329 ($[M+NH_4]^+$, 19%), 225 ($[M+NH_4-C_4H_4NO_3]^+$, 38%).

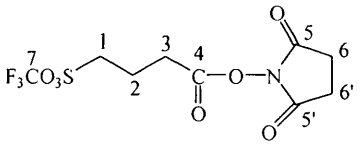
2.3.3.2 Synthesis of LiAE (3)

In a round bottom flask (5 ml), 2,6-dibromophenyl acridine-9-carboxylate (57.2 mg, 0.13 mmol) and succinimidyl 4-iodobutanoate (51.6 mg, 0.17 mmol) were ground and mixed fully. The flask was equipped with a stirrer bar and then flushed with nitrogen. The starting materials were heated in an oil bath (150 °C) for 0.5 h. Soon after, the mixture turned to dark brown. To the resulting mixture, acetonitrile (0.5 ml) was added to extract the product. The crude product was precipitated with diethyl ether (2 ml), and washed with ethyl acetate repeatedly (10 × 2 ml or so) until TLC showed that all the starting materials were removed. A red powder (1 mg) was

obtained and identified as LiAE by ^{1}HNMR spectrum. The residue was evaporated under vacuum and re-used for the reaction under same conditions, another 1 mg of the product could be obtained. The starting materials could be recycled for 2 times; LiAE (3.0 mg) was obtained with 3% yield in total. The ^{1}HNMR and ES^+ mass spectra are same with those of **compound 4** (see **section 2.3.4.2**).

2.3.4.1 Synthesis of succinimidyl 4-(trifluoromethanesulfonyloxy)butanoate (4b)

To a round bottom flask (25 ml) equipped with a stirrer bar, succinimidyl 4-iodobutanoate (145 mg, 0.466 mmol) and silver triflate (163 mg, 0.637 mmol) were added, and then the flask was flushed with N_2 for 5 minutes. Dry benzene (2 ml) was introduced with a syringe, and the mixture was stirred for 23 h. The resulting mixture was passed through a mini silica gel column, eluted by DCM. The fractions with Rf = 0.25 were pooled and evaporated on a rotary evaporator and then pumped overnight to leave a white solid, m.p. 59-60 °C (73 mg, 47% yield).



^{1}HNMR (400 MHz, chloroform-d, δ, ppm), 4.47 (2H, H_1, t, J = 7 Hz), 2.67 (4H, H_6, $H_{6'}$, s), 2.63 (2H, H_3, t, J = 7 Hz), 2.10 (2H, H_2, quintet, J = 7 Hz).

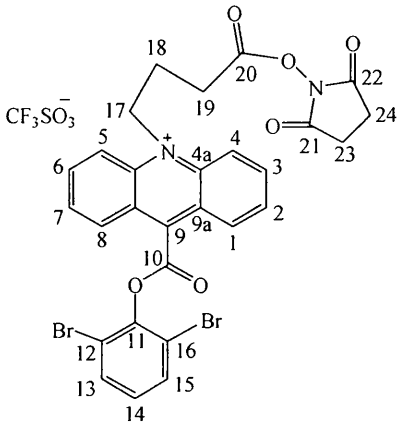
^{13}CNMR (400 MHz, chloroform-d, δ, ppm), 169.2 (C_5/$C_{5'}$), 167.7 (C_4), 120.6 (C_7, splitting into four peaks), 75.3 (C_1), 27.2 (C_3), 26.0 (C_6/$C_{6'}$), 24.9 (C_2).

IR (υ, cm^{-1}), 1738 (C=O).

2.3.4.2 Synthesis of LiAE' (4)

To a round bottom flask (5 ml), equipped with a stirrer bar and an air condenser, freshly prepared succinimidyl 4-(trifluoromethanesulfonyloxy)butanoate (56.9 mg, 0.17 mmol) and 2,6-dibromophenyl acridine-9-carboxylate (61.9 mg, 0.14 mmol)

were added and the flask was flushed with nitrogen. Then TCE (2 ml) was introduced with a syringe. The flask was placed into an oil bath (140 °C) for 2 h. The resulting mixture was stripped of solvent on a rotary evaporator, and then treated in the similar way for purification of LiAE. A brown solid (7 mg, 6.3% yield) was obtained.



^{1}HNMR (400 Hz, acetonitrile-d_3, δ, ppm), 8.93 (2H, H_4, H_5, d, J = 9 Hz), 8.62 (2H, H_1, H_8, d, J = 8 Hz), 8.40 (2H, H_3, H_6, t, J = 9 Hz), 8.01 (2H, H_2, H_7, t, J = 8 Hz); 7.73 (2H, H_{13}, H_{15}, d, J = 8 Hz); 7.20 (1H, H_{14}, t, J = 8 Hz); 5.33 (2H, H_{17}, t, J = 8 Hz); 3.02 (2H, H_{19}, t, J = 7 Hz); 2.69 (4H, H_{23}, H_{24}, s); 2.45 (2H, H_{18}, quintet).

IR (υ, cm^{-1}), 1746 (C=O).

MS (m/z), ES^+: 641 ([M-CF_3SO_3, $^{79}Br^{81}Br$ isotope peak]$^+$, 11%); ES^-: 149 ([CF_3SO_3]$^-$, 100%). Acc-MS: calculated mass: 638.9761 ([M-CF_3SO_3, $^{79}Br^{79}Br$ isotope peak]$^+$); measured mass: 638.9769.

2.3.5.1 Synthesis of 6-iodohexanoic acid (5a)

The sodium iodide (3.11 g, 20.7 mmol) in a round bottom flask (100 ml) was dried over an oil pump for a while. To the flask, 6-bromohexanoic acid (1.49 g, 7.64 mmol) was added, followed by a stirrer bar. Then the flask was equipped with a septum and flushed with N_2. Redistilled acetone (30 ml) was introduced with a syringe. The

mixture was stirred for 4 h. The resulting mixture was poured into water (15 ml), and the product was extracted with diethyl ether (4×20 ml). The organic phase was washed with saturated $Na_2S_2O_3$ (20 ml), dried over $MgSO_4$, filtered and evaporated over a rotary evaporator. The pale yellow crude product was chromatographed, eluted with hexane + EtOEt + formic acid (1:1:0.1%). The fractions with Rf = 0.35 were pooled and stripped of solvents to give white flakes (1.48 g, 80% yield), m.p. 40-41 °C (**Lit.** [9a], 43-43.5 °C).

1 2 3 4 5 6
$ICH_2CH_2CH_2CH_2CH_2COOH$

^{1}HNMR (400 MHz, chloroform-d, δ, ppm), 3.12 (2H, H_1, t, J = 7 Hz), 2.31 (2H, H_5, t, J = 7 Hz), 1.78 (2H, H_2, m, J = 7 Hz), 1.60 (2H, H_4, m, J = 7 Hz), 1.40 (2H, H_3, m, J = 7 Hz). H_6 was not been found, due to its chemical shift beyond 11.5 ppm or a too broad peak.

^{13}CNMR (400 MHz, chloroform-d, δ, ppm), 34.14 (C_5), 33.5 (C_2), 30.3 (C_3), 24.0 (C_4), 6.85 (C_1), the signal for carbonyl group was too weak to be found.

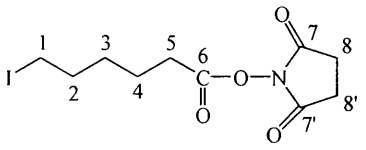
IR (υ, cm^{-1}), 1703 (C=O).

MS (m/z), ES^-: 483 ($[2M-H]^-$, 100%), 241.1 ($[M-H]^-$, 19%).

2.3.5.2 Synthesis of succinimidyl 6-iodohexanoate (5b)

In a round bottom flask (25 ml), equipped with a stirrer bar, flushed with nitrogen, 6-iodohexanoic acid (699.9 mg, 2.89 mmol) was dissolved in dry THF (10 ml), and cooled to 0 °C in an ice bath. *N*-Hydroxysuccinimide (433.8 mg, 3.77 mmol) in THF (2 ml) was added, followed by DCC (680.0 mg, 3.30 mmol). The mixture was stirred at 0 °C for 3 h, then at room temperature overnight. The resulting mixture was

filtered, the filtrate was condensed and loaded onto a silica column for chromatography, eluted with Tol + EtOAc (4: 1), and the fractions with Rf = 0.48 were combined and evaporated on a rotary evaporator to give a white solid (706 mg). The crude product was recrystallized in hexane + EtOEt at 65 °C. White needle crystals (448 mg, 46% yield,) were obtained, m.p. 74-75 °C.



^{1}HNMR (400 MHz, chloroform-d, δ, ppm), 3.13 (2H, H_1, t, J = 7 Hz), 2.78 (4H, H_8, $H_{8'}$, d, J = 2 Hz), 2.57 (2H, H_5, t, J = 7 Hz), 1.82 (2H, H_2, quintet, J = 7 Hz), 1.71 (2H, H_4, quintet, J = 7 Hz), 1.46 (2H, H_3, quintet, J = 7 Hz).

^{13}CNMR (400 MHz, chloroform-d, δ, ppm), 169.5 (C_7/$C_{7'}$), 168.8 (C_6), 33.3 (C_2), 31.1 (C_5), 30.0 (C_3), 26.0 (C_8/$C_{8'}$), 23.9 (C_4), 6.51 (C_1).

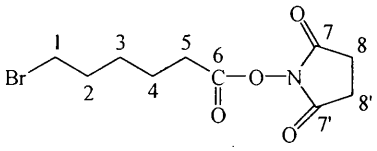
IR (υ, cm^{-1}), 1737 (C=O), 1653 (C=O).

MS (m/z), EI: 225 ($[M-C_4H_4NO_3]^+$, 18%); CI: 357 ($[M+NH_4]^+$, 13%).

2.3.5.2' Synthesis of succinimidyl 6-bromohexanoate (5b')

In a round bottom flask (25 ml), equipped with a stirrer bar, 6-bromohexanoic acid (318.0 mg, 1.63 mmol) in dry THF (10 ml) was cooled to 0 °C in an ice bath. *N*-Hydroxysuccinimide (290.4 mg, 2.52 mmol) in THF (2 ml) was added, followed by DCC (372.4 mg, 1.80mmol) in THF (5 ml). The flask was flushed with nitrogen, and the mixture was stirred at 0 °C for 3 h, afterwards at room temperature overnight. The resulting mixture was filtered, and the filtrate was evaporated to give a white solid. The crude product was chromatographed over a silica column, eluted with Tol

+ EtOAC (4: 1). The fractions with Rf = 0.48 were combined and evaporated on a rotary evaporator to give a white solid, purified further by recrystallisation (250.4 mg, 45% yield), m.p. 70-71 °C.



^{1}HNMR (400 MHz, chloroform-d, δ, ppm), 3.45 (2H, H_1, t, J = 7 Hz), 2.85 (4H, H_8, $H_{8'}$, s), 2.64 (2H, H_5, t, J = 7 Hz), 1.91 (2H, H_2, quintet, J = 7 Hz), 1.78 (2H, H_4, quintet, J = 7 Hz), 1.57 (2H, H_3, quintet, J = 7 Hz).

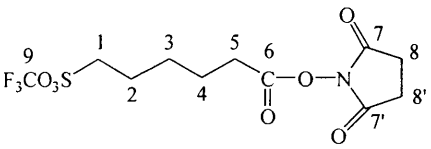
^{13}CNMR (400 MHz, chloroform-d, δ, ppm), 169.53 (C_7/$C_{7'}$), 168.8 (C_6), 33.6 (C_2), 32.6 (C_1), 31.2 (C_5), 27.7 (C_3), 26.0 (C_8/$C_{8'}$), 24.16 (C_4).

IR (υ, cm^{-1}), 1736 (C=O).

MS (m/z), EI: 177 ($[Br(CH_2)_5CO]^+$, 11%); CI: 309 ($[M+NH_4]^+$, 100%).

2.3.5.3 Synthesis of succinimidyl 6-(trifluoromethanesulfonyloxy)hexanoate (5c)

To a round bottom flask (5 ml) equipped with a stirrer bar, succinimidyl 6-iodohexanoate (109 mg, 0.322 mmol) was added, followed by silver triflate (166 mg, 0.648 mmol). The flask was flushed with nitrogen for 5min, and then re-distilled benzene (2 ml) was introduced with a syringe. The mixture was stirred at room temperature for 18 h. The resulting mixture was condensed on a rotary evaporator, and chromatographed over a silica column, eluted with DCM. The fractions with Rf = 0.28 were pooled, evaporated on a rotary evaporator, and then pumped overnight to give a white gum (54.8 mg, 47% yield).



^{1}HNMR (400 MHz, chloroform-d, δ, ppm), 4.45 (2H, H_1, t, J = 7 Hz), 2.75 (4H, H_8, $H_{8'}$, s), 2.56 (2H, H_5, t, J = 7 Hz), 1.79 (2H, H_2, quintet, J = 7 Hz), 1.73 (2H, H_4, quintet, J = 7 Hz), 1.48 (2H, H_3, quintet, J = 7 Hz).

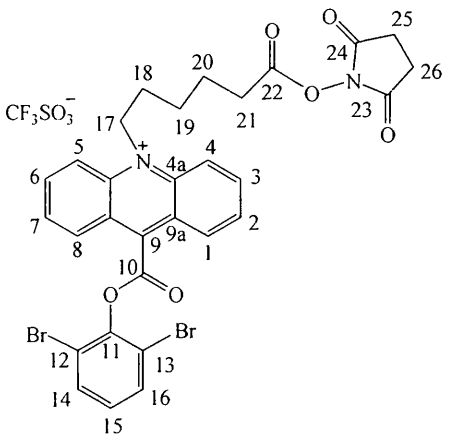
^{19}FNMR (400 MHz, chloroform-d_3, δ, ppm), -74.8.

^{13}CNMR (400 MHz, chloroform-d, δ, ppm), 169.5 (C_7/$C_{7'}$), 168.6 (C_6), 117.4 (C_9, splitting into 4 peaks), 77.4 (C_1), 31.0 (C_5), 29.2 (C_3), 26.0 (C_8/$C_{8'}$), 24.7 (C_4), 24.3 (C_2).

IR (υ, cm^{-1}), 1736 (C=O).

2.3.5.4 Synthesis of 9-(2,6-dibromophenoxycarbonyl)-10-(5-succinimidyloxycarbonylpentyl)acridinium trifluoromethanesulfonate (5)

In a round bottom flask (5 ml), equipped with a stirrer bar and a condenser, 2,6-dibromophenyl acridine-9-carboxylate (82.5 mg, 0.18 mmol) and freshly prepared succinimidyl 6-(trifluoromethanesulfonyloxy)hexanoate (70 mg, 0.19 mmol) were added. The flask was flushed with nitrogen, and then dry DCE (2 ml) was introduced with a syringe. The mixture was refluxed under N_2 for 21.5 h. The resulting mixture was stripped of solvent on a rotary evaporator, and then was taken up in DCM (1 ml). The product was precipitated with diethyl ether (3 ml), the precipitate was re-dissolved in DCM, and re-precipitated repeatedly, until TLC showed absence of the starting materials. A yellow solid (17 mg, 12% yield) was obtained, m.p. 96-98 °C.



^{1}HNMR (400 Hz, chloroform-d, δ, ppm): 9.07 (2H, H_4, H_5, d, J = 8 Hz), 8.96 (2H, H_1, H_8, d, J = 8 Hz), 8.64 (2H, H_3, H_6, t, J = 8 Hz), 8.13 (2H, H_2, H_7, d, J = 8 Hz), 7.81 (2H, H_{14}, H_{16}, d, J = 8 Hz), 7.30 (1H, H_{15}, t, J = 8 Hz), 5.70 (2H, H_{17}, t, J = 8 Hz), 2.88 (4H, H_{25}, H_{26}, s), 2.71-2.76 (2H, H_{21}, m), 2.42-2.48 (2H, m) and 1.97-2.03 (4H, m), (H_{18}, H_{19}, H_{20}).

^{13}CNMR (400 Hz, chloroform-d, δ, ppm), 169.8 (C_{23}/C_{24}), 168.9 (C_{22}), 161.5 (C_{10}), 147.2 (C_{11}), 145.8 (C_9), 141.7 (C_{4a}), 141.0 (C_{14}/C_{16}), 130.4 (C_{15}), 133.8, 129.9, 129.2, 119.8 (C_1/C_8, C_2/C_7, C_3/C_6, C_4/C_5), 123.9 (C_{9a}), 117.6 (C_{12}/C_{13}), 52.6 (C_{17}), 30.9 (C_{21}), 26.0 (C_{25}/C_{26}), 29.2, 25.5, 24.6 (C_{18}, C_{19}, C_{20}).

IR (υ, cm^{-1}), 1734 (C=O).

MS, ES^+: 723 (37%), 669 ([M-CF_3SO_3, $^{79}Br^{81}Br$ isotope peak]$^+$, 100%), 449 (56%), 79 (44%); ES^-: 149 ([CF_3SO_3]$^-$, 100%). Acc-MS: calculated mass: 667.0074 ([M-CF_3SO_3, $^{79}Br^{79}Br$ isotope peak]$^+$); measured mass: 667.0081.

2.3.6.1 Synthesis of 11-iodoundecanoic acid (6a)

The sodium iodide (3.04 g, 20.3 mmol) in a round bottom flask (100 ml) was dried over an oil pump for a while. Then, 11-bromoundecanoic acid (2.19 g, 8. 27 mmol)

was added, followed by a stirrer bar. The flask was flushed with N_2 *via* a septum. Redistilled acetone (20 ml) was added with a syringe. The mixture was stirred for 4 h at room temperature. The precipitate was removed by filtration. To the filtrate, dry NaI (1.50 g, 10.1 mmol) was added again, the mixture was stirred for further 4 h. The resulting mixture was poured into water (20 ml), and the product was extracted with diethyl ether (4 × 25 ml). The organic phase was washed with saturated $Na_2S_2O_3$ (20 ml), dried over $MgSO_4$, filtered, and evaporated over a rotary evaporator to give a pale pink solid (2.36 g), which was later chromatographed over a silica column and eluted with hexane + EtOEt + formic acid (1: 1: 0.1%). The fractions with Rf = 0.35 were pooled and evaporated to give white flakes (2.23 g, 83% yield), m.p. 62–63 °C (**Lit.** [9a], 64-65 °C).

1 2 3 4 5 6 7 8 9 10 11
I $CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOH$

^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 3.12 (2H, H_1, t, J = 7 Hz), 2.23 (2H, H_{10}, t, J = 7 Hz), 1.75 (2H, H_2, quintet, J = 7 Hz), 1.56 (2H, H_9, quintet, J = 7 Hz), 1.28-1.18 (12H, H_4, H_5, H_6, H_7, H_8, H_9, m).

^{13}CNMR (400 Hz, chloroform-d, δ, ppm), 179.9 (C_{11}), 34.3 (C_{10}), 33.9 (C_2), 30.8 (C_3), 29.7 (C_5, C_6, C_7), 29.4 (C_8), 28.9 (C_4), 25.0 (C_9), 7.53 (C_1).

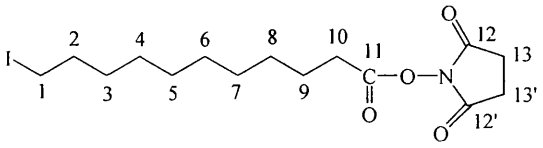
IR (υ, cm^{-1}), 1693 (s, CO).

MS (m/z), EI: 313 ($[M + H]^+$, 3%); CI: 330 ($[M + NH_4]^+$, 100%).

2.3.6.2 Synthesis of succinimidyl 11-iodoundecanoate (6b)

In a round bottom flask (50 ml), equipped with a stirrer bar, 11-iodoundecanoic acid (989 mg, 3.17 mmol) in dry THF (10 ml) was cooled to 0 °C in an ice bath.

N-Hydroxysuccinimide (655.5 mg, 5.70 mmol) in THF (5 ml) was added with a syringe, followed by DCC (760 mg, 3.69 mmol) in THF (5 ml). The flask was flushed with nitrogen, and the mixture was stirred at 0 °C for 3 h, then at room temperature overnight. The resulting mixture was filtered, and the filtrate was condensed for loading onto a silica column for chromatography, eluted with tol + EtOAC (4: 1). The fractions with Rf = 0.48 were combined and evaporated on a rotary evaporator to give a white solid. The crude product was further purified by recrystallization in hexane + EtOEt at 65 °C, to give white needle crystals (847 mg, 65% yield), m.p. 84 °C.



^{1}HNMR, (400 Hz, chloroform-d, δ, ppm), 3.16 (2H, H_1, t, J = 7 Hz), 2.81 (4H, H_{13}, $H_{13'}$, s), 2.57 (2H, H_{10}, t, J = 7 Hz), 1.79 (2H, H_2, quintet, J = 7 Hz), 1.71 (2H, H_9, quintet, J = 7 Hz), 1.35 (2H, H_3, quintet, J = 7 Hz), 1.35-1.23 (10H, H_4, H_5, H_6, H_7, H_8, m).

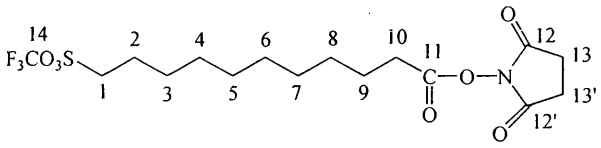
^{13}CNMR (400 Hz, chloroform-d, δ, ppm), 169.5 (C_{12}/$C_{12'}$), 169.0 (C_{11}), 35.3 (C_2), 33.9 (C_3), 31.3 (C_{10}), 30.8, 29.6, (C_5, C_6, C_7, two of them overlapped accidentally), 29.4 (C_8), 29.2 (C_4), 24.9 (C_9), 26.0 (C_{13}/$C_{13'}$), 7.6 (C_1).

IR (υ, cm^{-1}), 1725 (C=O).

MS (m/z), CI: 427 ($[M+NH_4]^+$, 15%), 301 ($[M+NH_4-I]^+$ 15%), 225 (16%), 149 (30%), 132 (100%).

2.3.6.3 Synthesis of succinimidyl 11-(trifluoromethanesulfonyloxy)undecanoate (6c)

To a round bottom flask (5 ml) equipped with a stirrer bar, succinimidyl 11-iodoundecanoate (147 mg, 0.359 mmol) was added, followed by silver triflate (266 mg, 1.03 mmol). The flask was flushed with nitrogen for 5min *via* a septum, and then dry benzene (2 ml) was introduced with a syringe. The nitrogen source was removed, the septum was sealed with nesco film, and the mixture was stirred at room temperature for 18 h. The resulting mixture was condensed on a rotary evaporator for chromatography over a silica column, eluted with DCM. The fractions with Rf = 0.31 were pooled and evaporated under vacuum to give a white gum (110.4 mg, 71% yield).



^{1}HNMR (400 MHz, chloroform-d_3, δ, ppm), 4.44 (2H, H_1, t, J = 7Hz), 2.74 (4H, H_{13}, $H_{13'}$, s), 2.51 (2H, H_{10}, t, J = 7 Hz), 1.73 (2H, H_2, quintet, J = 7Hz), 1.65 (2H, H_9, quintet, J = 7 Hz), 1.31 (2H, H_3, quintet), 1.27-1.18 (10H, H_4, H_5, H_6, H_7, H_8, m).

^{19}FNMR (400 MHz, chloroform-d, δ, ppm), -74.8.

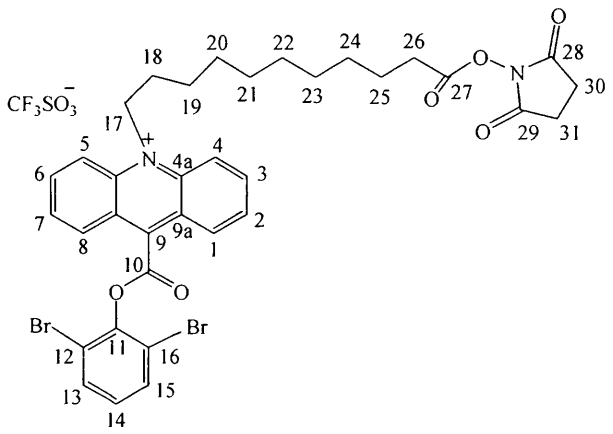
^{13}CNMR (400 MHz, chloroform-d, δ, ppm), 169.6 (C_{12}/$C_{12'}$), 169.0 (C_{11}), 118.2 (C_{14}, splitting into 4 peaks), 78.14, (C_1), 31.3 (C_{10}), 29.6, 29.5 (C_5, C_6, C_7), 29.3, 29.2 (C_4, C_8), 29.1 (C_3), 26.0 (C_{13}/$C_{13'}$), 25.4 (C_9), 24.9 (C_2).

IR (υ, cm^{-1}), 1725 (C=O).

2.3.6.4 Synthesis of 9-(2,6-dibromophenoxycarbonyl)-10-(10-succinimidyloxycarbonyldecyl)acridinium trifluoromethanesulfonate (6)

In a round bottom flask (5 ml), equipped with a stirrer bar and a condenser, 2,6-dibromophenyl acridine-9-carboxylate (93.2 mg, 0.20mmol) and freshly prepared

succinimidyl 11-(trifluoromethanesulfonyloxy)undecanoate (94.1 mg, 0.22 mmol) were added. The flask was flushed with nitrogen, and then redistilled TCE (2 ml) was introduced with a syringe. The mixture was heated at 140 °C under N_2 for 4 h. The product was isolated in a similar way used for purifying LiAE to give a yellow solid (28.0 mg, 15% yield), m.p. 165-166 °C.



^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 8.84 (2H, H_4, H_5, d, J = 8 Hz), 8.59 (2H, H_1, H_8, d, J = 9 Hz), 8.39 (2H, H_3, H_6, dd, J = 8, 7 Hz), 7.89 (2H, H_2, H_7, dd, J = 9, 7 Hz), 7.58 (2H, H_{13}, H_{15}, d, J = 8 Hz), 7.06 (1H, H_{14}, t, J = 8 Hz), 5.47 (2H, H_{17}, t, J = 8 Hz), 2.65 (4H, H_{30}, H_{31}, s), 2.41 (2H, H_{26}, t, J = 7 Hz), 2.07-1.98, (2H, H_{18}, m), 1.64-1.49 (4H, H_{19}, H_{25}, m) 1.31- 1.08 (10H, H_{20}, H_{21}, H_{22}, H_{23}, H_{24}, m).

^{13}CNMR (400 Hz, chloroform-d, δ, ppm), 169.7 (C_{28}/C_{29}), 169.1 (C_{27}), 161.5 (C_{10}), 147.1 (C_{11}), 145.9 (C_9), 141.7 (C_{4a}), 140.8 (C_{13}/C_{15}), 130.3 (C_{14}), 133.8, 129.9, 129.3, 119.8 (C_1/C_8, C_2/C_7, C_3/C_6, C_4/C_5), 123.9 (C_{9a}), 117.7 (C_{12}/C_{16}), 52.9 (C_{17}), 31.3 (C_{26}), 30.1, 29.6, 29.5 (2 lines), 29.3, 29.0, (C_{19}, C_{20}, C_{21}, C_{22}, C_{23}, C_{24}), 26.9 (C_{18}), 26.0 (C_{30}/C_{31}), 24.9 (C_{25}).

^{19}FNMR (400 Hz, chloroform-d, δ, ppm), -78.2.

IR (υ, cm^{-1}), 1728 (C=O).

MS, ES^+: 793 (48%), 739 ($[M\text{-}CF_3SO_3]^+$, $^{79}Br^{81}Br$ isotope peak]$^+$, 82%), 519 (100%), 361 (20%); ES^-: 149 ($[CF_3SO_3]^-$, 100%). Acc-MS: calculated mass: 737.0856 ($[M\text{-}CF_3SO_3$, $^{79}Br^{79}Br$ isotope peak]$^+$); measured mass: 737.0865.

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Chapter 3

Synthesis of 9-(substituted phenoxycarbonyl)-10-substituted acridinium derivatives

3.1 Introduction

Label 2[1] shown in **figure 3.1**, was reported to be a more stable acridinium ester (AE), compared with **label 1**[2], the first AE label reported in 1983. Furthermore it exhibits a two-fold improvement in the signal-to-noise ratio when used in solid phase specific binding assay and its IgG conjugate also exhibits a three-fold increase in light emitting efficiency.[1, 3] Steric hindrance could explain these characteristics since most of the compounds exhibiting this stability are substituted in the *ortho* position of the phenol by bulky groups (methyl) or atoms (Br) while the same substitution in another position is not efficient from this point of view.[4, 5] The presence of substituents at the *ortho* positions has the effect of improving the stability towards hydrolysis at the ester group, irrespective of the electronic nature of the substituents. The electronic effects are more important in determining the rate at which the chemiluminescent reaction occurs, thereby providing a range of labels, which are differentiated in the time over which light is emitted as well as being relatively stable to hydrolysis. This provides opportunities for choosing a label with properties appropriate for a particular application. Electron-withdrawing groups introduced in the phenyl ring increase both efficiencies and reaction rates while electron donating groups have the opposite effects. The dibromo compound flashes quickly while the dimethyl and dimethoxy compounds give off chemiluminescence slowly, as a result of the relative ease of expulsion of the corresponding phenoxide anions.[3-7]

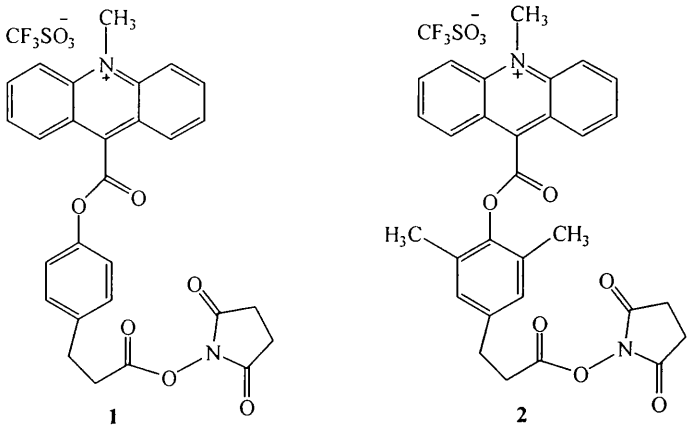
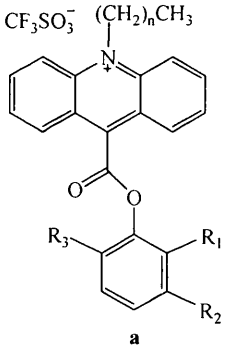


Figure 3.1, structures of **label 1** and **label 2** in literatures[1-3]

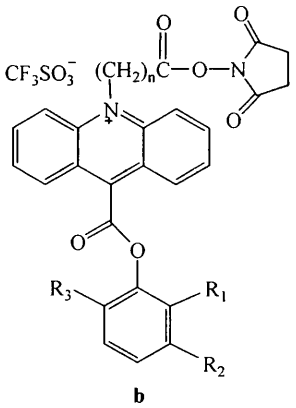
In this chapter, the protons of the phenoxyl ring are substituted by 2,5-methyl, 2,6-bis(trifluoromethyl), 2,6-dinitro groups, which are expected to influence both the

stability and the chemiluminescent kinetics by a combination of steric and electronic effects from the substituents. Studies of the chemiluminescent properties of the compounds will, therefore, enable some further understanding of the importance of such factors and allow multi-analysis, based on different decay kinetics of several acridinium derivatives. Such compounds have been designed for use as DNA probes, although these methods could probably also be applied for antibody based assays. In order to try to combine the advantages of an acridinium ester, which has substituents on the phenoxy ring and an acridinium ester, which has its linker and spacer moiety bonded to the nitrogen atom, several new target compounds were designed (**figure 3.2**). AE without linker groups are included in **group a,** to allow investigation of the chemiluminescent kinetics and stability; and compounds in **group b** would be used to investigate not only chemiluminescent kinetics and stability, but also the properties of their conjugates to oligonucleotides or antibodies.



a

n = 0, 11
$R_1 = R_2 = R_3 = H$; $R_1 = R_3 = NO_2$, $R_2 = H$;
$R_2 = R_3 = CH_3$, $R_1 = H$; $R_1 = R_3 = CF_3$, $R_2 = H$.



b

n = 3, 5, 10:
$R_1 = R_2 = R_3 = H$; $R_1 = R_3 = NO_2$, $R_2 = H$;
$R_2 = R_3 = CH_3$, $R_1 = H$; $R_1 = R_3 = CF_3$, $R_2 = H$.

Figure 3.2, the target compounds

The specific compounds intended to be made are shown in **table 3.1**.

Table 3.1. The structures of intended targets

Compound number	Type **a** or **b**	n	R_1	R_2	R_3
7	a	0	H	H	H
8	a	11	H	H	H
9	b	3	H	H	H
10	b	5	H	H	H
11	b	10	H	H	H
12	a	0	H	CH_3	CH_3
13	b	3	H	CH_3	CH_3
14	b	5	H	CH_3	CH_3
15	b	10	H	CH_3	CH_3
16	a	0	CF_3	H	CF_3
17	b	10	CF_3	H	CF_3
18	a	0	NO_2	H	NO_2
19	b	10	NO_2	H	NO_2
23	b	3	NO_2	H	NO_2
24	b	5	NO_2	H	NO_2
25	b	3	CF_3	H	CF_3
26	b	5	CF_3	H	CF_3

Note: Attempts to synthesize **compounds 23, 24, 25** and **26** were unsuccessful (see text), but all other compounds were prepared; and are reported later in this chapter.

The target compounds are classified into several groups according to the properties of the substituted groups on the phenoxy ring. The group of AEs with 2,6-dibromo (weakly electron-withdrawing) substitution on the phenyl ring reported in **chapter 2,** is expected to have a quicker and stronger chemiluminescence output than the corresponding phenyl ring unsubstituted group; the second group, with 2,5-dimethyl (electron-donating) substitution, is expected to have the opposite effect; the third group, with 2,6-bis(trifluoromethyl) or 2,6-dinitro (strongly electron-withdrawing) substitution on the phenyl ring *ortho* to the ester functionality, is expected to have the quickest and strongest chemiluminescence output; the unsubstituted AEs would

play a role as model compounds against which to test synthetic methods and provide a baseline for comparison of properties.

3.2 Results and discussion

The first requirement was to synthesize a range of target compounds. This work is described in the following sections.

3.2.1 The scheme for synthesis of 9-(phenoxycarbonyl)-10-methylacridinium trifluoromethanesulfonate (7)

9-(Phenoxycarbonyl)-10-methylacridinium trifluoromethanesulfonate (**7**), would be derived from phenyl acridine-9-carboxylate (**7a**), which would be methylated with methyl triflate to afford the product. The first task, therefore, was to synthesize **7a**.

3.2.1.1 Synthesis of phenyl acridine-9-carboxylate (7a)

Phenyl acridine-9-carboxylate (**7a**) was synthesized by stirring phenol and acridine-9-carboxylic acid chloride (**figure 3.3**). The synthesis was performed in a similar procedure to that used for synthesis of **compound 1c**. However, after reaction, column chromatography followed by pumping overnight was able to provide the pure product (63% yield). Phenol was not completely removed by the chromatography, but could be pumped away under vacuum, whereas for purification of **compound 1c**, the excess 2,6-dibromophenol could not be removed by the oil pump due to its high boiling point.

The ^{1}HNMR spectrum of **compound 7a** showed 7 peaks in the aromatic region integrating in the ratio 2:2:2:2:2:2:1. In the ^{13}CNMR spectrum, a peak at 163.0 ppm, attributed to the carbonyl group, and another 11 aromatic peaks for 11 different carbons were found. The IR spectrum showed a peak at 1755 cm^{-1} for the ester bond. The mass spectrum gave ions at m/z 229 ($[M]^{+}$), 206, and 178 (**figure 3.4**). It was therefore clear that **compound 7a** had been synthesized successfully.

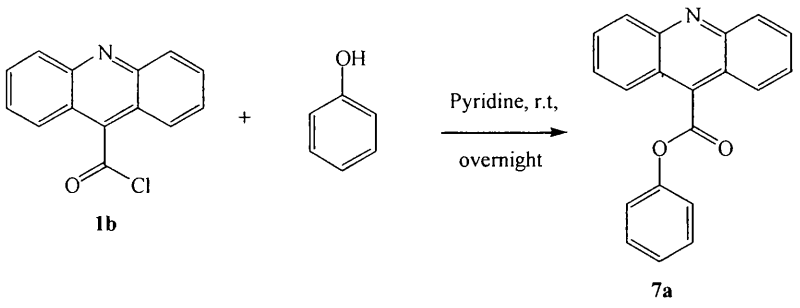


Figure 3.3, the synthesis of phenyl acridine-9-carboxylate (**7a**)

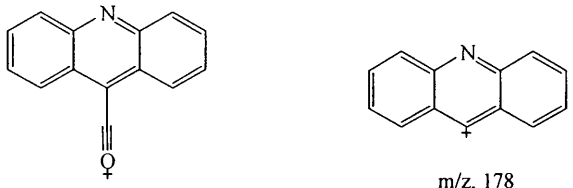


Figure 3.4, MS fragments for **compound 7a**

3.2.1.2 Synthesis of 9-(phenoxycarbonyl)-10-methylacridinium trifluoromethanesulfonate (7)[8-11]

The synthesis of **compound 7** (**figure 3.5**) was carried out in a way similar to that used for **compound 2 (section 2.2.2)**. The starting materials, **compound 7a** and methyl triflate, were stirred at room temperature for 3 h. The precipitate produced was filtered and washed with EtOEt to provide the product. In the ^{1}HNMR spectrum, there were 7 peaks in the aromatic region and a singlet at 5.33 ppm integrating in a ratio of 2:2:2:2:2:2:1:3. In the ^{13}CNMR spectrum, a peak at 164.9 ppm was attributed to the carbonyl group, a signal at 41.1 ppm appeared for a methyl group and another 11 aromatic carbons were found. The IR spectrum showed absorption at 1760 cm^{-1}. The mass spectrum showed cations at m/z 314 ($[M\text{-}CF_3SO_3]^+$) and 193 (**figure 3.6**) and an anion at m/z 149 ($[CF_3SO_3]^-$).

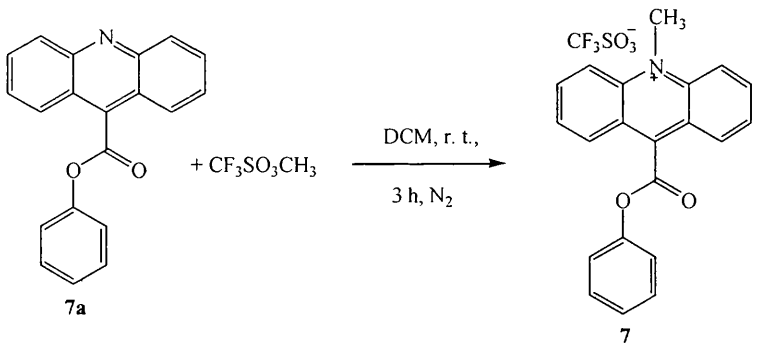


Figure 3.5, synthesis of 9-(phenoxycarbonyl)-10-methylacridinium trifluoromethanesulfonate (**7**)

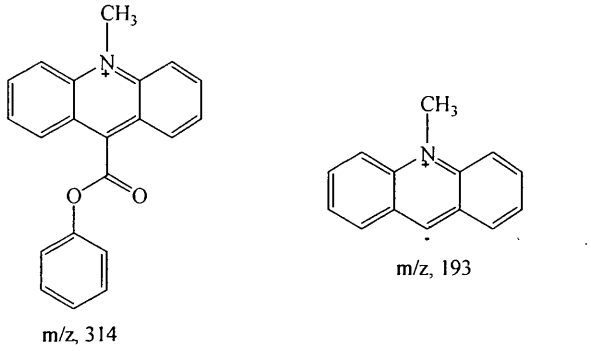
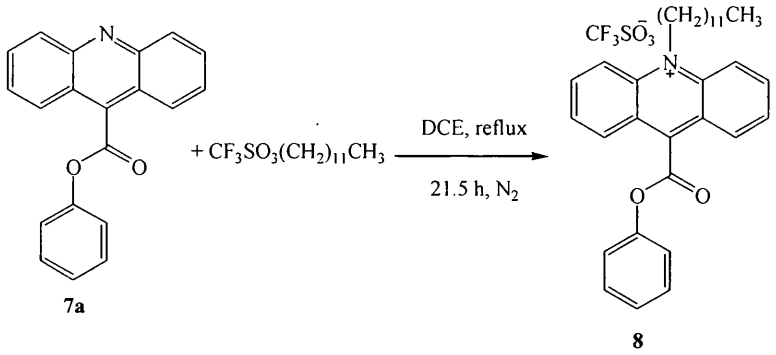


Figure 3.6, MS fragments for **compound 7**

3.2.2 Synthesis of 9-(phenoxycarbonyl)-10-dodecylacridinium trifluoromethanesulfonate (8)

Synthesis of **compound 8** was carried out by using freshly prepared dodecyl triflate (**1a**, **section 2.2.1.1**) and phenyl acridine-9-carboxylate (**7a**). Such reaction mixtures were heated in *p*-xylene, toluene, nitrobenzene and DCE respectively, at different temperatures for different lengths of time. The resulting mixtures were evaporated under vacuum and dissolved in chloroform-*d* and submitted for ^{1}HNMR spectrometry. The crude yield was calculated in each case from the ratio of the proton integrals at δ = 5.58 ppm (attributed to the $CH_2^{\bullet}$ group next to the N atom in the desired product) and $\delta >=$ 7.40 ppm (attributed to all protons in the aromatic region of the starting material and the product) in the ^{1}HNMR spectrum, multiplied by 13/2. The crude product was purified by the method used for purifying LiAE to give a bright yellow solid.[10] The results for syntheses of **compound 8** under different conditions are listed in **table 3.2**. From **table 3.2**, refluxing the starting materials in

DCE for 21.5 h afforded the best crude yield (39%). The crude yield was not obtained for the reaction conducted in nitrobenzene because the high boiling point of the solvent rendered it difficult to remove, and thus the ^{1}HNMR spectrum was too complicated. A slightly better yield was obtained for the synthesis of **compound 8** than for **compound 1** under similar conditions. **Compound 8** was isolated and purified in the same way as **compound 1 (section 2.2.1.4)**.



7a **8**

Figure 3.7, the synthesis of 9-(phenoxycarbonyl)-10-dodecylacridinium trifluoromethanesulfonate (**8**)

In the ^{1}HNMR spectrum, there were 7 peaks in the aromatic region and 6 peaks in the aliphatic region, integrating in a ratio of 2:2:2:2:2:2:1:2:2:4:2:12:3. A peak at 5.52 ppm was attributed to the CH_2 group next to the N atom and one at 0.80 ppm to the CH_3 group. The protons on the acridinium ring resonated at lower field, than for **compound 7a**. In the ^{13}CNMR spectrum, there were a signal at 163.3 ppm attributed to carbonyl group, another 11 aromatic peaks for the 11 different aromatic carbons, a peak at 52.6 ppm for the CH_2 group next to the N atom and another 11 peaks in aliphatic region. The mass spectrum showed a cation at m/z 468 ($[M\text{-}CF_3SO_3]^+$), and an anion at m/z 149 ($[CF_3SO_3]^-$), corroborating the structure of the product.

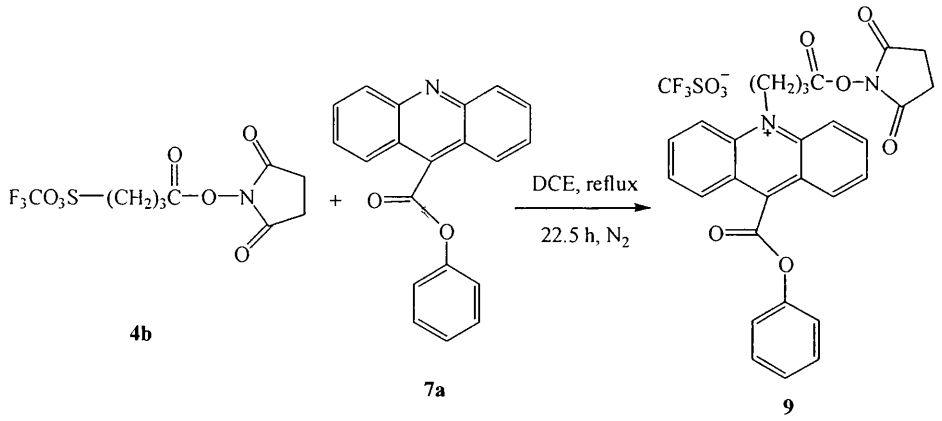
Table 3.2. The results of attempted synthesis of 9-(phenoxycarbonyl)-10-dodecylacridinium trifluoromethylsulfonate (**8**) under different conditions.

Solvent	Reaction time & temperature	The amount of **7a**	The amount of **1a**	Yield / %, Crude
DCE	45.5 h (reflux)	83.5 mg, 0.28 mmol	80 mg, 0.25 mmol	38
DCE	21.5 h (reflux)	95.5 mg, 0.32 mmol	80 mg, 0.25 mmol	39
Toluene	9 h (reflux)	74.3 mg, 0.25 mmol	100 mg, 0.31 mmol	8
p-xylene	7 h (reflux)	77.2 mg, 0.26 mmol	90 mg, 0.28 mmol	15
Nitrobenzene	2 h (140°C)	65.0 mg, 0.22 mmol	70 mg, 0.22 mmol	No pure product
	4 h (140°C)	59.5 mg, 0.20 mmol	60 mg, 0.19 mmol	

The starting materials, **compounds 7a** (phenyl acridine-9-carboxylate) and **1a** (dodecyl triflate) in a ratio of from 1.3 to 0.8, were heated in solvent (2 ml) for the stated time at the stated temperature respectively.

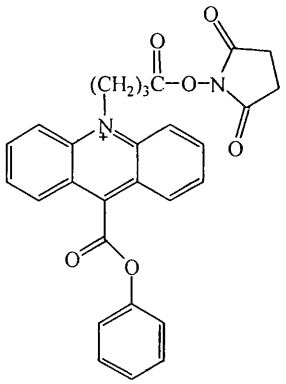
3.2.3 The scheme for synthesis of 9-(phenoxycarbonyl)-10-(3-succinimidyloxycarbonylpropyl)acridinium trifluoromethanesulfonate (9)

The route for synthesis of **compound 9** is shown in **figure 3.8**. A mixture of **compounds 7a** and **4b** was heated at reflux in DCE for 22.5 h under nitrogen. The product was isolated by the method used for isolating **compound 4** (**section 2.2.4.2**). The product was obtained in a 2.7% yield. A similar reaction carried out in refluxing nitrobenzene at higher temperature did not give a better yield. The ^{1}HNMR spectrum of **compound 9** showed 7 peaks in the aromatic region and 4 peaks in the aliphatic region, integrating in a ratio of 2:2:2:2:2:2:1:2:2:4:2. A typical signal at 5.63 ppm was attributed to the CH_2 group next to the N atom and a signal at 2.82 ppm was attributed to the protons of the succinimidyloxy group. The mass spectrum, showing cations at m/z 483 ($[M\text{-}CF_3SO_3]^+$) and 368 (**figure 3.9**), and an anion at m/z 149 ($[CF_3SO_3]^-$), further corroborated the structure of the product. The amount of the product was not enough for a ^{13}CNMR spectrum.



4b 7a 9

Figure 3.8, the synthetic pathway for **compound 9**



m/z, 483

Figure 3.9, MS fragment for **compound 9**

3.2.4 Synthesis of 9-(phenoxycarbonyl)-10-(5-succinimidyloxycarbonylpentyl)-acridinium trifluoromethanesulfonate (10)

Compound 10 was synthesized in a pathway similar to that used for **compound 5 (section 2.2.5.4)**, by refluxing phenyl acridine-9-carboxylate (**7a**) and freshly prepared succinimidyl 5-(trifluoromethanesulfonyloxy)hexanoate (**5c**) in redistilled DCE under nitrogen for 22.5 h (**figure 3.10**). The resulting mixture was evaporated under vacuum, and the residue was subjected to gradient chromatography on a silica column, eluted with DCM, then DCM+CH_3CN (4:1) and finally DCM+CH_3CN (2:1). The fractions with Rf = 0.15 developed in DCM+CH_3CN (4:1) were pooled and evaporated to give a bright yellow solid in 9% yield.

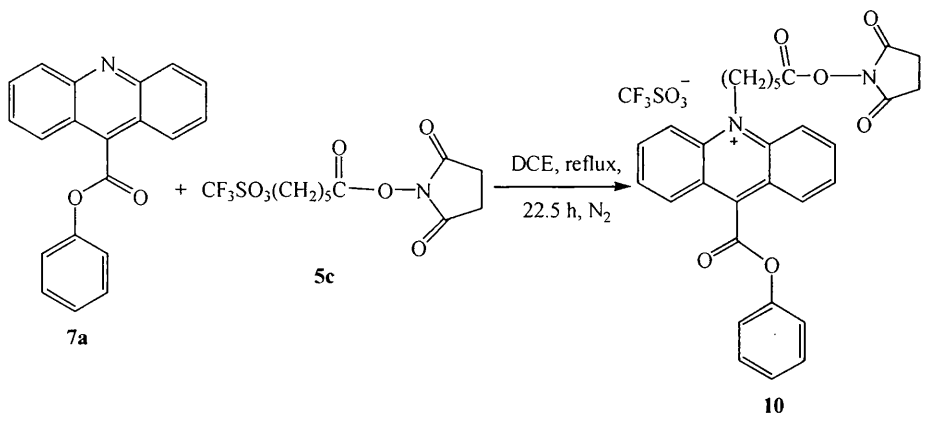


Figure 3.10, synthesis of 9-(phenoxycarbonyl)-10-(5-succinimidyloxycarbonylpentyl)-acridinium trifluoromethanesulfonate (**10**).

In the ^{1}HNMR spectrum, there were 7 peaks in the aromatic region and 6 peaks in the aliphatic region integrating in a ratio of 2:2:2:2:2:2:1:2:4:2:2:4. A triplet at 5.70 ppm was attributed to the CH_2 group next to the N atom and a singlet at 2.88 ppm was attributed to the protons of the succinimidyloxy moiety. The peaks for the protons on the acridinium ring appeared at relatively low field compared to the corresponding starting material. In the ^{13}CNMR spectrum, 3 signals for at 171.0, 170.0 and 164.8 ppm for the 3 different carbonyl groups, a typical peak at 52.6 ppm for the carbon next to the N atom, and a signal at 26.0 ppm for the CH_2 groups of the succinimidyloxy moiety were found. The IR spectrum showed absorption at 1734 cm^{-1} (C=O), presumably for the 3 overlapped carbonyl groups. The mass spectrum showed a cation at m/z 511 ($[M\text{-}CF_3SO_3]^+$) (**figure 3.11**) and an anion at m/z 149 ($[CF_3SO_3]^-$).

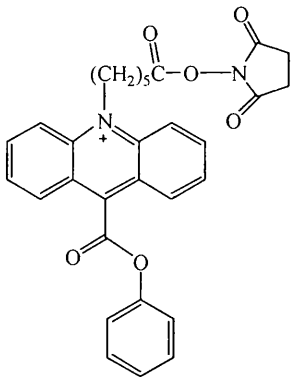


Figure 3.11, MS fragment for **compound 10**

3.2.5 Synthesis of 9-(phenoxycarbonyl)-10-(10-succinimidyloxycarbonyldecyl)-acridinium trifluoromethanesulfonate (11)

Compound 11 was synthesized by a procedure similar to that used for synthesis of **compound 6 (section 2.2.6.4)**, by refluxing phenyl acridine-9-carboxylate (**7a**) and succinimidyl 10-(trifluoromethanesulfonyloxy)undecanoate (**6c**) in DCE for 21 h under N_2 (**figure 3.12**). The product was isolated in 41% yield by column chromatography (silica), eluted by DCM, then DCM+CH_3CN (4:1), and finally DCM+CH_3CN (2:1). The fractions with Rf 0.19 (developed in DCM+CH_3CN (2:1)) were pooled and evaporated to give a yellow solid.

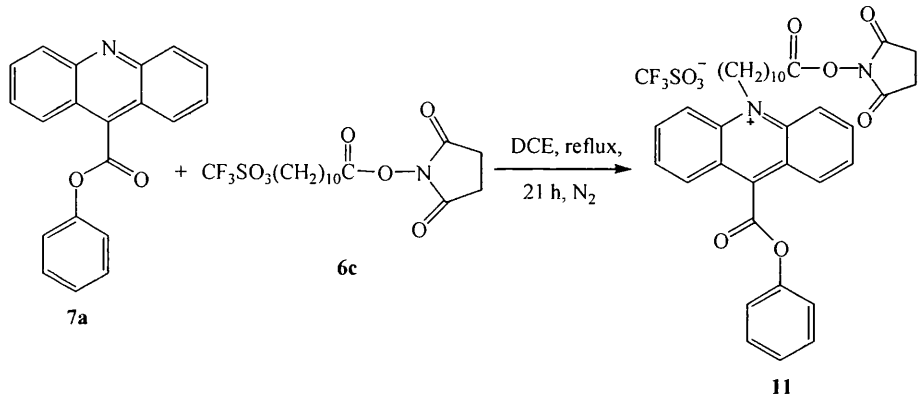


Figure 3.12, the synthesis of 9-(phenoxycarbonyl)-10-(10-succinimidyloxycarbonyldecyl)-acridinium trifluoromethanesulfonate (**11**)

In the ^{1}HNMR spectrum of the product, there were 7 peaks in the aromatic region and 7 peaks in the aliphatic region integrating in a ratio of 2:2:2:2:2:2:1:2:4:2:2:2:2:10. A typical triplet at 5.47 ppm was attributed to the CH_2 group next to the N atom, and a singlet at 2.65 ppm was for the protons of the succinimidyloxy moiety. The peaks attributed to the acridinium ring appeared at relatively low field compared with the starting material. In the ^{13}CNMR spectrum, peaks at 169.7, 169.1 and 161.5 ppm were attributed to the 3 different carbonyl groups. There were also 11 aromatic peaks for 11 different aromatic carbons, a typical peak at 52.6 ppm attributed to the carbon next to the N atom, and a peak at 26.0 ppm attributed to the 2 CH_2 groups of the succinimidyloxy moiety. The mass spectrum showed cations at m/z 581 ($[M\text{-}CF_3SO_3]^+$) and 506 (**figure 3.13**), and an anion at m/z 149 ($[CF_3SO_3]^-$).

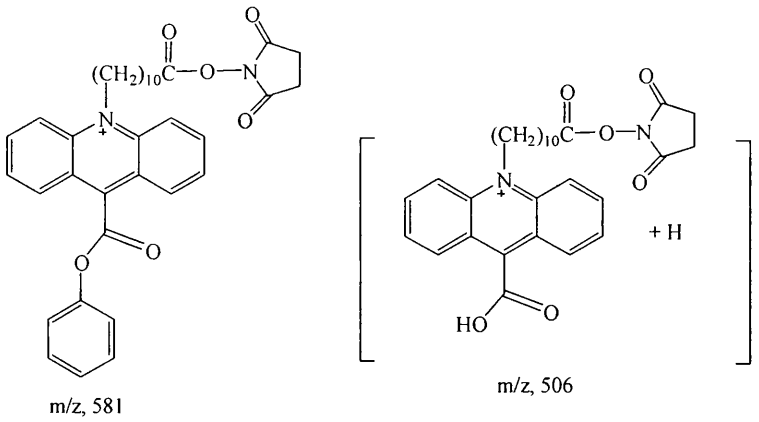


Figure 3.13, MS fragments for **compound 11**

3.2.6 The scheme of synthesis of 9-(2,5-dimethylphenoxycarbonyl)-10-methylacridinium trifluoromethanesulfonate (12)

There are 2 steps involved in synthesis of 9-(2,5-dimethylphenoxycarbonyl)-10-methyl acridinium trifluoromethanesulfonate (**12**). **Compound 12** would be derived from 2,5-dimethylphenyl acridine-9-carboxylate (**12a**), which would be obtained by esterification of **compound 1b** with 2,5-dimethylphenol. The synthetic pathway is illustrated in **figure 3.14**.

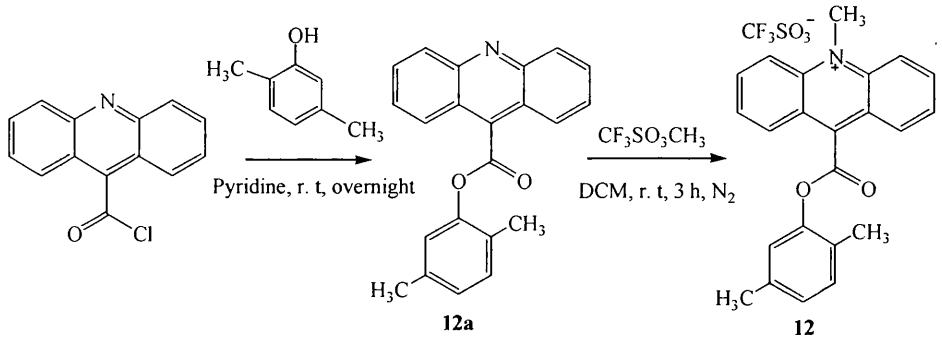


Figure 3.14, the synthesis of 9-(2,5-dimethylphenoxycarbonyl)-10-methylacridinium trifluoromethanesulfonate (**12**)

3.2.6.1 Synthesis of 2,5-dimethylphenyl acridine-9-carboxylate (12a)

Compound 12a was synthesized by stirring acridine-9-carboxylic acid chloride (**1b**) and 2,5-dimethylphenol in pyridine at room temperature overnight, in a similar

synthetic way as used for **compound 7a (section 3.2.1.1)**. The resulting mixture was evaporated under vacuum. The product was purified by column (silica) chromatography, eluted with DCM.

In the ^{1}HNMR spectrum of the product, there were 7 peaks in the aromatic region and 2 singlets at 3.39 and 2.29 ppm attributed to the 2 different methyl groups on the phenoxy group, integrating in a ratio of 2:2:2:2:1:1:1:3:3. In the ^{13}CNMR spectrum, a peak at 166.3 ppm was attributed to the carbonyl group, another 13 aromatic peaks for 13 different aromatic carbons, and 2 peaks in the aliphatic region, were found. The IR spectrum showed a peak at 1748 cm^{-1} for the ester bond. The mass spectrum gave fragments at m/z 328 ($[M+H]^+$), 327 (M^+), 206 (100%) and 178 (82%). The mass fragments at m/z 206 and 178 are the same as those from **compound 1c** and **compound 7a (figure 3.4)**.

3.2.6.2 Synthesis of 9-(phenoxycarbonyl)-10-methylacridinium trifluoromethanesulfonate (12)

Compound 12 was synthesized by stirring **compound 12a** and methyl triflate in dry DCM at room temperature for 3 h. The precipitate produced was filtered and washed with DCM and EtOEt to leave a yellow solid. The ^{1}HNMR spectrum showed 7 peaks in the aromatic region and 3 peaks in the aliphatic region integrating in a ratio of 2:2:2:2:1:1:1:3:3:3. A singlet at 4.99 ppm attributed to the methyl group next to the N atom and another 2 singlets at 2.30 and 2.16 ppm attributed to the 2 methyl groups on the phenoxy ring were found. The peaks for the protons on the acridinium ring resonated at relatively low field compared to the corresponding protons of the starting material. In the ^{13}CNMR spectrum, a peak at 165.1 ppm attributed to the carbonyl group, another 13 aromatic peaks for the 13 different aromatic carbons, a peak at 41.2 ppm attributed to the methyl group next to the N atom and 2 peaks at 21.5 ppm and 17.2 ppm attributed to the 2 methyl groups on the phenoxy group, were found. The IR spectrum showed a peak at 1747 cm^{-1} for the ester bond. The mass spectrum showed cations at m/z 342 ($[M-CF_3SO_3]^+$, 100%) and 193 (68%) (**figure 3.15**) and an anion at m/z, 149 ($[CF_3SO_3]^-$).

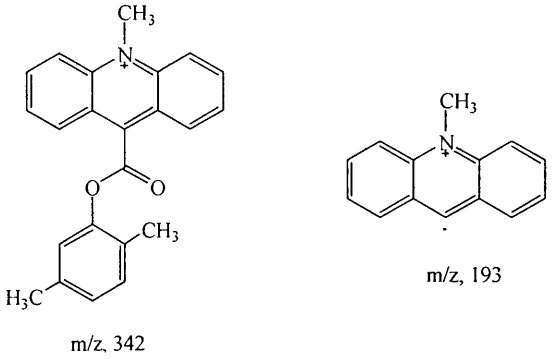


Figure 3.15, the MS fragments of **compound 12**

3.2.7 Synthesis of 9-(2,5-dimethylphenoxycarbonyl)-10-(3-succinimidyloxycarbonylpropyl)acridinium trifluoromethanesulfonate (13)

Compound 13 was synthesized by using 2,5-dimethylphenyl acridine-9-carboxylate (**12a**) and freshly prepared succinimidyl 4-(trifluoromethanesulfonyloxy)butanoate (**4b**), (**figure 3.16**). The starting materials were refluxed in DCE for 20 h. The resulting mixture was extracted with acetonitrile, and the extract showed three TLC spots (2 yellow and 1 colourless) was loaded onto a silica column for chromatography. The 1st yellow band was eluted with DCM, and then the column was eluted with DCM + MeOH (3:1), the fractions with Rf = 0.25 (developed in DCM + MeOH (3:1)) were pooled and evaporated to give a white solid. The solid was obtained in only small amount, but sufficient to get a ^{1}HNMR spectrum. ^{1}HNMR (400 Hz, chloroform-d, δ, ppm) showed 3 peaks, 9.37 (2H, m), 9.09 (1H, t, J = 8 Hz), 8.56 (2H, m). It is clear that this side product does not contain a 2,5-dimethylphenoxy moiety, and the succinimidyloxycarbonylpropyl group is not present. Furthermore, the white colour suggests that the product does not contain an acridine ring. It is possible that the acridine ring has been reduced to an acridan ring, but the mechanism for producing such a side product is unknown. The fractions containing the 3rd yellow band with Rf = 0.15 were pooled and evaporated to give a yellow solid. This proved to be the desired product as shown by ^{1}HNMR and mass spectrometry. It has not been characterized by ^{13}CNMR, since there was not enough sample to obtain a ^{13}CNMR spectrum.

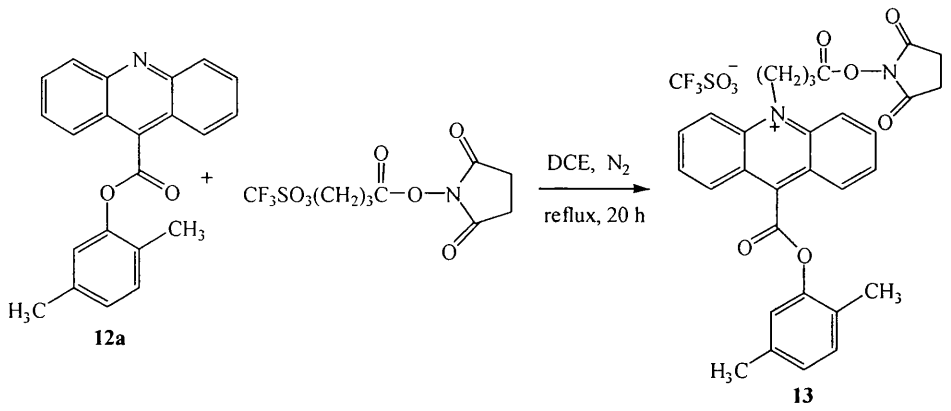


Figure 3.16, synthesis of 9-(2,5-dimethylphenoxycarbonyl)-10-(3-succinimidyloxycarbonylpropyl)acridinium trifluoromethanesulfonate (**13**)

In the ^{1}HNMR spectrum, there were 7 peaks in the aromatic region and 6 peaks in the aliphatic region, integrating in a ratio of 2:2:2:2:1:1:1:2:2:4:2:3:3. A triplet at 5.83 ppm was attributed to the CH_2 group next to the N atom. A singlet at 3.03 ppm attributed to the 2 identical CH_2 groups of the succinimidyloxy moiety, and 2 singlets at 2.59 and 2.46 ppm attributed to the 2 different methyl groups on the phenoxy group, were found. The peaks for the protons on the acridinium ring resonated at relatively low field compared to the corresponding protons of the starting material. The cations in the mass spectrum at m/z 511 ($[M\text{-}CF_3SO_3]^+$), 414 and 396 (**figure 3.17**), and an anion at m/z 149 ($[CF_3SO_3]^-$) further proved it was the desired structure.

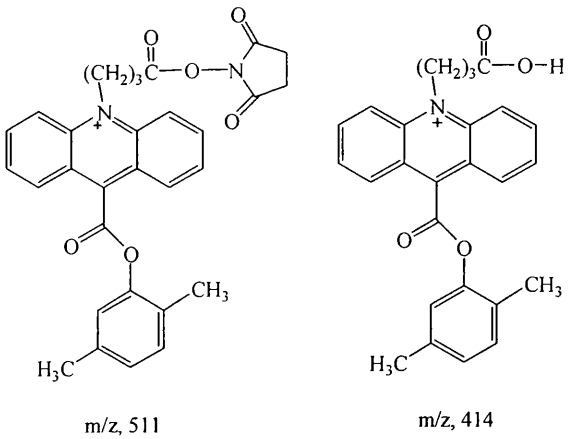


Figure 3.17, the MS fragments for **compound 13**

3.2.8 Synthesis of 9-(2,5-dimethylphenoxycarbonyl)-10-(5-succinimidyloxycarbonylpentyl)acridinium trifluoromethanesulfonate (14)

Compound 14 was synthesized in a pathway similar to those used for **compounds 5 (section 2.2.5.4)** and **10 (section 3.2.4),** by refluxing 2,5-dimethylphenyl acridine-9-carboxylate (**12a**) and succinimidyl 5-(trifluoromethanesulfonyloxy)hexanoate (**5c**) in DCE for 21 h, as illustrated in **figure 3.18**. The resulting mixture was stripped of solvents, and subjected to chromatography on a silica column, eluted with DCM+ MeOH (3:1). The fractions with Rf = 0.18 were pooled and evaporated to give a yellow solid.

In the ^{1}HNMR spectrum, there were 7 peaks in the aromatic region and 6 peaks in the aliphatic region, integrating in a ratio of 2:2:2:2:1:1:1:2:4:2:3:3:2:4. A triplet at 5.39 ppm was attributed to the CH_2 group next to the N atom. A singlet at 2.60 ppm attributed to the 2 identical CH_2 groups of the succinimidyloxy moiety, and 2 singlets at 2.24 and 2.11 ppm attributed to the 2 methyl groups on the phenoxy group, were found. The protons on the acridinium ring resonated at the relatively low field compared to those corresponding protons of the starting material. In the ^{13}CNMR spectrum, there were 3 peaks at 169.8, 168.9, and 163.2 ppm attributed to the 3 different carbonyl groups, and another 13 aromatic peaks for the 13 different aromatic carbons. A signal at 52.3 ppm was attributed to the CH_2 group next to the N atom and a peak at 26.0 ppm was attributed to the 2 CH_2 groups of the succinimidyl moiety. Two peaks at 21.5 and 17.2 ppm for the 2 methyl groups on the phenoxy group were found. The cations in the mass spectrum at m/z 539 ($[M\text{-}CF_3SO_3]^+$), 442 and 424 (**figure 3.19**); and an anion at m/z 149 ($[CF_3SO_3]^-$) further proved it was the desired structure.

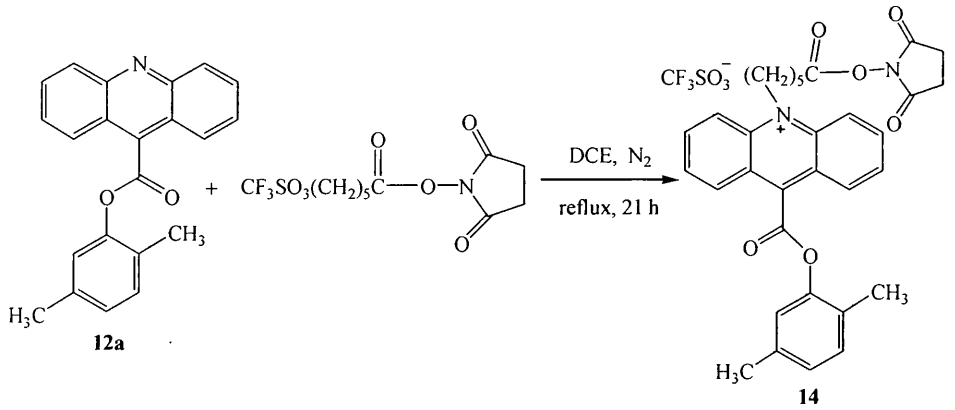


Figure 3.18, synthesis of 9-(2,5-dimethylphenoxycarbonyl)-10-(5-succinimidyloxycarbonylpentyl)acridinium trifluoromethanesulfonate **(14)**

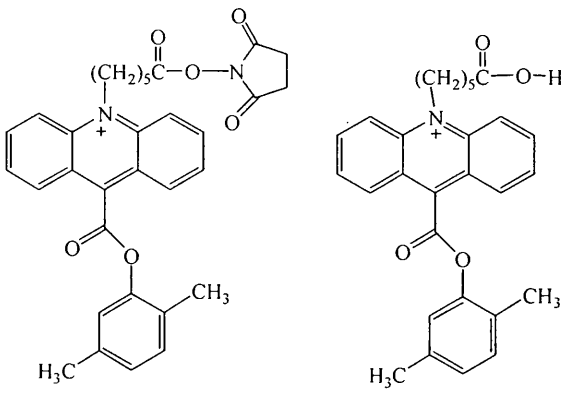


Figure 3.19, the MS fragments for **compound 14**

3.2.9 The synthesis of 9-(2,5-dimethylphenoxycarbonyl)-10-(10-succinimidyloxycarbonyldecyl)acridinium trifluoromethanesulfonate (15)

Compound 15 was synthesized in a synthetic pathway similar to those used for **compounds 6 (section 2.2.6.4)** and **11 (section 3.2.5),** by refluxing 2,5-dimethylphenyl acridine-9-carboxylate (**12a**) and succinimidyl 10-(trifluoromethanesulfonyloxy)undecanoate (**6c**) in DCE for 20 h (**figure 3.20**). The product was isolated by chromatography in 34% yield.

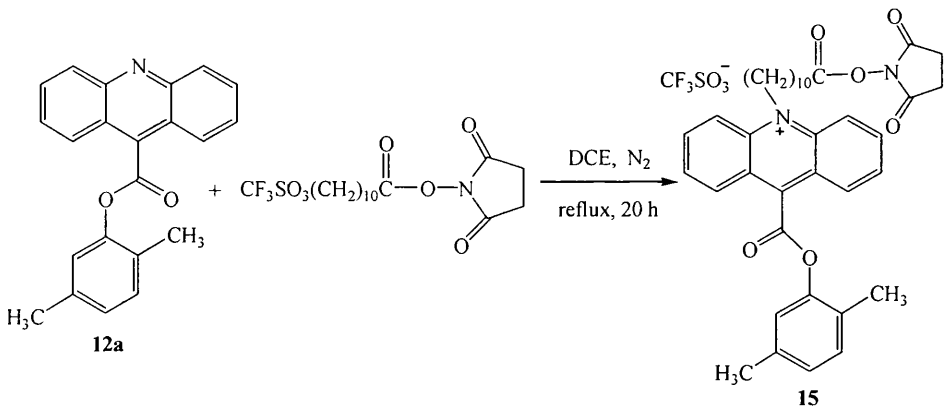


Figure 3.20, synthesis of 9-(2,5-dimethylphenoxycarbonyl)-10-(10-succinimidyloxycarbonyldecyl)acridinium trifluoromethanesulfonate (**15**)

In the ^{1}HNMR spectrum, there were 7 peaks in the aromatic region and 8 peaks in the aliphatic region, integrating in a ratio of 2:2:2:2:1:1:1:2:4:2:3:3:2:4:10. A triplet

at 5.59 ppm was attributed to the CH_2 group next to the N atom. A singlet at 2.83 ppm attributed to the 2 identical CH_2 groups of the succinimidyloxy moiety and 2 singlets at 2.44 and 2.31 ppm attributed to the 2 methyl groups on the phenoxy group were found. Peaks for the protons on the acridinium ring resonated at the relatively lower field than those for the corresponding protons of the starting material. In the ^{13}CNMR spectrum, peaks at 169.8, 169.1, and 163.3 ppm attributed to the 3 different carbonyl groups, and another 13 aromatic peaks for the 13 aromatic carbons were found. A signal at 52.3 ppm for the CH_2 group next to the N atom, a peak at 26.0 ppm for the 2 CH_2 groups of the succinimidyloxy moiety and 2 peaks at 21.5 and 17.2 ppm for the 2 methyl groups on the phenoxy moiety, were found. Cations in the mass spectrum at m/z 609 ($[M\text{-}CF_3SO_3]^+$) and 512 (**figure 3.21**), and an anion at m/z 149 ($[CF_3SO_3]^-$) further proved it was the desired product.

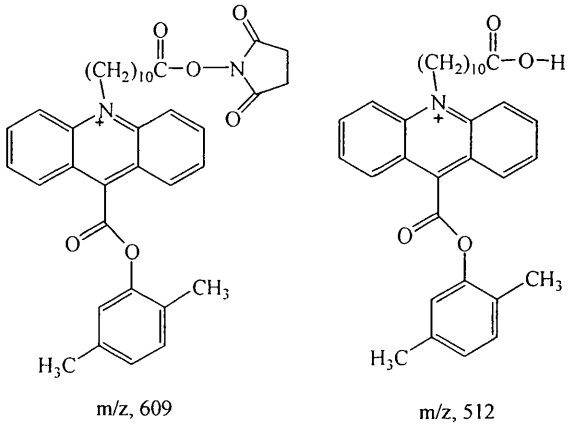


Figure 3.21, the MS fragments for **compound 15**

3.2.10 The scheme of synthesis of 9-(2,6-bis(trifluoromethyl)phenoxycarbonyl)-10-methylacridinium trifluoromethanesulfonate (16)

2,6-Bis(trifluoromethyl)phenyl acridine-9-carboxylate (**16e**) is an essential intermediate for **compound 16**. **Compound 16e** is an analogue of **compounds 5c**, **7a**, and **12a**. Therefore, it could be synthesized by using 2,6-bis(trifluoromethyl)phenol (**16d**) and **1b** in a synthetic pathway similar to that used for its analogues. However, 2,6-bis(trifluoromethyl)phenol (**16d**) is not commercially available. Therefore, 3 extra steps would be needed for the synthesis of 2,6-bis(trifluoromethyl)phenol (**16d**) from 2-trifluoromethylphenol (**16a**). The hydroxy

group of 2-trifluoromethylphenol (**16a**) would need to be protected by 3,4-dihydropyran to produce **16b**, which would be treated with n-BuLi followed by iodine in order to attach an iodide atom *ortho* to the hydroxy group to afford **compound 16c**. Finally, substituting the iodide atom on **16c** with a trifluoromethyl group would accomplish the synthesis of 2,6-bis(trifluoromethyl)phenol (**16d**).

3.2.10.1 Synthesis of 2-(trifluoromethyl)phenol tetrahydropyranyl ether (16b)

2-(Trifluoromethyl)phenol (**16a**) was refluxed with excess 3,4-dihydropyran (DHP) in EtOEt for 18 h, in the presence of a little *p*-toluenesulfonic acid monohydrate as catalyst, as shown in **figure 3.22**. The resulting mixture was poured into saturated $NaHCO_3$ solution and the product was extracted with EtOEt, dried over $MgSO_4$ and evaporated under vacuum to give the crude product, which was purified by chromatography on a silica column. The product was obtained in 89% yield.[12, 13] The mechanism[12, 13] is shown in **figure 3.23**.

The ^{1}HNMR spectrum of **16b** showed 4 peaks in the aromatic region and 6 apparent peaks in the aliphatic region, integrating in a reasonable overall ratio, 4:9. It is worth noting that an asymmetric center was produced (**figure 3.22**), so the protons of the CH_2 groups are in principle not identical. However, only those of the CH_2 group next to the oxygen atom gave 2 clearly separated signals, at 3.77ppm (apparent dt), and at 3.53 ppm (m).

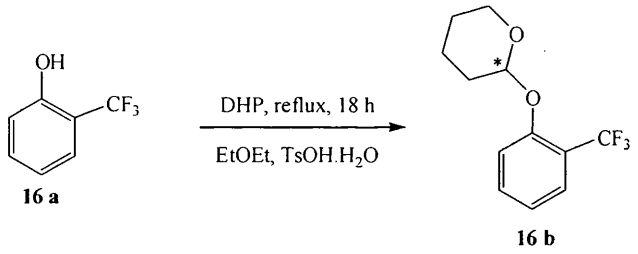


Figure 3.22, protection of hydroxyl group

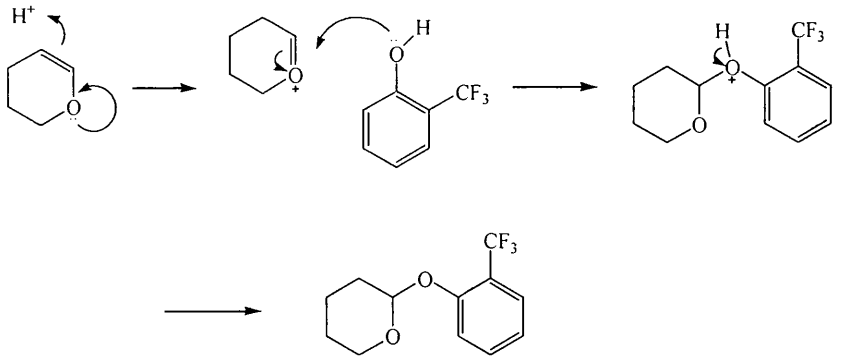
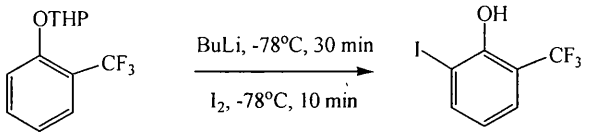


Figure 3.23, the mechanism of synthesis of 2-(trifluoromethyl)phenol tetrahydropyranyl ether

3.2.10.2 Synthesis of 2-trifluoromethyl-6-iodophenol (16c)

Methoxy groups are known to direct lithiation to the *ortho* position of an aromatic ring, so the presence of the ether bond should allow lithiation to occur at the *ortho* position. The lithium could then be replaced by iodine. **Compound 16b** in THF was treated with n-butyllithium at -78 °C. After the mixture was stirred for 30 minutes, a solution of iodine in THF was added and stirred for further 10 minutes before allowing the mixture to warm to room temperature. The resulting brown mixture was poured into aqueous Na_2SO_3 (20%) solution to remove the excess iodine, causing it to turn pale yellow after shaking. The product was extracted with EtOEt, dried over $MgSO_4$ and evaporated under vacuum to give a yellow oil. In its ^{1}HNMR spectrum, three peaks were found in the aromatic region from 7.88 to 6.80 ppm. The lack of a fourth peak indicated that one proton had been exchanged by iodine. The proton integrals in the aromatic region and aliphatic regions were in a ratio of approximately 3:9 revealing that the product should indeed be 2-(trifluoromethyl)-6-iodophenol tetrahydropyranyl ether (**16c'**). Although the ^{1}HNMR spectrum proved that the synthesis of **compound 16c'** was successful, a small amount of impurities was still present. The crude product was purified by short-path distillation using a Kugelrohr apparatus, according to the literature (b.p. 70 °C / 0.25 Torr[12]; b.p. 130 °C / 1 Torr[13]). It is interesting that 2-(trifluoromethyl)-6-iodophenol tetrahydropyranyl ether completely decomposed into its corresponding phenol 2-(trifluoromethyl)-6-iodophenol (**16c**) during short-path distillation at 130 °C (1 Torr), and decomposed to the extent of about 50% at 120 °C (0.7 Torr). The ^{1}HNMR spectrum of **16c** showed a

peak at 5.76 ppm, attributed to the proton of its hydroxyl group. However, there were also other peaks in the downfield region, attributed to impurities produced from hydrolysis (**figure 3.25**). The crude sample of **compound 16c** would correspond to a 94% yield, if it were assumed to be pure, but in reality, it was contaminated by about 15% of dihydropyran hydrate. The yield was lower than that reported in the literature (97% yield).[12] One possible explanation for this is that smaller quantities of the starting material were used, so loss in transfer and during distillation would be greater.



16 b **16 c**

Figure 3.24, synthesis of 2-(trifluoromethyl)-6-iodophenol (**16c**)

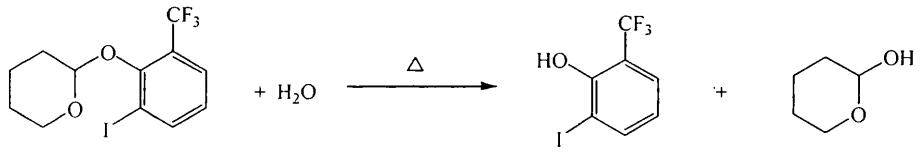


Figure 3.25, the possible hydrolysis of 2-(trifluoromethyl)-6-iodophenol tetrahydropyranyl ether

3.2.10.3 Synthesis of 2,6-bis(trifluoromethyl)phenol (16d)

2,6-Bis(trifluoromethyl)phenol (**16d**) was prepared from 2-(trifluoromethyl)-6-iodophenol by using the reactive organometallic compound '$CuCF_3$', which was produced by Burton's methodology,[13-15] shown in **figure 3.26**. The mechanism proposed for the formation of CF_3MX and the subsequent reaction to form CF_3Cu is shown in **figure 3.27**. A mixture of cadmium powder in DMF was cooled to 0 °C under nitrogen, and CF_2Br_2 was introduced by a syringe very slowly. Upon contact of the CF_2Br_2 with Cd powder, heat and gas were given off, indicating an exothermic reaction. The mixture was diluted with HMPA to stabilize the reactive component, and then CuBr was added at 0 °C. The resulting mixture was treated with 2-(trifluoromethyl)-6-iodophenol and heated at 65 °C for 1.5 h. After cooling, the

resulting mixture was poured into a mixture of HCl (3 mol L^{-1}) and EtOEt. Solids were removed by filtration, and the aqueous phase was extracted with EtOEt. The combined extract was washed with saturated NaCl to remove some of the DMF, dried over $MgSO_4$ and evaporated to give the crude product, which was purified by short-path distillation at 80 °C / 0.7 Torr (**lit.**, b.p. 130 °C / 1 Torr[13] and b.p. 70 °C / 0.25 Torr[12]), to give a colourless oil which turned to yellow on standing.

In the ^{1}HNMR spectrum of the product, two peaks at 7.70 ppm and 7.06 ppm were found in the aromatic region, integrating in a ratio 2:1. The spectrum also showed the peaks of DMF (corresponding to around 17% of the mass). The ^{13}CNMR spectrum gave 5 signals at 153.0, 131.0, 123.8 (q due to coupling to 3 F nuclei), 120.3, and 120.0 ppm for 5 different carbons. The mass spectrum showed the molecular ion at m/z 230 ($[M]^+$).

The short-path distillation could separate the product from the starting material, but DMF possesses a boiling point relatively close to the product, so could not be removed completely by this way. Column chromatography after short-path distillation was used by Dr. A.M. Holland to purify the product[13]. However, because the DMF would not affect the reaction in the next stage and the amount of the product obtained in this stage was not enough for the luxury of chromatography, the product containing 17% DMF was used for the next stage without further purification. The yield (38%) on this stage was low, due to the losses during extraction and short-path distillation.

16c **16d**

Figure 3.26, synthesis of 2,6-bis(trifluoromethyl)phenol

$$Cd + CF_2Br_2 \rightarrow Cd^+[CF_2Br_2]^- \rightarrow [CF_2Br]^- + Br^- + Cd^{2+}$$

$$[CF_2Br]^- \rightarrow [:CF_2] + Br^-$$

$$[:CF_2] + Me_2NCH{=}O \rightarrow CO + Me_2NCF_2H \leftrightarrow [Me_2N^+{=}CFH]F^-$$

$$F^- + [:CF_2] \leftrightarrow [CF_3^-] \rightarrow CF_3CdBr + (CF_3)_2Cd + Br^-$$

$$CF_3CdBr + (CF_3)_2Cd + CuBr \rightarrow [CF_3Cu]$$

Figure 3.27, suggested mechanism for formation of $[CF_3Cu]$[12, 13]

3.2.10.4 Synthesis of 2,6-bis(trifluoromethyl)phenyl acridine-9-carboxylate (16e)

2,6-Bis(trifluoromethyl)phenyl acridine-9-carboxylate (**16e**) was synthesized in a way similar to those used for **compounds 1c (section 2.2.1.3)**, **7a (section 3.2.1.1)** and **12a (section 3.2.6.1)** by stirring 2,6-bis(trifluoromethyl)phenol (**16d**) and acridine-9-carboxylic acid chloride (**1b**) in pyridine overnight at room temperature (**figure 3.28**). The product was isolated by chromatography. The ^{1}HNMR spectrum of the product showed 6 peaks in the aromatic region, integrating in a ratio of 2:2:2:2:2:1. In the ^{13}CNMR spectrum, a signal at 164.6 ppm attributed to the carbonyl group; a peak at 122.8 ppm (q due to coupling to 3 F nuclei) attributed to the CF_3 group and another 11 aromatic peaks for 11 the different carbons, were found, while there were 7 different aromatic carbons for **compound 1b**. The IR spectrum showed an absorption at 1758 cm^{-1}. The CI mass spectrum showed pseudo molecular ion peaks at m/z 435 ($[M]^+$) and 436 ($[M+H]^+$), giving the corroboratory evidence for the desired product. The other MS fragments were similar to those of **compounds 1c** and **7a (figure 3.4)**.

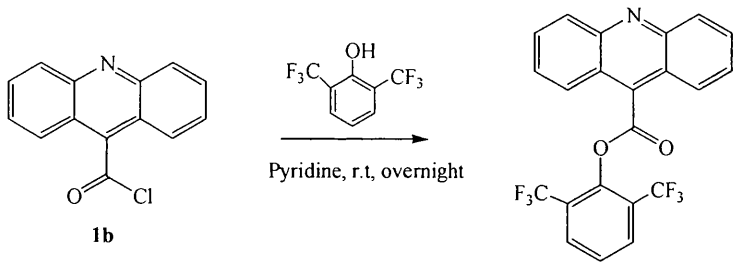


Figure 3.28, synthesis of 2,6-bis(trifluoromethyl)phenyl acridine-9-carboxylate (**16e**)

3.2.10.5 Synthesis of 9-(2,6-bis(trifluoromethyl)phenoxycarbonyl)-10-methylacridinium trifluoromethanesulfonate (16)

The synthesis of **compound 16** was carried out by stirring 2,6-

bis(trifluoromethyl)phenyl acridine-9-carboxylate (**16e**) and methyl triflate in a way similar to that used for **compounds 2 (section 2.2.2)**, **7 (section 3.2.1.2)**, and **12 (section 3.2.6.2)**, as illustrated in **figure 3.29**. The precipitate in the resulting mixture was filtered, and washed with EtOEt to provide the product (**16**).

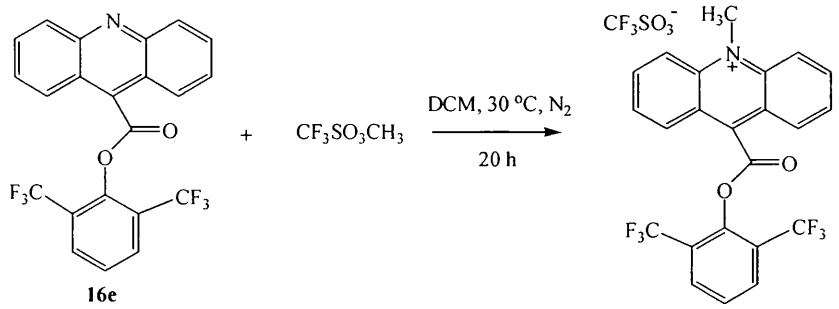


Figure 3.29, synthesis of 9-(2,6-bis(trifluoromethyl)phenoxycarbonyl)-10-methylacridinium trifluoromethanesulfonate (**16**)

In the ^{1}HNMR spectrum of the product, there were 6 peaks in the aromatic region and another one singlet at 4.95 ppm attributed to the methyl group next to the N atom, integrating in a ratio of 2:2:2:2:2:1:3. Peaks for the protons on the acridinium ring resonated at the relatively low field compared to those for the corresponding protons of the starting material. In the ^{13}CNMR spectrum, a peak at 161.7 ppm was attributed to the carbonyl group and a peak at 40.1 ppm was attributed to the methyl group next to the N atom. Another 11 aromatic peaks for the 11 different aromatic carbons were found. However, the signal for CF_3 groups was too weak to be seen. The mass spectrum showing cations at m/z 450 ($[M\text{-}CF_3SO_3]^+$), 221 and 193 (**figure 3.30**), and an anion at m/z, 149 ($[CF_3SO_3]^-$), corroborated the structure of the product.

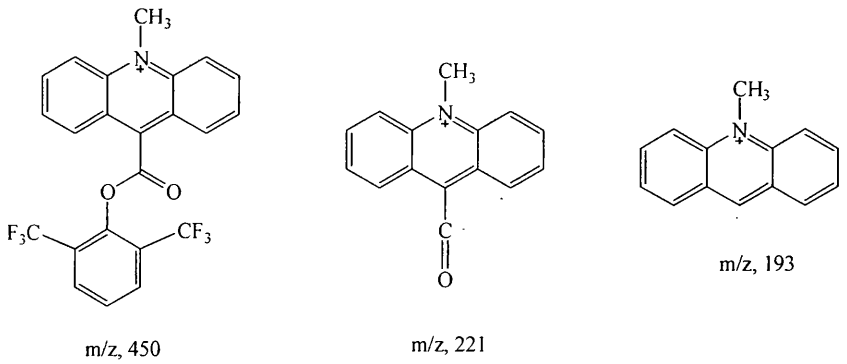


Figure 3.30, MS fragments for **compound 16**

3.2.11 Synthesis of 9-(2,6-bis(trifluoromethyl)phenoxycarbonyl)-10-(10-succinimidyloxycarbonyldecyl)acridinium trifluoromethanesulfonate (17)

Compound 17 was synthesized in a synthetic pathway similar to that used for **compounds 6 (section 2.2.6.4)**, **11 (section 3.2.5)** and **15 (section 3.2.9)**, by refluxing 2,6-bis(trifluoromethyl)phenyl acridine-9-carboxylate (**16e**) and succinimidyl 10-(trifluoromethanesulfonyloxy)undecanoate (**6c**) in DCE for 21 h under N_2, as illustrated in **figure 3.31**. The resulting mixture was evaporated under vacuum, then DCM was used to extract the residue, and EtOEt to precipitate the product. The procedure of re-dissolving and re-precipitating was repeated several times in a way for isolating **compounds 3, 4, 5** and **6.** The ^{1}HNMR spectrum showed impurities in the aromatic region. This suggested that some side reaction occurred, and the side product has similar Rf values to those of **compound 17**. Therefore HPLC (ODS column; mobile phase: CH_3CN + H_2O (90:10); UV detector) was employed to isolate the product. The fractions with R_t (retention time) = 1.79 minutes or so were collected, condensed and injected back, and only one peak appeared as expected. But the ^{1}HNMR showed the signal for the CH_2 group close to the N atom was not around 5.6 ppm as its other analogues, but at 4.57 ppm, so the compound collected from HPLC was not the desired product.

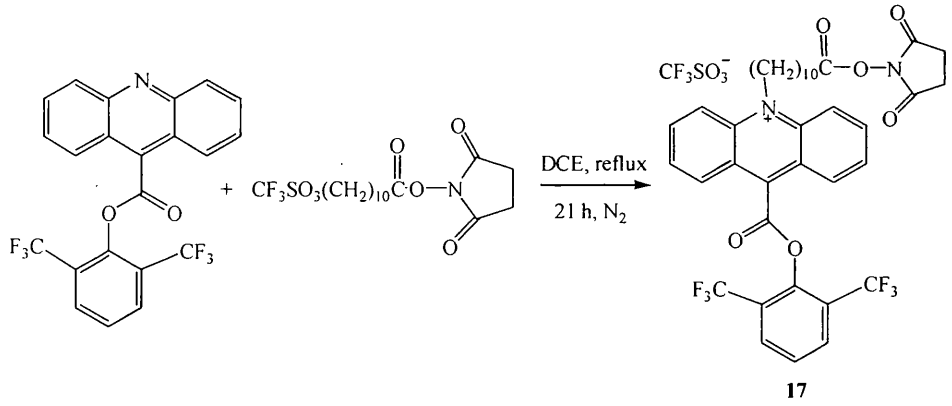
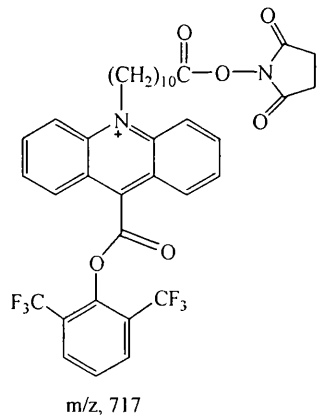


Figure 3.31, synthesis of 9-(2,6-bis(trifluoromethyl)phenoxycarbonyl)-10-(10-succinimidyloxycarbonyldecyl)acridinium trifluoromethanesulfonate (**17**)

Therefore, the crude product was purified by flash chromatography (silica column), eluted with DCM, and then DCM + acetonitrile (3:1). The fractions with Rf 0.19 developed in DCM + acetonitrile (3:1), were pooled and evaporated to give a bright yellow solid in 11% yield. In the ^{1}HNMR spectrum of the product, there were 6

peaks in the aromatic region and 7 peaks in the aliphatic region, integrating in a reasonable ratio of 2:2:2:2:2:1:2:4:2:2:4:10. A triplet at 5.69 ppm was attributed to the CH_2 group next to the N atom, and a singlet at 2.85 ppm was attributed to the protons of the succinimidyloxy moiety. Peaks for the protons on the acridinium ring resonated at the relatively low field compared to the corresponding protons of the starting material. In the ^{13}CNMR spectrum, 3 peaks at 169.7, 169.1 and 161.8 ppm were attributed to the 4 carbons of the 3 different carbonyl groups. Another 11 peaks in total in the aromatic region for 11 carbons, and a peak at 53.3 ppm attributed to the CH_2 group next to the N atom, were found. A peak at 26.0 ppm was attributed to the CH_2 groups of succinimidyloxy moiety. However, no signal for CF_3 groups was found. The coupling of the carbon nucleus with the three F nuclei resulting in 4 split peaks possibly accounted for the weakness of the signal. The ^{19}FNMR spectrum showed 2 peaks at -78.4 ppm ($[CF_3SO_3]^-$) and -59.8 ppm (the 2 CF_3 substituents on the phenoxy group). The mass spectrum showed a cation at m/z 717 ($[M\text{-}CF_3SO_3]^+$) (**figure 3.32**), and an anion at m/z 149 ($[CF_3SO_3]^-$).



m/z, 717

Figure 3.32, the MS fragment for **compound 17**

3.2.12 Attempt to synthesize 9-(2,6-bis(trifluoromethyl)phenoxycarbonyl)-10-(3-succinimidyloxycarbonylpropyl)acridinium trifluoromethanesulfonate (23)

The attempt to synthesize 9-(2,6-bis(trifluoromethyl)phenoxycarbonyl)-10-(3-succinimidyloxycarbonylpropyl)acridinium trifluoromethanesulfonate was performed by using 2,6-bis(trifluoromethyl)phenyl acridine-9-carboxylate (**16e**) (53 mg, 0.12 mmol) and freshly prepared succinimidyl 3-(trifluoromethanesulfonyloxy)-

butanoate (**4b**) (36 mg, 0.11 mmol). The starting materials were refluxed in DCE for 21 h, as illustrated in **figure 3.33**. Unlike the reaction for **compound 17** and other analogues, the mixture turned black after heating for a few hours. TLC showed a yellow spot possessing an Rf value similar to that of **compound 17** and other analogues. An attempt was made to separate the product in a way similar to that used for LiAE, but no pure product was obtained, possibly due to the yield being too poor and side reactions having happened. Due to the strongly electron-withdrawing substituents on the phenoxy group, the electron density on the phenoxy moiety decreases and electrons flow from the acridine ring to the phenoxy group, resulting in the electron density on the acridine ring decreasing too. Therefore, the reactivity of **compound 16e** toward electrophiles decreases and furthermore **compounds 16e** and **4b** have several easily broken moieties, so side reactions are more likely. The reaction was not tried in TCE at higher temperature because of the probability of side reactions.

3.2.13 Attempt to synthesize 9-(2,6-bis(trifluoromethyl)phenoxycarbonyl)-10-(5-succinimidyloxycarbonylpentyl)acridinium trifluoromethanesulfonate (24)

The attempted synthesis of 9-(2,6-bis(trifluoromethyl)phenoxycarbonyl)-10-(5-succinimidyloxycarbonylpentyl)acridinium trifluoromethanesulfonate was performed by refluxing 2,6-bis(trifluoromethyl)phenyl acridine-9-carboxylate (**16e**) (90 mg, 0.21 mmol) and freshly prepared succinimidyl 5-(trifluoromethanesulfonyloxy)hexanoate (**5c**) (75 mg, 0.21 mmol) in DCE for 21 h under N_2 (**figure 3.33**; n = 5; $R_1 = R_2 = CF_3$) . The mixture turned to black after 0.5 h. Although TLC showed a yellow spot with Rf = 0.19 (a Rf value similar to that of **compound 17** and its analogues), when developed in DCM + CH_3CN (3:1), due to a very low yield, no pure product was obtained or characterized. The reaction was repeated for 4 times, but on no occasion was a pure product isolated due to the same reason with for compound **23**.

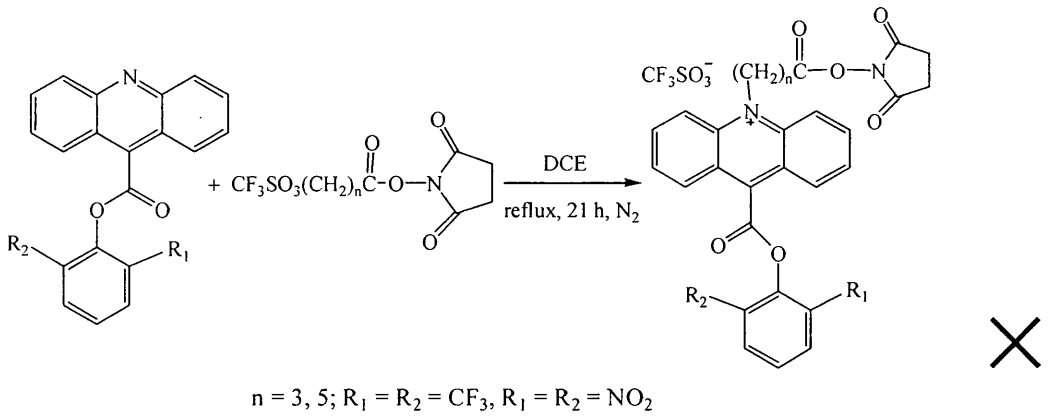


Figure 3.33, the reactions for conjunction of **compounds 16e** and **18a** separately with **4b** and **5c**

3.2.14 The scheme of synthesis of 9-(2,6-dinitrophenoxycarbonyl)-10-methylacridinium trifluoromethanesulfonate (18)

There are 2 steps involved in the synthesis of **compound 18**, by using methyl triflate and 2,6-dinitrophenyl acridine-9-carboxylate (**18a**). **Compound 18a** was prepared from 2,6-dinitrophenol and acridine-9-carboxylic acid chloride (**1b**).

3.2.14.1 Synthesis of 2,6-dinitrophenyl acridine-9-carboxylate (18a)

Compounds 1c, **7a**, **12a** and **16e,** synthesized by stirring acridine 9-carboxylic acid chloride (**1b**) with the corresponding phenols in pyridine for more than 20 h, are analogous to **18a**. Commercial 2,6-dinitrophenol contains 20% water, and stirring the commercial material with **1b** in pyridine would result in the hydrolysis of **1b** into the carboxylic acid **1a**, giving none of the desired product (**18a**). Therefore, the water should first be removed from 2,6-dinitrophenol. The commercial 2,6-dinitrophenol was dissolved in DCM and treated with $MgSO_4$ followed by filtration and evaporation under vacuum. The 2,6-dinitrophenol obtained was used in the next stage without further purification.

A mixture of acridine-9-carboxylic acid chloride (**1b**) and dry 2,6-dinitrophenol dissolved in pyridine was stirred at room temperature (**figure 3.34**), during which, some yellow solid floated in the upper solution, while the by-product, pyridinium

chloride, precipitated at the bottom. The product was isolated by chromatography, but the yield of 2,6-dinitrophenyl acridine-9-carboxylate (**18a**) was only 9%, far lower than that of 2,6-bis(trifluoromethyl)phenyl acridine-9-carboxylate (**16e**) under the same conditions. Therefore, the synthesis was carried out in a different way, by using acridine-9-carboxylic acid (**1a**) and 2,6-dinitrophenol with DCC as dehydrating initiator in THF.[8-11, 13] TLC showed a new spot different from the starting materials, but no pure product was isolated after chromatography, due to the yield being too poor. The reaction was modified by using compound **1b** and 2,6-dinitrophenol, in the presence also of DCC as a dehydrating initiator. This time the product was isolated in 22% yield. The low yield may have resulted not only from hydrolysis, but also possibly as a result of the steric hindrance of the two bulky NO_2 groups. It is also possible that the difficulties with isolation rather than fundamental problems with the reaction itself, resulted in the poor yield. In particular, the product had chromatographic characteristics very close to those of the side product, DCU. The Rf value of **18a** was lower than those of its analogues, compounds **1c**, **7a**, **12a** and **16e**, which made it quite difficult to separate cleanly from DCU.

A possible way to improve the yield would be to add the 2,6-dinitrophenol during the conversion of acridine-9-carboxylic acid (**1a**) to acridine-9-carboxylic acid chloride (**1b**) by refluxing in $SOCl_2$, so that the water in 2,6-dinitrophenol could be removed by the $SOCl_2$ and hydrolysis would be prevented. In such circumstance, there would be no need to have DCC involved in the reaction, and the difficult purification would be avoided. However, this idea has not been put into practice, because the product obtained was enough for use in the following stages.

In the ^{1}HNMR spectrum of **18a**, there were 5 peaks in the aromatic region, integrating in a ratio of 2:2:2:2:3. It seems that two signals overlapped accidentally. In the ^{13}CNMR spectrum, there was a signal at 163.9 ppm, attributed to the carbonyl group; 11 aromatic peaks accounted for 11 different carbons, while there were only 7 different aromatic carbons for the **compound 1b**. The IR spectrum showed a peak at 1775 cm^{-1}. The EI/CI mass spectrum showed a molecular ion cluster at m/z 389 ($[M]^+$) and 390 ($[M+H]^+$), giving the corroboratory evidence for the desired product. The other MS fragments at m/z 206 and 178 were similar to those of **compounds 1c** and **7a,** shown in **figure 3.4**.

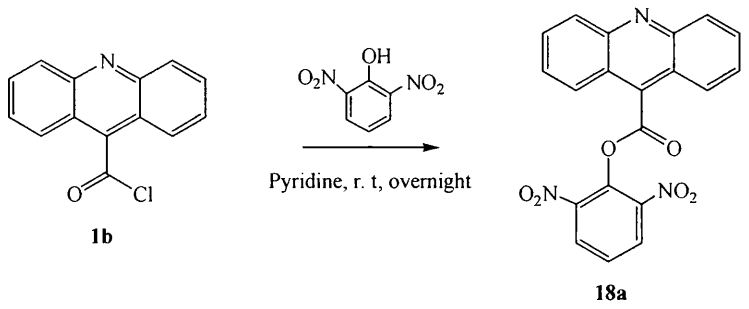


Figure 3.34, synthesis of 2,6-dinitrophenyl acridine-9-carboxylate (**18a**)

3.2.14.2 Synthesis of 9-(2,6-dinitrophenoxycarbonyl)-10-methylacridinium trifluoromethanesulfonate (18)

The synthesis of **compound 18** was carried out by refluxing 2,6-dinitrophenyl acridine-9-carboxylate (**18a**) and methyl triflate in DCE, in a way similar to that used to prepare **compound 2** in **section 2.2.2**, **compound 7** in **section 3.2.1.2** and **compound 12** in **section 3.2.6.2**. The synthetic pathway is illustrated in **figure 3.35**. The product was isolated by filtration followed by washing with EtOEt to give a yellow solid in 46% yield.

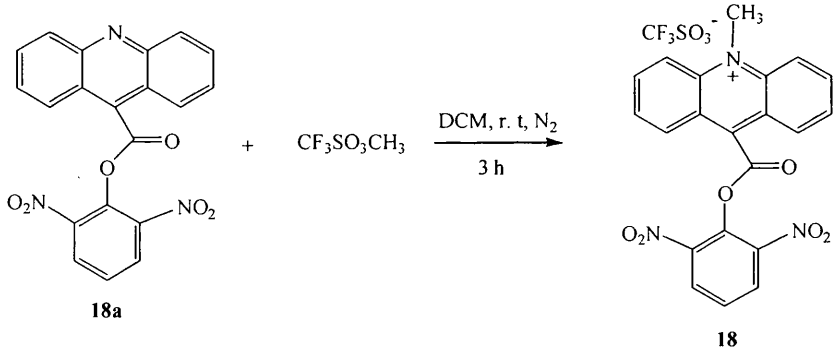


Figure 3.35, synthesis of 9-(2,6-dinitrophenoxycarbonyl)-10-methylacridinium trifluoromethanesulfonate (**18**)

In the ^{1}HNMR spectrum, there were 6 peaks in the aromatic region and a singlet at 4.95 ppm attributed to the methyl group next to the N atom, integrating in a ratio of 2:2:2:2:2:1:3. The peaks for the protons on the acridinium ring resonated at relatively low field compared to those of the starting material. In the ^{13}CNMR spectrum, a peak at 160.4 ppm was attributed to the carbonyl group, 11 aromatic peaks was attributed to the 11 different aromatic carbons, and a peak at 42.0 ppm was attributed to the

methyl group next to the N atom. The IR spectrum gave the absorption at 1767 cm^{-1}. The mass spectrum showed cations at m/z 404 ($[M\text{-}CF_3SO_3]^+$) and 193 (**figure 3.36**), and an anion at m/z 149 ($[CF_3SO_3]^-$).

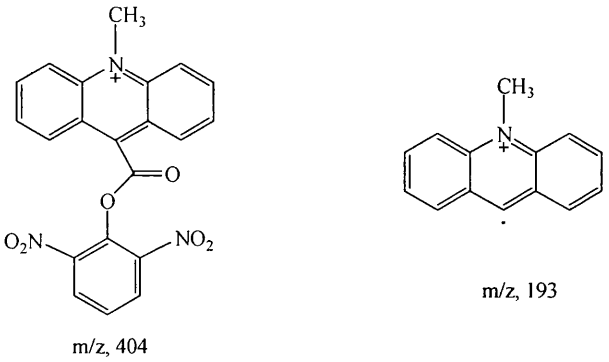


Figure 3.36, the MS fragments for **compound 18**

3.2.15 Synthesis of 9-(2,6-dinitrophenoxycarbonyl)-10-(10-succinimidyloxycarbonyldecyl)acridinium trifluoromethanesulfonate (19)

Compound 19 was synthesized by a synthetic pathway similar to that used for **compound 6** in **section 2.2.6.4**, **compound 11 (section 3.2.5)**, **compound 15 (section 3.2.9)** and **compound 17 (section 3.2.11)** by refluxing 2,6-dinitrophenyl acridine-9-carboxylate (**18a**) and succinimidyl 10-(trifluoromethanesulfonyloxy)undecanoate (**6c**) in DCE for 20 h under N_2, as illustrated in **figure 3.37**. The product was isolated by chromatography in 14% yield.

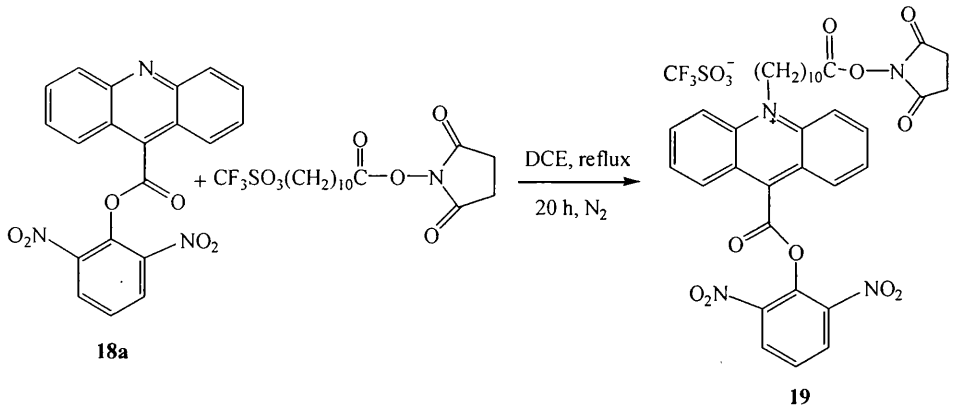
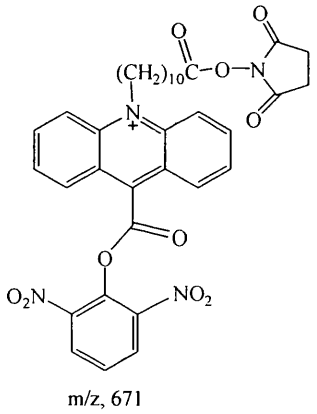


Figure 3.37, synthesis of 9-(2,6-dinitrophenoxycarbonyl)-10-(10-succinimidyloxycarbonyldecyl)acridinium trifluoromethanesulfonate (**19**)

In the ^{1}HNMR spectrum, there were 5 peaks in the aromatic region, two of them overlapped accidentally, and 6 peaks in the aliphatic region, all integrating in a ratio of 4:2:2:2:1:2:4:2:2:4:10. A triplet at 5.48 ppm and a singlet at 2.69 ppm were attributed to the CH_2 group linked to the N atom and the succinimidyloxy moiety, respectively. The peaks for the protons on the acridinium ring resonated at relatively low field compared to those of the starting material. In the ^{13}CNMR spectrum, 3 peaks at 169.7, 169.1 and 161.1 ppm were attributed to the 4 carbons of the 3 different carbonyl groups; there were also 11 peaks in total in the aromatic region, a typical peak at 53.1 ppm for the CH_2 group next to the N atom, and a peak at 26.0 ppm for the CH_2 groups of succinimidyloxy moiety. The IR spectrum showed one overlapped absorption for carbonyl groups at 1735 cm^{-1}. The mass spectrum showed a cation at m/z 671 ($[M\text{-}CF_3SO_3]^+$) (**figure 3.38**) and an anion at m/z 149 ($[CF_3SO_3]^-$), as expected.



m/z, 671

Figure 3.38, MS fragment for **compound 19**

3.2.16 Attempts to synthesize 9-(2,6-dinitrophenoxycarbonyl)-10-(3-succinimidyloxycarbonylpropyl)acridinium trifluoromethanesulfonate (25) and 9-(2,6-dinitrophenoxycarbonyl)-10-(5-succinimidyloxycarbonylpentyl)-acridinium trifluoromethanesulfonate (26)

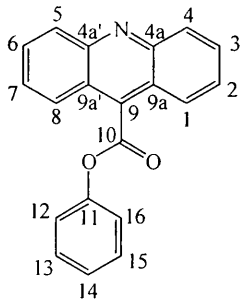
2,6-Dinitrophenyl acridine-9-carboxylate (**18a**) and freshly prepared succinimidyl 5-(trifluoromethanesulfonyloxy)hexanoate ester (**5c**) (33 mg, 0.084 mmol) were refluxed in DCE in a round bottom flask (5 ml) under N_2. The resulting mixture turned black after heating for 1 hour or so. The experiment was attempted several times and isolation of the product was tried by chromatography, or by the way used

to purify LiAE (involving solvent washes), but no pure product was obtained in any case, due to the small amount of the sample and too poor yield. A similar result was obtained for the attempted synthesis of 9-(2,6-dinitrophenoxycarbonyl)-10-(3-succinimidyloxycarbonylpropyl)acridinium trifluoromethanesulfonate by using the corresponding starting materials. The attempted synthetic route is illustrated in **figure 3.33** (n = 3 or 5; $R_1 = R_2 = NO_2$) in **section 3.2.13**.

3.3 Experimental details and charaterization

3.3.1.1 Synthesis of phenyl acridine-9-carboxylate (7a)

In a round bottom flask (25 ml) equipped with a $CaCl_2$ drying tube and a magnetic stirrer bar, acridine-9-carboxylic acid chloride (290 mg, 1.20 mmol) in pyridine (15 ml) was heated to 50 °C. After the solid had dissolved completely, the solution was cooled to room temperature, phenol (141 mg, 1.50 mmol) was added and the mixture was stirred vigorously overnight. The resulting mixture was stripped of pyridine to leave a brown solid. DCM (10 ml) was used to extract the product and the extract was subjected to column chromatography, eluted with toluene + EtOAc (4:1). The fractions with Rf = 0.51 were collected and evaporated to give a pale yellow solid (226 mg, 63% yield), m.p. 192 - 193 °C (**lit.**[13], 193 °C).



^{1}HNMR (400 MHz, chloroform-d, δ, ppm), 8.26 (2H, H_1, H_8, d, J = 9 Hz), 8.18 (2H, H_4, H_5, d, J = 9 Hz), 7.79 (2H, H_3, H_6, dd, J = 9, 7 Hz), 7.61 (2H, H_2, H_7, dd, J = 9, 7 Hz), 7.49 (2H, H_{13}, H_{15}, t, J = 9 Hz), 7.41 (2H, H_{12}, H_{16}, d, J = 9 Hz), 7.32 (1H, H_{14}, t, J = 9 Hz).

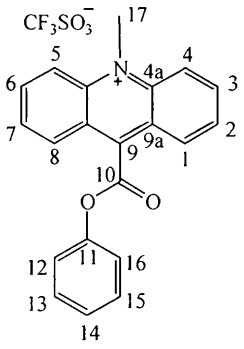
^{13}CNMR (400 MHz, chloroform-d, δ, ppm), 166.3 (C_{10}), 150.9 (C_{11}), 149.0 (C_{4a}/$C_{4a'}$), 135.6 (C_9), 130.9, 130.4, 130.3 (C_4/C_5, C_3/C_6, C_{13}/C_{15}), 128.0 (C_2/C_7), 127.1 (C_1/C_8), 125.3 (C_{14}), 122.8 (C_{9a}/$C_{9a'}$), 121.9 (C_{12}/C_{16}).

IR (υ, cm^{-1}), 1747 (C=O).

MS (m/z), EI: 299 (M^+, 78%), 206 (97%), 178 (100%).

3.3.1.2 Synthesis of 9-(phenoxycarbonyl)-10-methylacridinium trifluoromethanesulfonate (7)

To a round bottom flask (5 ml), equipped with a stirrer bar, phenyl acridine-9-carboxylate (65 mg, 0.22 mmol) was added. The flask was flushed with nitrogen. Afterwards dry dichloromethane (2 ml) and methyl triflate (90 µl, 130 mg, 0.80 mmol) were introduced with syringes respectively. The mixture was stirred at room temperature for 3 h. The resulting mixture was filtered, and the precipitate was washed with DCM (1 ml), EtOAC (1 ml), and then EtOEt (1 ml) to give a yellow solid (83 mg, 50% yield), m.p. 234 °C.



^{1}HNMR (400 MHz, DMSO-d_6, δ, ppm), 9.22 (2H, H_4, H_5, d, J = 9 Hz), 8.89 (2H, H_1, H_8, dd, J = 8, 1 Hz), 8.79 (2H, H_3, H_6, ddd, J = 9, 7, 1 Hz), 8.41 (2H, H_2, H_7, dd, J = 8, 7 Hz), 7.91 (2H, H_{12}, H_{16}, d, J = 8 Hz), 7.81 (2H, H_{13}, H_{15}, apparent t, J = 8 Hz), 7.65 (1H, H_{14}, t, J = 8 Hz).

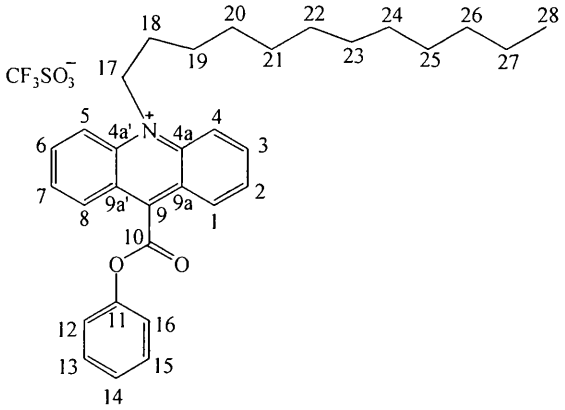
^{13}CNMR (400 MHz, DMSO-d_6, δ, ppm), 164.9 (C_{10}), 151.4 (C_{11}), 149.3 (C_9), 143.9 (C_{4a}/$C_{4a'}$), 140.9, 131.5, 131.2, 129.2 128.9 (C_{13}/C_{15}, C_4/C_5, C_3/C_6, C_2/C_7, C_1/C_8), 124.2 (C_{9a}/$C_{9a'}$), 123.2 (C_{14}), 121.3 (C_{12}/C_{16}). 41.1 (C_{17}).

IR (υ, cm^{-1}), 1760 (C=O).

MS (m/z), ES^+: 314 ($[M\text{-}CF_3SO_3]^+$, 100%), 193 (71%); ES^-: 149 ($[CF_3SO_3]^-$, 100%). Acc-MS: calculated mass: 314.1176 ($[M\text{-}CF_3SO_3]^+$); measured mass: 314.1175.

3.3.2 Synthesis of 9-(phenoxycarbonyl)-10-dodecylacridinium trifluoromethanesulfonate (8)

To a round bottom flask (5 ml) equipped with a stirrer bar and a condenser (25 ml), phenyl acridine-9-carboxylate (96 mg, 0.32 mmol) and dodecyl triflate (80 mg, 0.25 mmol) were added. The flask was flushed with N_2 for 5 min. Then DCE was introduced with a syringe. The mixture was gently refluxed for 21.5 h. The volatiles were evaporated under vacuum. To the residue, DCM (0.6 ml) was added to extract the product. The extract was precipitated with diethyl ether (3 ml), and the upper solution was removed with a pipette carefully. The yellow precipitate was re-dissolved in DCM (0.6 ml), and re-precipitated with diethyl ether repeatedly (x 6) to give a greenish yellow solid (30.3 mg, 19.5% yield), m.p. 110-111 °C.



^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 8.72 (2H, H_4, H_5, d, J = 9 Hz), 8.47 (2H, H_3, H_6, ddd, J = 9, 7, 1 Hz), 8.34 (2H, H_1, H_8, dd, J = 8, 1 Hz), 8.00 (2H, H_2, H_7, dd, J = 8, 7 Hz), 7.49 (2H, t, H_{13}, H_{15}, J = 8 Hz), 7.39 (2H, H_{12}, H_{16}, t, J = 8 Hz), 7.34 (1H, H_{14},

t, J = 8 Hz), 5.52 (2H, H_{17}, t, J = 8 Hz), 2.09 (2H, H_{27}, m), 1.69-1.59 (4H, m), 1.39-1.30 (2H, m) and 1.27-1.12 (12H, m), (H_{18}, H_{19}, H_{20}, H_{21}, H_{22}, H_{23}, H_{24}, H_{25}, H_{26}), 0.64 (3H, H_{28}, t, J = 7 Hz).

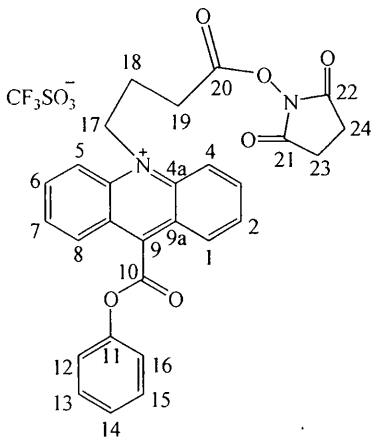
^{13}CNMR (400 Hz, chloroform-d, δ, ppm), 163.3 (C_{10}), 150.2 (C_{11}), 148.7 (C_9), 141.7 (C_{4a}/$C_{4a'}$), 140.6, 130.6, 130.1, 128.3, 128.0, (C_{13}/C_{15}, C_2/C_7, C_4/C_5, C_6/C_3, C_1/C_8), 123.3 (C_{9a}/$C_{9a'}$), 121.4 (C_{14}), 119.9 (C_{12}/C_{16}), 52.6 (C_{17}), 32.3 (C_{26}), 30.1, 30.0 (2 lines), 29.9, 29.8 (2 lines), 29.7 (C_{19}, C_{20}, C_{21}, C_{22}, C_{23}, C_{24}, C_{25}), 27.0 (C_{18}), 23.1 (C_{27}), 14.5 (C_{28}).

IR (υ, cm^{-1}), 1771 (C=O).

MS (m/z), ES^+: 468.5 ($[M\text{-}CF_3SO_3]^+$, 100%); ES^-: 149 ($[CF_3SO_3^-]$, 100%). Acc-MS: calculated mass: 468.2897 ($[M\text{-}CF_3SO_3]^+$); measured mass: 468.2900.

3.3.3 Synthesis of 9-(phenoxycarbonyl)-10-(3-succinimidyloxycarbonylpropyl) acridinium trifluoromethanesulfonate (9)

To a round bottom flask (5 ml), equipped with a stirrer bar and a condenser, freshly prepared succinimidyl 4-(trifluoromethanesulfonyloxy)butanoate (51 mg, 0.15 mmol) and phenyl acridine-9-carboxylate (78 mg, 0.26 mmol) were added. The flask was flushed with nitrogen. Then dry DCE (2 ml) was introduced with a syringe. The mixture was gently refluxed for 22.5 h. The volatiles were evaporated under vacuum. DCM (0.6 ml) was added to the residue to extract the product. The extract was precipitated with diethyl ether (3 ml), and the upper solution was removed with a pipette carefully. The yellow precipitate was re-dissolved in DCM (0.6 ml), and re-precipitated with diethyl ether repeatedly (× 7) to give a greenish yellow solid (2.6 mg, 2.7% yield), m.p. 110-111 °C.



^{1}HNMR (400 Hz, acetonitrile-d_3, δ, ppm), 9.03 (2H, H_4, H_5, d, J = 8 Hz), 8.52 (2H, H_3, H_6, ddd, J = 8, 7, 1 Hz), 8.36 (2H, H_1, H_8, dd, J = 8, 1 Hz), 8.00 (2H, H_2, H_7, dd, J = 8, 7 Hz), 7.50 (2H, H_{13}, H_{15}, t, J = 8 Hz), 7.38 (2H, H_{12}, H_{16}, d, J = 8 Hz), 7.36 (1H, H_{14}, t, J = 8 Hz), 5.63 (2H, H_{17}, t, J = 8 Hz), 3.24 (2H, H_{19}, t, J = 7 Hz), 2.82 (4H, H_{23}, H_{24}, s), 2.55 (2H, H_{18}, approx quintet).

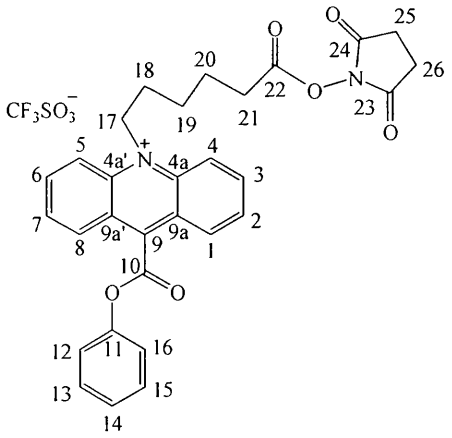
IR (υ, cm^{-1}), 1734 (C=O).

MS (m/z), ES^+: 483 ($[M\text{-}CF_3SO_3]^+$, 100%), 368 ($[M\text{-}CF_3SO_3\text{-}C_4H_5NO_3]^+$, 25%); ES^-: 149 ($[CF_3SO_3]^-$, 100%). Acc-MS: calculated mass: 483.1551 ($[M\text{-}CF_3SO_3]^+$); measured mass: 483.1553.

3.3.4 Synthesis of 9-(phenoxycarbonyl)-10-(5-succinimidyloxycarbonylpentyl) acridinium trifluoromethanesulfonate (10)

To a round bottom flask (5 ml), equipped with a stirrer bar and a condenser, phenyl acridine-9-carboxylate (77 mg, 0.26 mmol) and freshly prepared succinimidyl 6-(trifluoromethanesulfonyloxy)hexanoate (88 mg, 0.25 mmol) were added. The flask was flushed with nitrogen, and then dry DCE was introduced with a syringe. The mixture was refluxed under N_2 for 22.5 h. The resulting mixture was stripped of solvent on a rotary evaporator to leave a sticky yellow gum, which was chromatographed on a silica column, eluted with DCM firstly, then, DCM + CH_3CN (4:1), and finally DCM + CH_3CN (3:1). The fractions with Rf = 0.15 developed in

DCM + CH_3CN (3:1) were evaporated under vacuum to give a yellow solid (15 mg, 9% yield) m.p. 201-202 °C.



^{1}HNMR (400 Hz, acetone-d_6, δ, ppm): 8.88 (2H, H_4, H_5, d, J = 8 Hz), 8.55 (2H, H_1, H_8, d, J = 8 Hz), 8.46 (2H, H_3, H_6, dd, J = 8, 7 Hz), 8.08 (2H, H_2, H_7, dd, J = 8, 7 Hz), 7.53 (2H, H_{12}, H_{16}, d, J = 8 Hz), 7.46 (2H, H_{13}, H_{15}, apparent t, J = 8 Hz), 7.31 (1H, H_{14}, t, J = 8 Hz), 5.57 (2H, H_{17}, t, J = 8 Hz), 2.71 (4H, H_{25}, H_{26}, s), 2.55 (2H, H_{21}, t, J = 7 Hz), 2.32-2.20 (2H, H_{18}, m), 1.83-1.71 (4H, H_{19}, H_{20}, m).

^{13}CNMR (400 Hz, acetone-d_6, δ, ppm), 171.0 (C_{23}/C_{24}), 170.0 (C_{22}), 164.8 (C_{10}), 151.4 (C_{11}), 149.8 (C_9), 143.2 (C_{4a}/$C_{4a'}$), 141.2, 131.5, 131.1, 129.5, 128.8, (C_{13}/C_{15}, C_1/C_8, C_3/C_6, C_4/C_5, C_2/C_7), 124.5 (C_{9a}/$C_{9a'}$), 123.0 (C_{14}), 120.7 (C_{12}/C_{16}), 53.0 (C_{17}), 31.4 (C_{21}), 30.0 (C_{19}), 26.7 (C_{25}/C_{26}), 26.6, 25.5 (C_{18}, C_{20}).

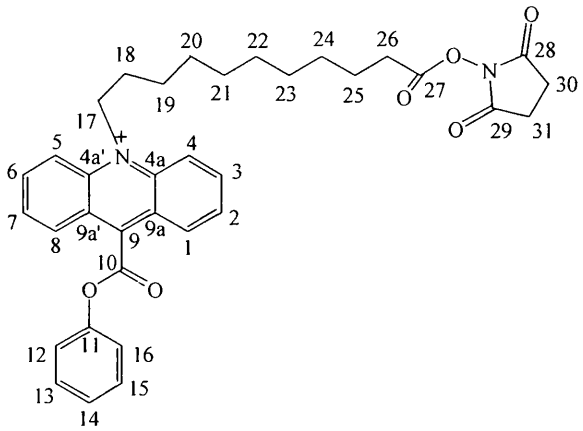
IR (υ, cm^{-1}), 1733 (C=O).

MS, ES^+: 565 (17%), 511 ($[M\text{-}CF_3SO_3]^+$, 100%); ES^-: 149 ($[CF_3SO_3]^-$, 100%). Acc-MS: calculated mass: 511.1864 ($[M\text{-}CF_3SO_3]^+$); measured mass: 511.1863.

3.3.5 Synthesis of 9-(phenoxycarbonyl)-10-(10-succinimidyloxycarbonyldecyl) acridinium trifluoromethanesulfonate (11)

To a round bottom flask (5 ml), equipped with a stirrer bar and a condenser, phenyl acridine-9-carboxylate (65 mg, 0.22 mmol) and freshly prepared succinimidyl 11-(trifluoromethanesulfonyloxy)undecanoate (43 mg, 0.10 mmol) were added. The

flask was flushed with nitrogen, and then dry DCE was introduced with a syringe. The starting materials were refluxed under N_2 for 21 h. The solvent was evaporated on a rotary evaporator to leave a sticky yellow gum, which was chromatographed on a silica column, eluted with DCM firstly, and then DCM + CH_3CN (3:1). The fractions with Rf = 0.15 developed in DCM + CH_3CN (4:1) were evaporated under vacuum to give a yellow solid (30 mg, 41% yield), m.p. 88-89 °C.



^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 8.78 (2H, H_4, H_5, d, J = 8 Hz), 8.54 (2H, H_3, H_6, dd, J = 8, 7 Hz), 8.42 (2H, H_1, H_8, d, J = 8 Hz), 8.09 (2H, H_2, H_7, dd, J = 8, 7 Hz), 7.55 (2H, H_{13}, H_{15}, apparent t, J = 8 Hz), 7.46 (2H, H_{12}, H_{16}, d, J = 8 Hz), 7.40 (1H, H_{14}, t, J = 8 Hz), 5.58 (2H, H_{17}, t, J = 8 Hz), 2.83 (4H, H_{30}, H_{31}, s), 2.58 (2H, H_{26}, t, J = 7 Hz), 2.21-2.10 (2H, m), 1.77-1.66 (4H, m), 1.46-1.30 (4H, m), 1.33-1.24 (6H, m), (H_{18}, H_{19}, H_{20}, H_{21}, H_{22}, H_{23}, H_{24}, H_{25}).

^{13}CNMR (400 Hz, chloroform-d, δ, ppm), 169.7 (C_{28}/C_{29}), 169.1 (C_{27}), 163.4 (C_{10}), 150.1 (C_{11}), 148.7 (C_9), 141.6 (C_{4a}/$C_{4a'}$), 140.8, 130.6, 130.3, 128.3, 127.9, (C_{13}/C_{15}, C_1/C_8, C_2/C_7, C_3/C_6, C_4/C_5), 123.3 (C_{9a}/$C_{9a'}$), 121.4 (C_{14}), 119.8 (C_{12}/C_{16}), 52.6 (C_{17}), 31.3 (C_{26}), 30.0, 29.6, 29.5, 29.4, 29.2, 29.0, 26.9, (C_{18}, C_{19}, C_{20}, C_{21}, C_{22}, C_{23}, C_{24}), 26.0 (C_{30}/C_{31}), 24.9 (C_{25})

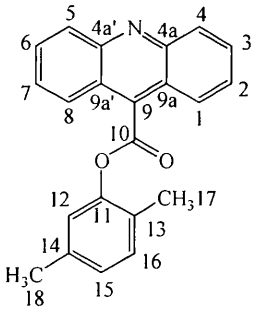
^{19}FNMR (400 Hz, chloroform-d, δ, ppm), -78.2.

IR (υ, cm^{-1}), 1733 (C=O).

MS (m/z), ES^+: 667 (68%), 635 (58%), 613 (76%), 581 ($[M\text{-}CF_3SO_3]^+$, 100%), 506 ($[M\text{-}CF_3SO_3\text{-}Ph+2H]^+$, 98%); ES^-: 149 ($[CF_3SO_3]^-$, 100%). Acc-MS: calculated mass: 581.2646 ($[M\text{-}CF_3SO_3]^+$); measured mass: 581.2645.

3.3.6.1 Synthesis of 2,5-dimethylphenyl acridine-9-carboxylate (12a)

In a round bottom flask (25 ml) equipped with a $CaCl_2$ drying tube and a magnetic stirrer bar, acridine-9-carboxylic acid chloride (425 mg, 1.90 mmol) in pyridine was heated to 50 °C, after the solid dissolved completely, the solution was cooled to room temperature, and then 2,5-dimethylphenol (267 mg, 2.18 mmol) was added. The mixture was stirred vigorously at room temperature overnight. The resulting mixture was stripped of pyridine to leave a brown residue. The residue was taken up in DCM (10 ml) and filtered, and the filtrate was condensed and loaded onto a column (silica) for chromatography, eluted with toluene + EtOAc (4:1). The fractions with Rf = 0.51 were pooled and evaporated under vacuum to give a pale yellow solid (424 mg, 68% yield), m.p. 187 °C.



^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 8.26, (2H, H_1, H_8, d, J = 8 Hz), 8.22 (2H, H_4, H_5, d, J = 8 Hz), 7.79 (2H, H_3, H_6, dd, J = 8, 7 Hz), 7.62 (2H, H_2, H_7, dd, J = 8, 7 Hz), 7.20 (1H, H_{12}, s), 7.18 (1H, H_{16}, d, J = 8 Hz), 7.03 (1H, H_{15}, d, J = 8 Hz), 2.39 (3H, H_{17} or H_{18}, s), 2.29 (3H, H_{17} or H_{18}, s).

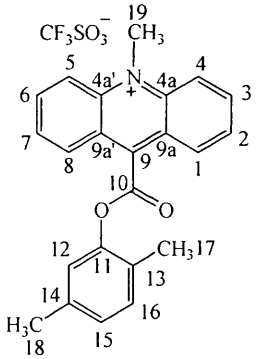
^{13} CNMR (400 MHz, chloroform-d, δ, ppm), 166.3 (C_{10}), 149.4 (C_{11}), 149.1 ($C_{4a}/C_{4a'}$), 137.8 (C_{14}), 136.5 (C_9), 131.8 (C_{16}), 130.8 (C_4/C_5), 130.6 (C_3/C_6), 128.0 (C_{15}), 127.9 (C_2/C_7), 127.1 (C_{13}), 125.3 (C_1/C_8), 122.9 ($C_{9a}/C_{9a'}$), 122.7 (C_{12}), 21.4 (C_{18}), 16.9 (C_{17}).

IR (υ, cm^{-1}), 1748 (C=O).

MS (m/z), EI: 327 ($[M]^+$, 5%), 206 (100%), 178 (82%), 77 (78%); CI: 328 ($[M+H]^+$, 52%), 206 (18%), 180 (100%).

3.3.6.2 Synthesis of 9-(2,5-dimethylphenoxycarbonyl)-10-methylacridinium trifluoromethanesulfonate (12)

To a round bottom flask (5 ml), equipped with a stirrer bar, 2,5-dimethylphenyl acridine-9-carboxylate (140 mg, 0.43 mmol) was added. The flask was flushed with nitrogen via a septum for 5min, and then dry DCM (2 ml) and methyl triflate (130 ul, 188.5 mg, 1.15 mmol) were introduced with syringes respectively. The mixture was stirred at room temperature for 3 h. The resulting mixture was filtered, and the precipitate was washed with DCM + EtOEt 3:1 (5 × 4 ml) to leave a yellow solid (144 mg) in 68% yield, m.p. ≧221 °C (oil appeared at 221 °C and turned to black, but the compound didn't melt completely until 229 °C).



^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 8.73 (2H, H_4, H_5, d, J = 9 Hz), 8.35 (2H, H_3, H_6, dd, J = 9, 7 Hz), 8.32 (2H, H_1, H_8, d, J = 8 Hz), 7.91 (2H, H_2, H_7, dd, J = 8, 7 Hz), 7.11 (1H, H_{12}, s), 7.08 (1H, H_{16}, d, J = 8 Hz), 6.97 (1H, H_{15}, d, J = 8 Hz), 4.99 (3H, H_{19}, s), 2.30 (3H, H_{17} or H_{18}, s), 2.16 (3H, H_{17} or H_{18}, s).

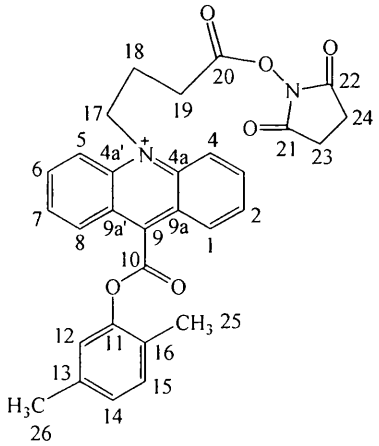
^{13}CNMR (400 MHz, acetonitrile-d_3, δ, ppm), 165.1 (C_{10}), 150.1 (C_{11}), 149.1 (C_9), 144.0 (C_{4a}/$C_{4a'}$), 141.3, 131.4, 129.4, 120.9 (C_1/C_8, C_2/C_7, C_3/C_6, C_4/C_5), 139.6 (C_{14}), 133.3 129.9, 123.9, (C_{12}, C_{15}, C_{16},), 128.4 (C_{9a}/$C_{9a'}$), 124.6 (C_{13}), 41.2 (C_{19}), 21.5 (C_{18}), 17.2 (C_{17}).

IR (υ, cm^{-1}), 1747 (C=O).

MS (m/z), ES^+: 396 (9%), 342 ($[M\text{-}CF_3SO_3]^+$, 100%), 252 (21%), 220 (21%), 193 (68%); ES^-: 149 ($[CF_3SO_3]^-$, 100%). Acc-MS: calculated mass: 342.1489 ($[M\text{-}CF_3SO_3]^+$); measured mass: 342.1489.

3.3.7 Synthesis of 9-(2,5-dimethylphenoxycarbonyl)-10-(3-succinimidyloxy-carbonylpropyl)acridinium trifluoromethanesulfonate (13)

To a round bottom flask (5 ml), equipped with a stirrer bar and a condenser, 2,5-dimethylphenyl acridine-9-carboxylate (43.2 mg, 0.13 mmol) and freshly prepared succinimidyl 4-(trifluoromethanesulfonyloxy)butanoate (30.0 mg, 0.09 mmol) were added. The system was flushed with nitrogen, and then DCE was introduced with a syringe. The mixture was refluxed under N_2 for 20 h. The resulting mixture was stripped of solvent on a rotary evaporator to leave a brown residue, which was taken up in DCM (1 ml), and then precipitated with diethyl ether (3 ml). The precipitate was re-dissolved in DCM, and re-precipitated repeatedly, until TLC showed absence of the starting materials (× 6). A yellow solid (1.6 mg, 2.7% yield) was obtained, m.p. 98-99 °C.



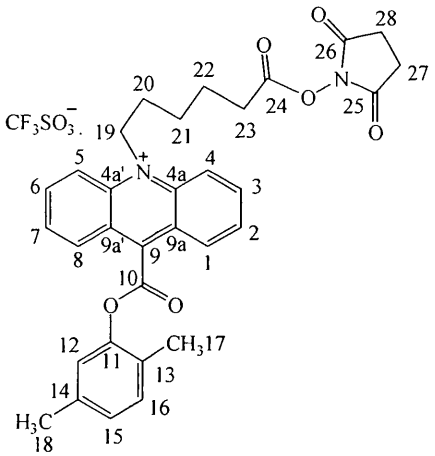
^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 9.27 (2H, H_4, H_5, d, J = 9 Hz), 8.60 (2H, H_1, H_8, d, J = 9 Hz), 8.75 (2H, H_3, H_6, apparent t, J = 8 Hz), 8.23 (2H, H_2, H_7, apparent t, J = 8 Hz), 7.41 (1H, H_{12}, s), 7.39 (1H, d, H_{15}, J = 8 Hz), 7.27 (1H, H_{14}, d, J = 8 Hz), 5.90-5.76 (2H, H_{17}, t, J = 8 Hz), 3.46 (2H, H_{19}, t, J = 8 Hz), 3.03 (4H, H_{23}, H_{24}, s), 2.79 (2H, H_{18}, apparent quintet), 2.59 (3H, H_{25} or H_{26}, s), 2.46 (3H, H_{25} or H_{26}, s).

IR (υ, cm^{-1}), 1737 (C=O).

MS (m/z), ES^+: 565 (53%), 543 (58%), 511 ($[M\text{-}CF_3SO_3]^+$, 100%), 436 (57%), 414 (68%); ES^-: 149 ($[CF_3SO_3]^-$, 100%). Acc-MS: calculated mass: 511.1864 ($[M\text{-}CF_3SO_3]^+$); measured mass: 511.1867.

3.3.8 Synthesis of 9-(2,5-dimethylphenoxycarbonyl)-10-(5-succinimidyloxycarbonylpentyl)acridinium trifluoromethanesulfonate (14)

To a round bottom flask (5 ml), equipped with a stirrer bar and a condenser, 2,5-dimethylphenyl acridine-9-carboxylate (36.1 mg, 0.11 mmol) and freshly prepared succinimidyl 6-(trifluoromethanesulfonyloxy)hexanoate (35.6 mg, 0.099 mmol) were added. The system was flushed with nitrogen, and then dry DCE was introduced with a syringe. The mixture was refluxed under N_2 for 21 h. The resulting mixture was stripped of solvent on a rotary evaporator, and then was taken up in DCM (0.5 ml) for loading onto a mini silica (0.8 g) column for chromatography, eluted with DCM firstly until TLC showed all starting materials were washed through out of the column, then with DCM + methanol (2:1). The fractions with Rf = 0.29 developed in DCM + methanol (2:1) were pooled and evaporated to give a bright yellow solid (13.4 mg, 20% yield), m.p. 87-88 °C.



^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 8.67 (2H, H_4, H_5, d, J = 9 Hz), 8.36 (2H, H_3, H_6, dd, J = 9, 7, 1 Hz), 8.25 (2H, H_1, H_8, dd, J = 9, 1 Hz), 7.89 (2H, H_2, H_7, dd, J = 9, 7 Hz), 7.07 (1H, H_{12}, s), 7.03 (1H, H_{16}, d, J = 8 Hz), 6.90 (1H, H_{15}, d, J = 8 Hz), 5.39 (2H, H_{19}, t, J = 8 Hz), 2.60 (4H, H_{27}, H_{28}, s), 2.41-2.47 (2H, H_{23}, t, J = 7 Hz), 2.24

(3H, H_{17} or H_{18}, s), 2.11 (3H, H_{17} or H_{18}, s), 2.09-2.00 (2H, m) and 1.69 (4H, m), (H_{20}, H_{21}, H_{22}).

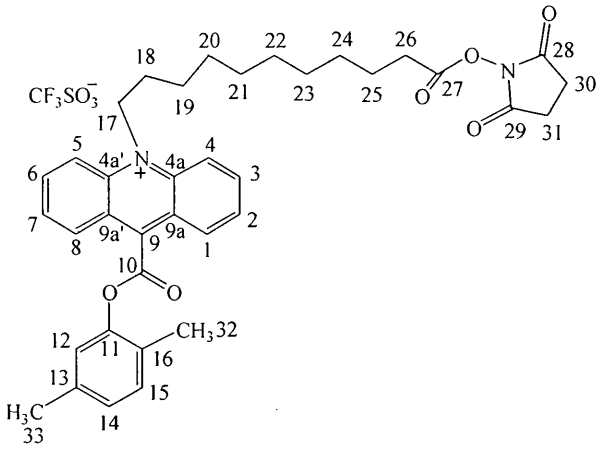
^{13}CNMR (400 MHz, chloroform-d, δ, ppm), 169.8 (C_{25}/C_{26}), 168.9 (C_{24}), 163.2 (C_{10}), 149.1 (C_{11}), 148.7 (C_9), 141.7 (C_{4a}/$C_{4a'}$), 140.9, 132.1, 130.2, 128.8, 128.1, 122.1, 120.0 (C_1/C_8, C_2/C_7, C_3/C_6, C_4/C_5, C_{12}, C_{15}, C_{16}) 138.3 (C_{14}), 126.6 (C_{13}), 123.4 (C_{9a}/$C_{9a'}$), 52.3 (C_{19}), 30.9 (C_{23}), 29.2 (C_{21}), 26.0 (C_{27}/C_{28}), 25.5 (C_{20}), 24.6 (C_{22}), 21.4 (C_{18}), 16.8 (C_{17}).

IR (υ, cm^{-1}), 1737 (C=O).

MS (m/z), ES^+: 593 (32%), 539 ($[M\text{-}CF_3SO_3]^+$, 100%), 464 (33%), 442 (22%), 424 (14%); ES^-: 149 ($[CF_3SO_3]^-$, 100%). Acc-MS: calculated mass: 539.2177 ($[M\text{-}CF_3SO_3]^+$); measured mass: 539.2180.

3.3.9 Synthesis of 9-(2,5-dimethylphenoxycarbonyl)-10-(10-succinimidyloxycarbonyldecyl)acridinium trifluoromethanesulfonate (15)

To a round bottom flask (5 ml), equipped with a stirrer bar and a condenser, freshly prepared succinimidyl 11-(trifluoromethanesulfonyloxy)undecanoate (76.0 mg, 0.18 mmol) was added, followed by 2,5-dimethylphenyl acridine-9-carboxylate (80.0 mg, 0.25 mmol). The flask was flushed with nitrogen, and then dry DCE was introduced with a syringe. The mixture was refluxed under N_2 for 20 h. The resulting mixture was stripped of solvent under vacuum to leave an orange gum, which was taken up in DCM (1 ml), and then precipitated with EtOEt (3 ml). The upper solution was removed with a pipette, the precipitate was re-dissolved in DCM and re-precipitated with EtOEt repeatedly (×6), until TLC showed only 1 spot, a bright yellow sticky oil which solidified on standing was obtained (46 mg, 34% yield), m.p. 56-60 °C.



^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 8.80 (2H, H_4, H_5, d, J = 9 Hz), 8.57 (2H, H_1, H_8, dd, J = 9, 1 Hz), 8.47 (2H, H_3, H_6, ddd, J = 9, 7, 1 Hz), 8.12 (2H, H_2, H_7, dd, J = 9, 7 Hz), 7.29 (1H, H_{12}, s), 7.23 (1H, H_{15}, d, J = 8 Hz), 7.11 (1H, H_{14}, d, J = 8 Hz), 5.59 (2H, H_{17}, t, J = 8 Hz), 2.83 (4H, H_{30}, H_{31}, s), 2.58 (2H, H_{26}, t, J = 7 Hz), 2.44 (3H, H_{32} or H_{33}, s), 2.31 (3H, H_{32} or H_{33}, s), 2.26-2.02 (2H, m), 1.77-1.67 (4H, m) and 1.49-1.25 (10H, m), (H_{18}, H_{19}, H_{20}, H_{21}, H_{22}, H_{23}, H_{24}, H_{25}).

^{13}CNMR (400 Hz, chloroform-d, δ, ppm), 169.8 (C_{28}/C_{29}), 169.1 (C_{27}), 163.3 (C_{10}), 149.0 (C_{11}), 148.7 (C_9), 141.6 (C_{4a}/$C_{4a'}$), 140.8, 132.1, 130.2, 128.7, 128.2, 122.2, 119.9 (C_1/ C_8, C_2/ C_7, C_3/ C_6, C_4/C_5, C_{12}, C_{14}, C_{15}), 138.3 (C_{13}), 126.6 (C_{16}), 123.4 (C_{9a}/$C_{9a'}$), 52.6 (C_{17}), 31.3 (C_{26}), 30.0, 29.6, 29.5, 29.4, 29.2, 29.0, 26.9, 24.9 (C_{18}, C_{19}, C_{20}, C_{21}, C_{22}, C_{23}, C_{24}, C_{25}), 26.0 (C_{30}/C_{31}), 21.4 (C_{33}), 16.8 (C_{32}).

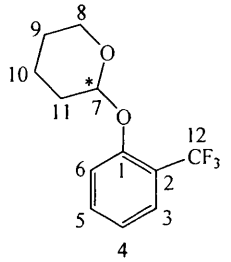
^{19}FNMR (400 MHz, chloroform-d, δ, ppm), -78.2.

IR (υ, cm^{-1}), 1734 (C=O).

MS (m/z), ES^+: 663 (7%), 609 ($[M\text{-}CF_3SO_3]^+$, 100%), 512 (9%), 481 (10%); ES^-: 149 ($[CF_3SO_3]^-$, 100%). Acc-MS: calculated mass: 609.2959 ($[M\text{-}CF_3SO_3]^+$); measured mass: 609.2965.

3.3.10.1 Synthesis of 2-(trifluoromethyl)phenol tetrahydropyranyl ether (16b)

To a round bottom flask (50ml), equipped with a stirrer bar and a condenser, 2-trifluoromethylphenol (1.00 g, 6.17 mmol) and 3,4-dihydropyran (2 ml) were added, followed by a few crystals of toluenesulfonic acid monohydrate ($TsOH.H_2O$). Then diethyl ether (25 ml) was introduced, and the mixture was refluxed for 19 h. The resulting mixture was poured into saturated $NaHCO_3$ (20 ml) aqueous solution, the aqueous layer was extracted with diethyl ether (25 ml × 3), the combined extracts were washed with saturated NaCl, dried over $MgSO_4$ and stripped of solvents to leave a colourless oil, which was purified by chromatography (silica column, EtOAC (5%) + hexane (95%) + Et_3N (0.5%)) to afford the product (1.35 g) in 89% yield.



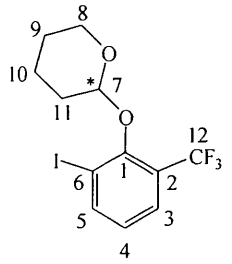
^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 7.50 (1H, H_3, d, J = 8 Hz), 7.38 (1H, H_5, apparent t, J = 8 Hz), 7.18 (1H, H_6, d, J = 8 Hz), 6.94 (1H, H_4, t, J = 8 Hz), 5.48 (1H, H_7, dd, J = 5, 2 Hz), 3.77 (1H, H_8^a, dt, J = 10, 3 Hz), 3.55 (1H, H_8^b, m), 2.01-1.46 (6H, H_{11}, H_9, H_{10}, m).

^{13}CNMR (400 Hz, chloroform-d, δ, ppm), 155.5 (C_1), 133.8 (C_5), 127.5 (C_2), 127.4 (C_3), 121.2 (C_4), 120.1 (C_{12}), 116.1 (C_6), 96.6 (C_7), 62.2 (C_8), 30.7 (C_{11}), 25.8 (C_9), 20.4 (C_{10}).

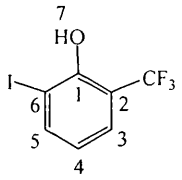
3.3.10.2 Synthesis of 2-trifluoromethyl-6-iodophenol (16c)

Compound 16b was dissolved in THF (8.5 ml) in a flask (25 ml). The system was flushed with nitrogen and then cooled in a acetone-dry ice bath. *n*-BuLi (3 ml, 2.5 M) was introduced with a syringe. After stirring at –78 °C for 30 min, the reaction mixture was treated with a solution of iodine (2.20 g, 8.67 mmol) in THF (8.5 ml), stirred for 10 min, and then allowed it to warm to room temperature. The resulting mixture was poured into Na_2SO_3 (20%) aqueous solution and vigorously shaken.

The aqueous layer was extracted with diethyl ether and the combined extract was washed with saturated NaCl, dried over $MgSO_4$, filtered, and evaporated to leave a yellow-red oil. Its ^{1}HNMR spectrum showed the oil was 2-(trifluoromethyl)-6-iodophenol tetrahydropyranyl ether (**compound 16c'**). The oil was subjected to short-path distillation, during which, the tetrahydropyranyl ether decomposed, giving the product, 2-(trifluoromethyl)-6-iodophenol (1.67 g, 94% yield if assumed to be pure, but in reality contaminated by about 15% of dihydropyran hydrate).



^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 7.88 (1H, H_5, dd, J = 8, 1 Hz), 7.46 (1H, H_3, d, J = 8, 1 Hz), 6.80 (1H, H_4, apparent t, J = 8 Hz), 5.25 (1H, H_7, dd, J = 5, 2 Hz), 4.04 (1H, H_8^a, m), 3.43 (1H, H_8^b, m), 2.01-1.42 (6H, m, H_9, H_{10}, H_{11}).

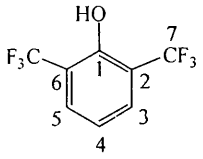


^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 7.77 (1H, H_5, d, J = 8 Hz), 7.47 (1H, H_3, d, J = 8 Hz), 6.70 (1H, H_4, apparent t, J = 8 Hz), 5.85 (1H, H_7, s).

3.3.10.3 The synthesis of 2,6-bis(trifluoromethyl)phenol (16d)

To a round bottom flask (50 ml) equipped with a stirrer bar, Cd powder (3.057 g, 27.2 mmol) and dimethyl formamide (DMF, 10 ml) were added. The flask was flushed with N_2 and then cooled to 0 °C in an ice bath. CBr_2F_2 (1ml + 1ml + 0.5ml, 27.17 mmol in total) was added slowly in portions by syringe, then the mixture was allowed to warm up to room temperature and stirred for an additional 30 min. The mixture was diluted with hexamethylphosphoramide (HMPA, 10 ml) and cooled to 0 °C. CuBr (1.80 g, 12.6 mmol) was added and the mixture was stirred for 10 min.

To the mixture containing $CuCF_3$, 2-trifluoromethyl-6-iodophenol (937.1 mg, 3.25 mmol) was added and the mixture was heated over an oil bath (65 °C) with stirring for 2 h. The resulting mixture was cooled to room temperature, poured into a mixture of HCl (3 mol L^{-1}, 30 ml) and diethyl ether (30 ml), and the precipitate was removed from the mixture by filtration. The aqueous phase was extracted with diethyl ether (25 ml × 5), and the combined extract was washed with saturated NaCl and then dried over $MgSO_4$, filtered and evaporated over a rotary evaporator to leave a brown residue, which was subjected to short path distillation. The fraction (b.p. 80 °C / 0.7 Torr), was collected to afford a colourless oil (283.4 mg) in 38% yield.



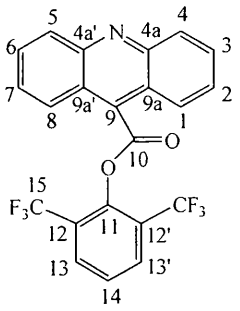
^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 7.69 (2H, H_3, H_5, d, J = 8 Hz), 7.06 (1H, H_4, t, J = 8 Hz), H_1 has not been found, because it was too broad.

^{13}CNMR (400 Hz, chloroform-d, δ, ppm), 153.0 (C_1), 131.0 (C_3/C_5), 123.8 (C_7, q due to coupling to 3 F nuclei), 120.3 (C_4), 120.0 (C_2/C_6).

MS (m/z), EI: 230 ($[M]^+$, 18%), 213 ($[M\text{-}OH]^+$, 28%), 179 (23%), 163 ($[M\text{-}CF_3\text{+}2H]^+$, 30%), 135 (100%).

3.3.10.4 Synthesis of 2,6-bis(trifluoromethyl)phenyl acridine-9-carboxylate (16e)[4]

In a flask (5ml) equipped with a stirrer bar, a condenser, and a $CaCl_2$ drying tube, acridine-9-carboxylic acid chloride (131.6 mg, 0.544 mmol), 2,6-bis-(trifluoromethyl)phenol (130 mg, 0.565 mmol), and pyridine (3 ml), were stirred vigorously at 40 °C for 1 day. Then the volatiles were evaporated and the residue was extracted with DCM. The extract was subjected to column chromatography (silica, Tol + EtOAc 4:1). The fractions with Rf = 0.49 were collected and evaporated to give an orange solid (137.5 mg, 58% yield), m.p. 171 °C.



^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 8.51 (2H, H_1, H_8, d, J = 9 Hz), 8.32 (2H, H_4, H_5, d, J = 9 Hz), 7.96 (2H, H_{13}, $H_{13'}$, d, J = 8 Hz), 7.79 (2H, H_3, H_6, dd, J = 9, 7 Hz), 7.61 (2H, H_2, H_7, dd, J = 9, 7 Hz), 7.56 (1H, H_{14}, t, J = 8 Hz).

^{13}CNMR ((400 Hz, chloroform-d, δ, ppm), 164.6 (C_{10}), 148.6 (C_{4a}/$C_{4a'}$), 146.3 (C_{11}), 131.9 (C_{13}/$C_{13'}$), 131.1 (C_4/C_5), 130.0 (C_3/C_6), 128.5 (C_2/C_7), 127.8 (C_1/C_8), 126.5 (C_9), 126.2 (C_{9a}/$C_{9a'}$), 125.8 (C_{14}), 123.8 (C_{12}/$C_{12'}$), 122.8 (C_{15}, q due to coupling to 3 F nuclei).

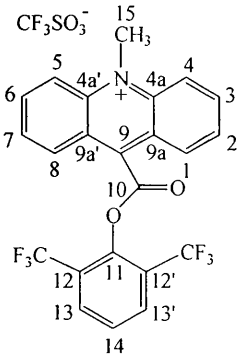
IR ((υ, cm^{-1}), 1758 (C=O).

MS (m/z), EI: 435 ($[M]^+$, 10%), 206 (82%), 178 (100%); CI, m/z, 436 ($[M+H]^+$, 39%), 180 (100%).

3.3.10.5 Synthesis of 9-(2,6-bis(trifluoromethyl)phenoxycarbonyl)-10-methylacridinium trifluoromethanesulfonate (16)

To a flask (10ml), equipped with a stirrer bar, 2,6-bis(trifluoromethyl)phenyl acridine-9-carboxylate (110 mg, 0.252 mmol) was added. The flask was flushed with N_2, and then dry DCM (3 ml) and methyl triflate (160 ul, 1.42 mmol) were introduced with syringes respectively. No precipitate appeared apparently as expected after the mixture was stirred at room temperature for 2 h. The flask was immersed into an oil bath (30 °C). A few minutes later, precipitate appeared. The mixture was maintained at 30 °C overnight. The resulting mixture was filtered, the solid was washed with ether and the filtrate was precipitated with diethyl ether. All

the solids were combined, washed with diethyl ether (2 ml) and pumped overnight to give a yellow solid (67.8 mg, 46% yield), m.p. 231-232 °C.



^{1}HNMR (400 Hz, acetonitrile-d_3, δ, ppm), 8.78 (2H, 2H, H_4, H_5, d, J = 9 Hz), 8.76 (2H, H_1, H_8, d, J = 9 Hz), 8.53 (2H, H_3, H_6, dd, J = 9, 7 Hz), 8.28 (2H, H_{13}, $H_{13'}$, d, J = 8 Hz), 8.15 (2H, H_2, H_7, dd, J = 9, 7 Hz), 7.91 (1H, H_{14}, t, J = 8 Hz), 4.95 (3H, H_{15}, s).

^{13}CNMR (400 Hz, acetonitrile-d_3, δ, ppm), 161.7 (C_{10}), 144.1 (C_9), 144.0 (C_{11}), 142.2 (C_{4a}/$C_{4a'}$), 138.9 (C_{13}/$C_{13'}$), 132.2, 129.4, 129.2, 127.0 (C_3/C_6, C_4/C_5, C_1/C_8, C_2/C_7), 124.5 (split into 2 lines, C_{12}/$C_{12'}$), 123.7 (C_{9a}/$C_{9a'}$), 119.0 (C_{14}), 40.1 (C_{15}).

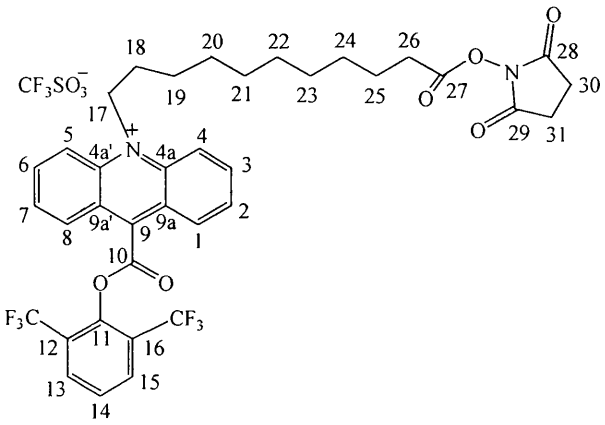
IR ((υ, cm^{-1}), 1769 (C=O).

MS (m/z), ES^+: 450 ($[M\text{-}CF_3SO_3]^+$, 100%), 252 (22%), 224 (17%), 193 (15%); ES^-: 149 ($[CF_3SO_3]^-$, 100%). Acc-MS: calculated mass: 450.0923 ($[M\text{-}CF_3SO_3]^+$); measured mass: 450.0928.

3.3.11 Synthesis of 9-(2,6-bis(trifluoromethyl)phenoxycarbonyl)-10-(10-succinimidyloxycarbonyldecyl)acridinium trifluoromethanesulfonate (17)

To a flask (5 ml) previously flushed with nitrogen, 2,6-bis(trifluoromethyl)phenyl acridine-9-carboxylate (74.9 mg, 0.172 mmol), and succinimidyl 11-(trifluoromethanesulfonyloxy)undecanoate (74.8 mg, 0.174 mmol) were added. The flask was re-flushed with N_2, and then dry DCE (1.6 ml) was introduced with a

syringe. The mixture was refluxed for 21 h and then stripped of solvents on a rotary evaporator to leave a yellow residue. The residue was extracted with DCM (1 ml), and the extract was subjected to column chromatography (silica gel), eluted with DCM + CH_3CN (3:1). The fractions with Rf = 0.19 was pooled and evaporated to give a yellow solid (15.9 mg, 11% yield), m.p. 164-165 °C.



^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 8.78 (2H, H_4, H_5, d, J = 9 Hz), 8.71 (2H, H_1, H_8, d, J = 9 Hz), 8.49 (2H, dd, H_3, H_6, J = 9, 7 Hz), 8.06 (2H, H_{13}, H_{15}, d, J = 8 Hz), 7.98 (2H, H_2, H_7, dd, 9, 7 Hz), 7.12 (1H, H_{14}, t, J = 8 Hz), 5.60 (2H, H_{17}, t, J = 8 Hz), 2.76 (4H, H_{30}, H_{31}, s), 2.51 (2H, H_{26}, t, J = 7 Hz), 2.29-2.18 (2H, m), 1.84-1.69 (4H, m) and 1.51-1.26 (10H, m), (H_{18}, H_{19}, H_{20}, H_{21}, H_{22}, H_{23}, H_{24}, H_{25}).

^{13}CNMR (400 Hz, chloroform-d, δ, ppm), 169.7 (C_{28}/C_{29}), 169.1 (C_{27}), 161.8 (C_{10}), 145.0 (C_9), 144.9 (C_{11}), 141.7 (C_{4a}/$C_{4a'}$), 140.6 (C_{13}/C_{15}), 132.4, 130.0, 129.0, 128.5, (C_1/C_8, C_3/C_6, C_4/C_5, C_2/C_7), 125.9 (splitting into 2 peaks, C_{12}/C_{16}), 124.3 (C_{9a}/$C_{9a'}$), 119.8 (C_{14}), 53.3 (C_{17}), 26.0 (C_{30}/C_{31}), 31.3, 30.1, 29.6, 29.5 (2 signals), 29.3, 29.0, 26.9, 24.9 (C_{18}, C_{19}, C_{20}, C_{21}, C_{22}, C_{23}, C_{24}, C_{25}, C_{26}), (the CF_3 signals were not seen as the signal to noise ratio was too low).

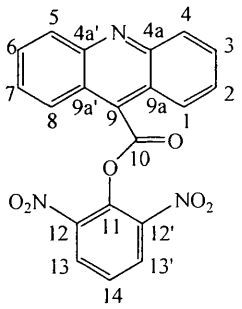
^{19}FNMR (400 Hz, chloroform-d, δ, ppm), -78.4 ($CF_3SO_3^-$), -59.8 (CF_3).

IR (υ, cm^{-1}), 1733 (C=O).

MS (m/z), ES^+: 717 ($[M\text{-}CF_3SO_3]^+$, 100%), 519 (17%), 505 (8%), 491 (9%); ES^-: 149 ($[CF_3SO_3]^-$, 100%). Acc-MS, calculated: 717.2394 ($[M\text{-}CF_3SO_3]^+$); measured: 717.2390.

3.3.12.1 Synthesis of 2,6-dinitrophenyl acridine-9-carboxylate (18a)

In a round bottom flask (5 ml) equipped with a stirrer bar, acridine-9-carboxylic acid chloride (1.08 mmol) and anhydrous 2,6-dinitrophenol (235 g, 1.28 mmol) were dissolved in pyridine. The flask was flushed with N_2, and then DCC (250 mg, 1.23 mmol) in THF (3 ml) was introduced with a syringe. The yellow colour of the mixture faded slightly and some precipitate appeared. The mixture was stirred for 2 days at room temperature. The resulting mixture was filtered to remove DCU and pyridinium chloride. The filtrate was evaporated on a rotary evaporator and the residue was re-dissolved in DCM for loading onto a silica column for chromatography. The column was eluted with DCM. The fractions with Rf = 0.19 were pooled and evaporated under vacuum to give a yellow solid (93 mg, 22% yield), m.p. 203-204 °C.



^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 8.47 (2H, H_1, H_8, d, J = 9 Hz), 8.31 (2H, H_{13}, $H_{13'}$, d, J = 8 Hz), 8.26 (2H, H_4, H_5, d, J = 9 Hz), 7.79 (2H, H_3, H_6, dd, J = 9, 7 Hz), 7.68-7.57 (3H, H_2, H_7, H_{14}, m).

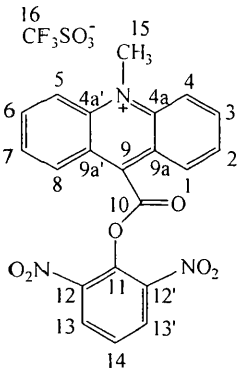
^{13}CNMR (400 Hz, chloroform-d, δ, ppm), 163.9 (C_{10}), 149.1 ($C_{4a}/C_{4a'}$), 144.6 (C_{11}), 138.2 ($C_{12}/C_{12'}$), 131.8 (C_9), 130.8, 130.6, 130.4 ($C_{13}/C_{13'}$, C_4/C_5, C_3/C_6), 128.6 (C_2/C_7), 127.9 (C_{14}), 125.7 (C_1/C_8), 123.5 ($C_{9a}/C_{9a'}$).

IR (υ, cm^{-1}), 1775 (C=O).

MS (m/z), EI: 389 ($[M]^+$, 17%); 206 (96%), 178 (100%); CI: 390 ($[M+H]^+$, 11%), 180 (100%).

3.3.12.2 Synthesis of 9-(2,6-dinitrophenoxycarbonyl)-10-methylacridinium trifluoromethanesulfonate (18)

To a flask (5 ml) equipped with a stirrer bar, 2,6-dinitrophenyl acridine-9-carboxylate (33 mg, 0.085 mmol) was added. The flask was flushed with N_2, and then dry DCM (2 ml) and methyl triflate were introduced, respectively. The mixture was stirred for 3 h. The resulting mixture was transferred into a vial (12 ml) and then diethyl ether (4 ml) was added. The precipitate was filtered and washed with diethyl ether until TLC gave only 1 yellow spot. The yellow solid was collected and pumped (21.4 mg, 46% yield) overnight, m.p. 199-200 °C.



^{1}HNMR (400 Hz, acetonitrile-d_3, δ, ppm), 8.83 (2H, H_4, H_5, d, J = 9 Hz), 8.74 (2H, H_1, H_8, d, J = 9 Hz), 8.60-8.48 (4H, H_3, H_6, H_{13}, $H_{13'}$, m), 8.14 (2H, H_2, H_7, apparent t), 7.94 (1H, H_{14}, t, J = 8 Hz), 4.96 (3H, H_{15}, s).

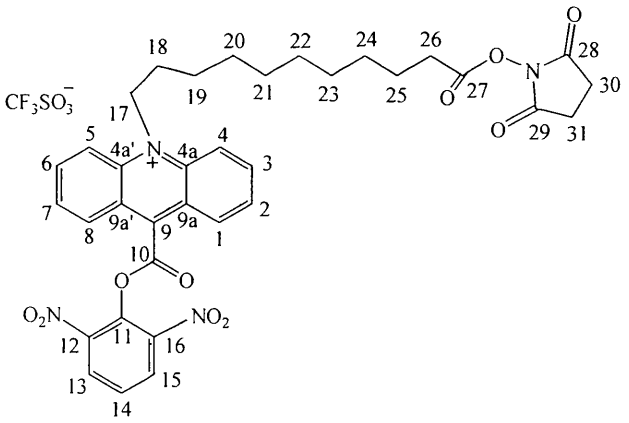
^{13}CNMR (400 Hz, acetonitrile-d_3, δ, ppm), 160.4 (C_{10}), 143.9 (C_9), 142.7 (C_{11}), 139.2 ($C_{4a}/C_{4a'}$), 135.4 ($C_{12}/C_{12'}$), 141.5, 130.4, 129.2, 128.9, 127.5, 118.6 (C_1/C_8, C_3/C_6, C_2/C_7, C_4/C_5, $C_{13}/C_{13'}$, C_{14}), 121.2 (C_{16}), 123.0 ($C_{9a}/C_{9a'}$), 42.0 (C_{15}).

IR (υ, cm^{-1}), 1767 (C=O).

MS (m/z), ES^+: 404 ($[M\text{-}CF_3SO_3]^+$, 100%), 252 (19%), 224 (29%), 193 (78%); ES^-: 149 ($[CF_3SO_3]^-$, 100%). Acc-MS: calculated mass: 404.0877 ($[M\text{-}CF_3SO_3]^+$); measured mass: 404.0882.

3.3.13 Synthesis of 9-(2,6-dinitrophenoxycarbonyl)-10-(10-succinimidyloxycarbonyldecyl)acridinium trifluoromethanesulfonate (19)

To a flask (5 ml) equipped with a stirrer bar and a condenser, succinimidyl 11-(trifluoromethanesulfonyloxy)undecanoate (57 mg, 0.13 mmol) was added, followed by 2,6-dinitrophenyl acridine-9-carboxylate (52.6 mg, 0.13 mmol). The system was flushed with N_2. Then DCE (2 ml) was introduced and the mixture was refluxed for 20 h. The resulting mixture was stripped of solvent, taken up in DCM (1 ml), and subjected to chromatography on a silica column (0.8 g), eluted with DCM firstly and then DCM + CH_3CN (3:1). The fractions with Rf = 0.17 were pooled and evaporated under vacuum to leave a yellow compound (oil like, 15.8 mg, 14% yield).



^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 8.71-8.60 (4H, H_1, H_8, H_4, H_5, m), 8.41 (2H, H_3, H_6, dd, J = 8, 7 Hz), 8.34 (2H, H_{13}, H_{15}, d, J = 8 Hz), 7.92 (2H, H_2, H_7, dd, J = 9, 7 Hz), 7.78 (1H, H_{14}, t, J = 8 Hz), 5.48 (2H, H_{17}, t, J = 8 Hz), 2.69 (4H, H_{30}, H_{31}, s), 2.45 (2H, H_{26}, t, J = 7 Hz), 2.08 (2H, m), 1.68-1.53 (4H, m) and 1.35-1.10 (10H, m) (H_{18}, H_{19}, H_{20}, H_{21}, H_{22}, H_{23}, H_{24}, H_{25}).

^{13}CNMR (400 Hz, chloroform-d, δ, ppm), 169.7 (C_{28}/C_{29}), 169.1 (C_{27}), 161.1 (C_{10}), 144.8 (C_9), 143.9 (C_{11}), 141.6 (C_{4a}/$C_{4a'}$), 136.6 (C_{12}/C_{16}), 140.7, 131.1, 130.2, 129.7, 128.9, 119.6 (C_1/C_8, C_2/C_7, C_3/C_6, C_4/C_5, C_{13}/C_{15}, C_{14}), 124.1 (C_{9a}/$C_{9a'}$), 53.1 (C_{17}),

31.3, 30.1, 29.6, 29.5, 29.4, 29.2, 29.0, 26.9, 24.9 (C_{18}, C_{19}, C_{20}, C_{21}, C_{22}, C_{23}, C_{24}, C_{25}, C_{26}), 26.0 (C_{30}/C_{31}).

IR (υ, cm^{-1}), 1735 (C=O).

MS (m/z), ES^+: 671 ($[M\text{-}CF_3SO_3]^+$, 100%), 519 (15%), 505 (23%), 491 (42%), 361 (77%), 246 (44%), ES^-: 149 ($[CF_3SO_3]^-$, 100%), 80 (41%). Acc-MS: calculated mass: 671.2348 ($[M\text{-}CF_3SO_3]^+$); measured mass: 671.2352.

3.4 Conclusions

The targets in this chapter were synthesized in a similar synthetic pathway to the corresponding compounds in **Chapter 2**. 2,5-Dimethyl (electron-donating) AEs and unsubstituted AEs (model compounds) were synthesized successfully and all gave better yields compared with the 2,6-dibromo compounds (**1-6**). However, for the targets with the phenoxy moiety substituted with 2,6-bis(trifluoromethyl) and 2,6-dinitro groups (strongly electron-withdrawing), only 4 compounds were synthesized successfully (**compounds 16-19**) and another 4 compounds (**23-26**) could not be synthesized. The yield for synthesis of 2,6-dibromo AEs in **Chapter 2**, was slightly lower compared with the model compounds, although the reaction conditions were already relatively harsh. This situation was exacerbated even more by the strongly electron-withdrawing nitro and trifluoromethyl groups.

For the simple AEs without a linker group, the yield for methylation of the acridine is higher than dodecylation, and the reaction conditions are milder. This is consistent with the reference.[16]

For syntheses of AEs with the linker and spacer attached to the N atom, with the length of the linkers increasing, the necessary reaction conditions became milder, and the yields were improved, due to the inductive effect from the ester group. Also as the length of the linkers increased, the polarity of the AEs decreased, so that for compounds with long linker groups, column chromatography proved to be a better purification method than that simple solvent washing, whereas for compounds with only three CH_2 groups in the linker chain, chromatography doesn't work so well. In

this case, the product could be separated from the starting materials due to their different solubility in DCM, diethyl ether and EtOAc.

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Chapter 4

Synthesis of acridinium derivatives with substituents on the acridinium ring

4. 1 Introduction

The only factor affecting the wavelength of the emitted light involves changes made on the acridine ring. Substitutions on the acridinium ring (emitter) not only can change the wavelength of the light emitted, but also can affect the kinetics as well as stability of an acridine ester, in a similar way to modifications to the phenoxy ring.[1] **Compound 20** shown in **figure 4.1,** is a simple model compound to test synthetic methods that might be used for synthesis of a substituted acridine ring in an acridinium ester. If such methods prove successful, **compound 21** (**figure 4.1**), designed to have a spacer and a linker group on the acridine ring rather than have them linked with the phenoxy ring as in **compound 22** (shown in **figure 4.1**) or with the N atom of the acridinium ring (**compounds 3, 4, 5, 6, 9, 10, 11, 13, 14, 15, 17, 19**; see **chapter 2 & 3**), would be synthesized.

9-(4-(2-Benzyloxycarbonylethylphenoxycarbonyl)-2,7-dimethoxy-10-methyl-acridinium triflate (**compound 22'**, **figure 4.1**) had been synthesized by Dr. Li before, and gave a longer wavelength of emitted light than that of the AE with the acridinium ring unsubstituted, because the conjugation system is enlarged by an n-π conjugation effect between the methoxy group and the acridinium ring. Therefore, this compound, together with a standard acridinium ester, could allow multi-analysis based on different wavelengths of chemiluminescence. Consequently, **Compound 22** was also designed and was expected to have the same chemiluminescent wavelength as **compound 22'**, but the presence of a linker group would enable it to be used as a label for oligonucleotides.

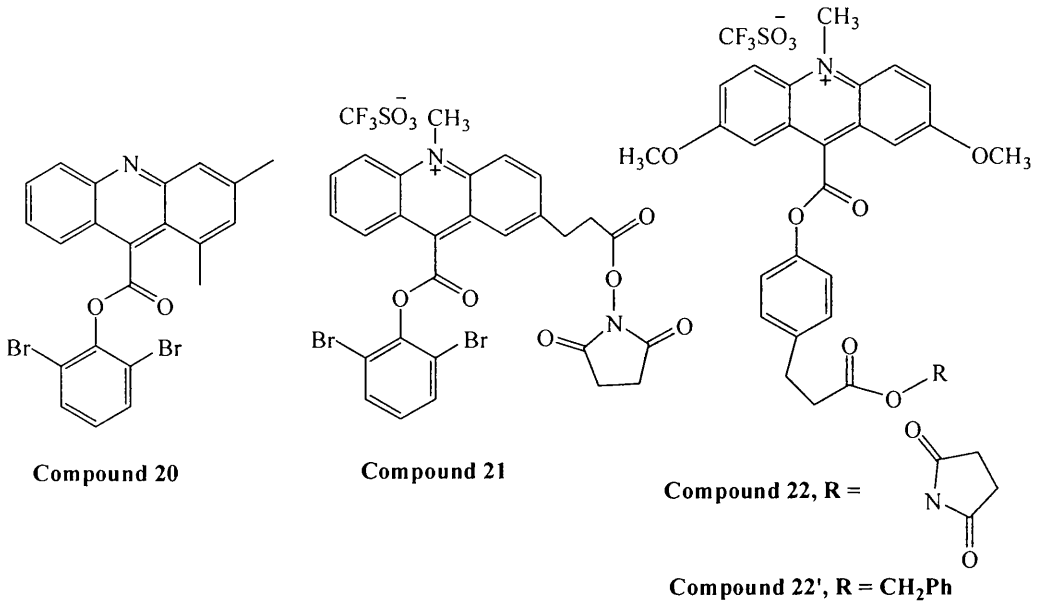


Figure 4.1, targets in chapter 4

4. 2 Results and discussion

4.2.1 A simple model compound 2,6-dibromophenyl 1,3-dimethylacridine-9-carboxylate (20)

Compound 20 would be synthesized in a synthetic pathway similar to that used for **compound 7a**, by converting 1,3-dimethylacridine-9-carboxylic acid (**20d**) to its corresponding acid chloride **20e**, and then coupling **20e** with 2,6-dibromophenol to afford the product **20** (**figure 4.2**). Formation of the substituted acridine ring is a key stage in synthesis of the product, and was expected to involve the following steps (**figure 4.2**): (i) coupling *p*-bromoxylene and formanilide to give *N*-(3,5-dimethylphenyl)formanilide (**20a**); (ii) hydrolysis of **20a** to provide *N*-(3,5-dimethylphenyl)aniline (**20b**); (iii) coupling **20b** with phosgene, $(COCl)_2$, followed by treating with a strong Lewis acid, $AlCl_3$, to afford a substituted isatin, 3,5-dimethyl-*N*-phenylisatin (**20c**); (iv) rearrangement of **20c** in refluxing KOH solution to form **20d**; and (v) conversion of compound **20d** into the desired compound **20.** Indeed, as described in the following sections, the synthesis was successful under the conditions recorded in **figure 4.2**.

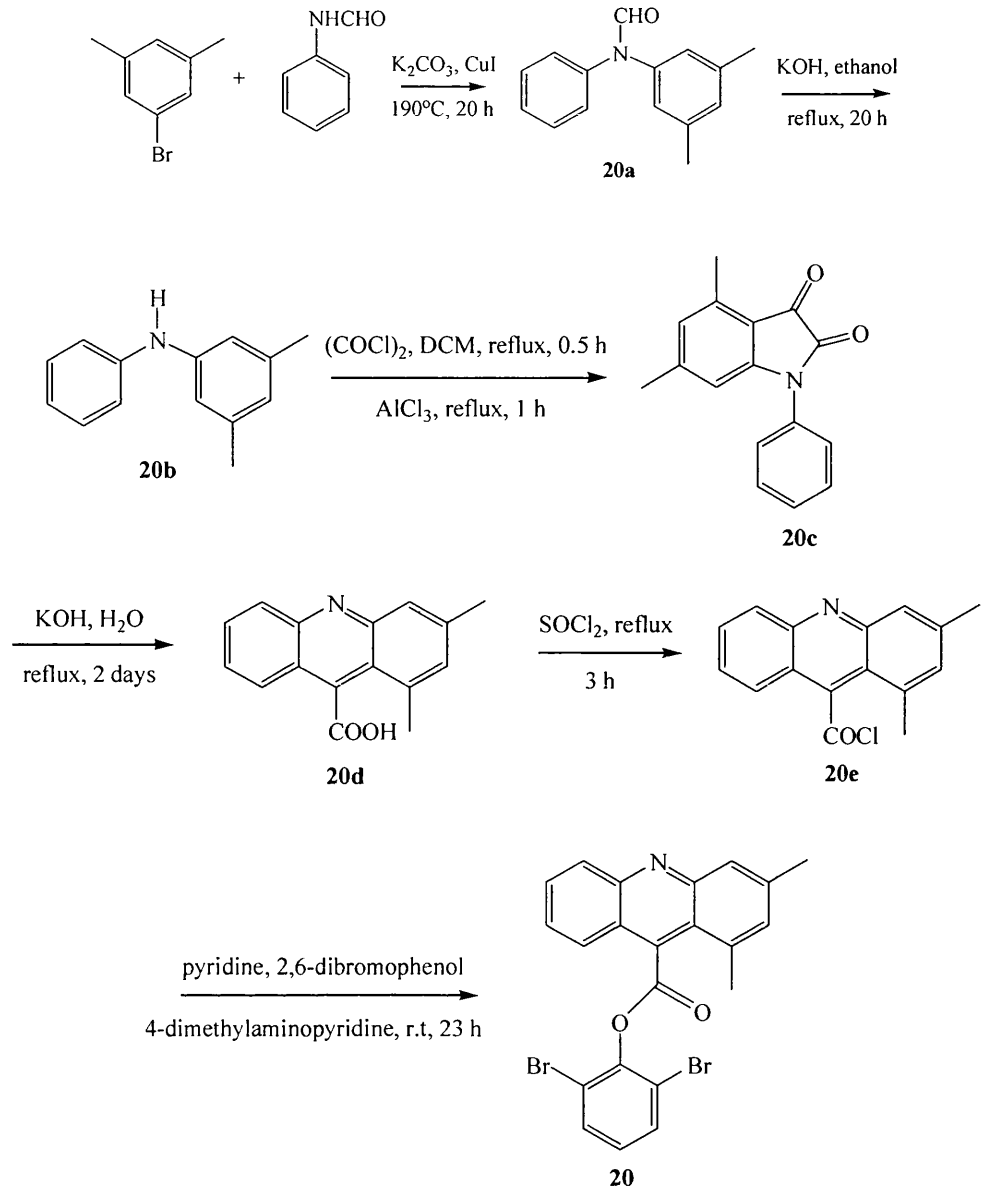


Figure 4.2, synthesis of 2,6-dibromophenyl 1,3-dimethylacridine-9-carboxylate (**20**)

4.2.1.1 Synthesis of *N*-(3,5-dimethylphenyl)formanilide (20a)

Synthesis of *N*-(3,5-dimethylphenyl)formanilide (**20a**) was performed according to Ahmed.[1] *p*-Bromoxylene and formanilide were heated with K_2CO_3 at 190 °C for 17 h, using CuI as a catalyst. The resulting mixture was taken up in DCM and then filtered to remove the inorganic materials. The filtrate was evaporated to give a crude product, showing 2 components by TLC. The crude product was purified by chromatography (silica column), eluted with DCM:hexane (1:1). The 1st component (Rf = 0.73) was shown to be a side product (white crystals, m.p. 144°C, 20% yield).

It was characterised by its ^{1}HNMR spectrum (400 Hz, chloroform-d, δ, ppm), 7.15 (2H, dd, J = 8, 7 Hz), 6.96 (2H, d, J = 8 Hz), 6.89 (1H, t, J = 7 Hz), 6.63 (4H, s), 6.59 (2H, s), 2.16 (12H, s); ^{13}CNMR spectrum (400 Hz, chloroform-d, δ, ppm), 148.7, 148.3, 139.1, 129.4, 125.0, 124.2, 122.7, 122.4, 21.7. The IR spectrum did not show any absorption from 1600 to1800 cm^{-1} (C=O) or absorption around 3400 cm^{-1} (N-H). The EI/CI mass spectrum showed the molecular ion peak at m/z 301 (M^{+}), together with other ions at m/z 285 (46%) and 302 ($[M+H]^{+}$, 100%). The spectra above suggested that the product had the structure shown in **figure 4.3**. This product could be understood as resulting from deformylation of **20a**, followed by further reaction with a second mole of *p*-bromoxylene.

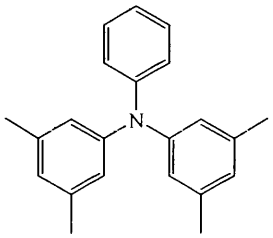


Figure 4.3, the structure of a side product formed during preparation of **20a**

The 2nd component of the product mixture (Rf, 0.30) isolated in a 67% yield was shown to be the desired product. In the ^{1}HNMR spectrum of **20a**, peaks in the aromatic (including CHO) and aliphatic regions integrated in a ratio of 9:6. Due to restricted rotation around the nitrogen-carbonyl bond, two sets of signals were observed, including two aldehyde peaks at 8.59 ppm and 8.55 ppm. In the ^{13}CNMR spectrum, the signals for each carbon atom in the aromatic region, were similarly split into two close lines, and a signal at 162 ppm attributed to the carbonyl group was found. The EI/CI mass spectrum showed ions at m/z 225 (M^{+}), 243 ($[M+NH_4]^{+}$) and 226 ($[M+H]^{+}$, 86%). The successful usage of the product in the next stage corroborated its structure.

Although the side product could be removed by chromatography, changing the reaction to employ slightly lower temperature, a 1:1 molar ratio of the starting materials and control of the amount of the catalyst should allow improvement in the yield of the desired product and decrease the amount of the side product. However, a similar reaction had been performed by McCapra's group, using nitrobenzene as solvent.[2] Therefore, the reaction was performed under McCapra's conditions, which

were similar to those of Ahmed, apart from the use of the solvent. The resulting mixture was first evaporated to remove nitrobenzene, then purified by chromatography. A 65% yield of the desired product was obtained. However, an additional side product (brown oil in 4% yield) was obtained. In the ^{1}HNM R spectrum of the brown oil, it did not show any methyl group in the aliphatic region, and all peaks were in the low field, integrating in a ratio of 1:1:1:1:2:4:4. The ^{13}CNMR spectrum showed the carbonyl group. The EI/CI mass spectrum showed molecular ion peaks at m/z 242 ($[M]^{+}$, twice of the formanilide), 121 (fromanilide), 260 ($[M+NH_4]^{+}$), 198, and 139. The accurate mass spectrum gave an ion at m/z at 241.1087 while the M-H peak for a compound with twice the molecular mass is 241.1084. It is thought to be the structure shown in **figure 4.4**, formed via a mechanism similar to the aldol condensation.[3] The reaction being carried out at extremely high temperature 200 °C for 17 h might account for the formation of this product. ^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 8.84 (1H, s), 8.72 (1H, d), 8.34 (1H, s), 7.97 (1H, s), 7.57 (2H, d, J = 8 Hz), 7.44-7.31 (4H, m), 7.25-7.10 (4H, m). ^{13}CNMR (400 Hz, chloroform-d, δ, ppm), 163.4, 159.8, 137.4, 137.2, 130.2, 129.5, 125.7, 125.2, 120.5, 119.2. IR (υ, cm^{-1}), 3262 (-OH, -NH), 1669 (C=O).

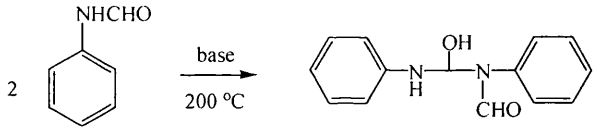


Figure 4.4, a possible side reaction during preparation of **20a**.

4.2.1.2 Synthesis of *N*-(3,5-dimethylphenyl)aniline (20b)

Compound **20a** was refluxed in ethanol with KOH (10%) for 20 h, as shown in **figure 4.2**. The resulting mixture was evaporated, and DCM was added to take up the organic compounds. The crude product was purified by chromatography on a silica column, eluted with DCM. The fractions with Rf = 0.78 were pooled and evaporated to give crystals with a dark brown colour on the surface and white in the core (68% yield). In the ^{1}HNMR spectrum of the product **20b**, there were 6 peaks in the aromatic region and a singlet in the aliphatic region, integrating in a ratio of 8:6. The singlet was attributed to the 2 CH_3 groups. In the ^{13}CNMR spectrum, there were only

7 peaks in the aromatic region, but it was believed that there were 8 different aromatic carbons, due to accidental overlaps. A peak at 21.8 ppm was attributed to the 2 CH_3 groups. As expected, no signal for carbonyl group was found, indicating loss of the formyl group.. The IR spectrum showed a strong sharp absorption at 3400 cm^{-1}, attributed to υ_{N-H}; no absorption for a carbonyl group was found. The EI-CI mass spectrum showed pseudo molecular ion peaks at m/z 197 ($[M^+]$) and 198 ($[M+H]^+$), corroborating the structure of the product.

4.2.1.3 Synthesis of 3,5-dimethyl-*N*-phenylisatin (20c)

In the literature of both F. McCapra and Z. Ahmed,[1, 2] CS_2 was used as solvent. Because CS_2 is toxic and has a poor solubility for the starting material **20b**, therefore, DCM was used in place of CS_2 and worked well. To oxalyl chloride in DCM, *N*-(3,5-dimethylphenyl)aniline (**20b**) in DCM was added slowly and then the mixture was gently refluxed for 0.5 h. The excess oxalyl chloride and solvent were removed by evaporation. DCM was added again, followed by aluminium chloride in portions. The mixture was then refluxed for 1 h. The resulting mixture was evaporated. To the black residue, hydrochloric acid (1 mol L^{-1}) was added carefully (a lot of heat was generated) to destroy the reactive components. Then the product was extracted with EtOEt and purified by chromatography on silica column, eluted with DCM. The fractions with Rf = 0.61 were pooled and evaporated to give an orange solid, **20c**.

According to the accepted mechanism, the oxalyl chloride couples to the N atom first, and then the carboxylic acid chloride attacks the carbon *ortho* to the N atom (a Friedel-Crafts reaction involving intramolecular cyclisation). In the process of cyclisation there are 2 carbons *ortho* to the N atom, so the attack toward C atom can occur in two ways (**figure 4.5**). Therefore, 2 isomers (**20c** and **20c'**) would be expected to form. However, this was not the case. The ^{1}HNMR spectrum showed 2 triplets or apparent triplets, 1 doublet and 2 singlets in the aromatic region, and 2 singlets in the aliphatic region, integrating in a ratio of 2:1:2:1:1:3:3. The ^{1}HNMR and ^{13}CNMR spectra suggested that the compound obtained should be isomer **20c** (**figure 4.5**), which means that the attack occured only by one route (**figure 4.5**). The richer electron on the dimethylphenyl ring compared to the phenyl ring may account

for this result. In the ^{13}CNMR spectrum, there were 10 peaks in the aromatic region. There were 2 signals at 182.9 and 158.4 ppm attributed to 2 carbonyl groups, and another 2 signals at 23.2 and 18.6 ppm attributed to 2 methyl groups. The IR spectrum showed absorptions at 1745 and 1721 cm^{-1}, attributed to 2 carbonyl groups. The EI/CI mass spectrum gave ion peaks at m/z 251 (M^+) and 269 ($[M+NH_4]^+$).

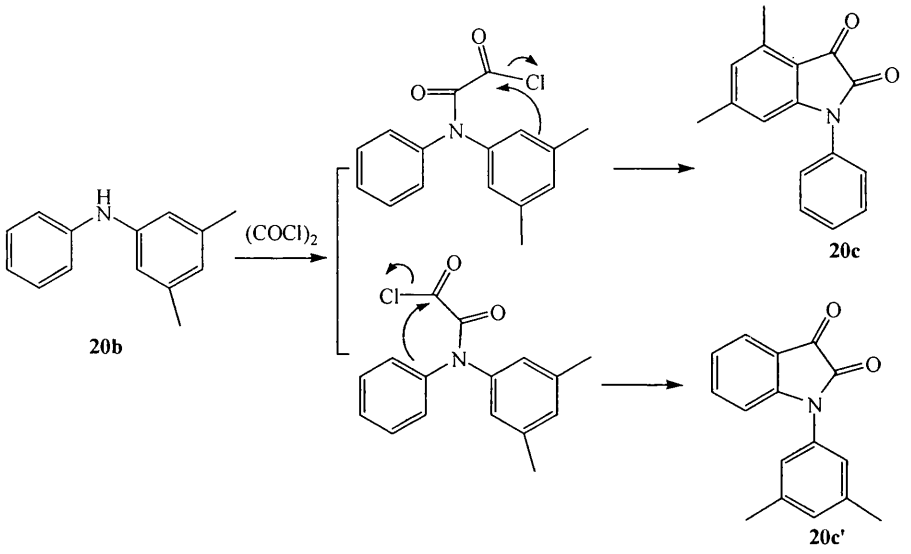


Figure 4.5, the 2 possible attack directions in synthesis of isatin from diarylamine.

4.2.1.4 An attempt at synthesis of *N*-(3,5-dimethylphenyl)isatin (20c')

Compound 20c and **20c'** would give the same product, **compound 20d** in the next stage. However, synthesis of **compound 20c'** by the route shown in **figure 4.6**, would save two steps (compared to **sections 4.2.1.1** to **4.2.1.3**). Furthermore, if successful, the experience gained might be useful for syntheses of other precursors of substituted acridine rings.

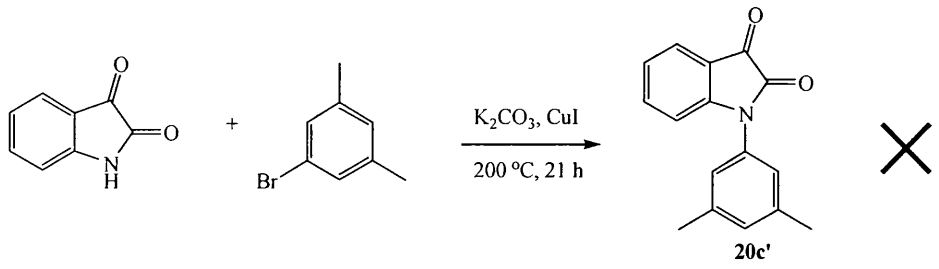
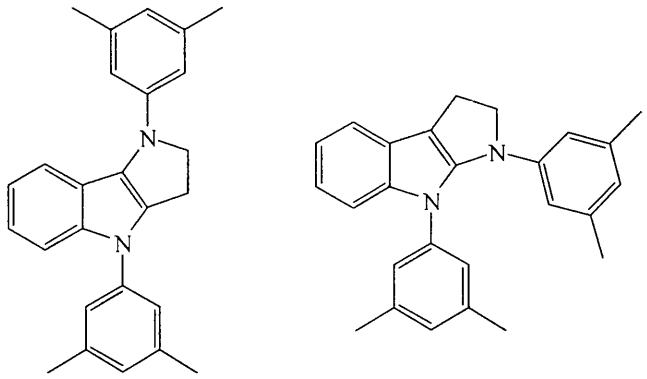


Figure 4.6, an attempt to synthesize *N*-(3,5-dimethylphenyl)isatin

The attempted synthesis of *N*-(3,5-dimethylphenyl)isatin (**20c'**) was performed by using isatin (2.407 g, 13.9 mml) and *p*-bromoxylene (3 g, 16.2 mmol) under conditions similar to those used for the cross-coupling reaction (**section 4.2.1.1**). After the starting materials were heated with Na_2CO_3 (2.558 g, 18.5 mmol) at 200 °C using CuI (0.753 g, 3.96 mmol) as a catalyst for 21 h, the mixture was extracted with DCM (100 ml), and the extract was concentrated and chromatographed. TLC showed 5 components when developed with DCM or 6 components when developed in hexane : DCM (1:10). The 1st component with Rf = 0.90 (DCM) was a white solid, m.p. 241-244 °C, (85 mg, 1% yield). The ^{1}HNMR spectrum showed 6 peaks in the aromatic region and 1 singlet at 2.26 ppm in a ratio of 1:1:2:1:1:1:6. ^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 7.43 (1H, d, J = 8 Hz), 7.36 (1H, s, broad), 7.16 (2H, s), 7.04 (1H, s, broad), 6.92 (1H, d, J = 7 Hz), 6.88 (1H, s), 2.26 (6H, s). The ^{13}CNMR spectrum showed 12 peaks in total but did not give any signal for a carbonyl group. ^{13}CNMR (400 Hz, chloroform-d, δ, ppm), 141.3, 139.7, 139.1, 128.7, 127.0, 123.8, 122.8, 119.7, 118.9, 116.1, 111.4, 21.9. The EI/CI mass spectrum gave ions at m/z 414 ($[M^+]$), 415 ($[M+H]^+$), and 309 further, which suggested the possible structure shown in **figure 4.7** (several isomeric structures are possible, of which the most likely is drawn). IR did not show any absorption for a carbonyl group, but showed a strong peak at 1590 cm $^{-1}$. The 2nd, 3rd, and 4th spots were very close, and have not been separated. They have a slightly higher Rf value than the simple isatin and one of them could be the desired product, possibly. The ^{1}HNMR spectrum of the mixture of the 2nd, 3rd, and 4th components was too messy to give useful information. The Ullman methodology (C-N cross coupling conditions) is clearly not very suitable for the isatin analogues, possibly because the isatin is an amide and not an amine, leading to lower reactivity for the coupling. It has been reported in the literature,[4] that isatin can react in DMSO in the presence of NaH and benzene with dimethyl or diethyl sulphate, a variety of alkyl halides, acyl halides and anhydrides to give *N*-alkyl and *N*-acylisatins.[5] However no reaction with aromatic halides has been reported.



a b

Figure 4.7, possible side product from the attempted synthesis of *N*-(3,5-dimethylphenyl)isatin

Given the difficulties associated with this reaction, it was decided to continue with compound **20c** for the next stage of the synthesis.

4.2.1.5 Synthesis of 1,3-dimethylacridine-9-carboxylic acid (20d)[1]

3,5-Dimethyl-*N*-phenylisatin (**20c**) was refluxed in KOH aqueous solution (10%) for 39 h, as illustrated in **figure 4.2**. The resulting mixture was acidified with concentrated HCl, and then filtered. The precipitate was re-dissolved in K_2CO_3 aqueous solution (10%), carefully re-precipitated by acidification with concentrated HCl, filtered, and dried to give a yellow solid in 57% yield.[1] The ^{1}HNMR spectrum showed 1 doublet, 1 mutiplet, 2 singlets and 1 apparent triplet in the aromatic region, and 2 singlets in the aliphatic region, integrating in a ratio of 1:2:1:1:1:3:3. Due to the poor solubility in DMSO, signals in the ^{13}CNMR spectrum were too weak to betray any useful information. The IR spectrum gave a broad strong peak around 3127 cm^{-1} (OH/NH) and a peak at 1714 cm^{-1} (C=O). Pseudo molecular ion peaks at m/z 251 ($[M]^+$) and 252 ($[M+H]^+$) in the EI/CI mass spectrum and its successful usage in the next step further corroborated the structure of the product.

4.2.1.6 Synthesis of 2,6-dibromophenyl 1,3-dimethylacridine-9-carboxylate (20)

Synthesis of 2,6-dibromophenyl 1,3-dimethylacridine-9-carboxylate (**20**) was first attempted in a synthetic pathway similar to that used for **1c**, **7a**, **12a** and **16e** (see

chapter 3). However, the product was obtained in a yield of only 3%, due to steric hindrance from the methyl group at position 1 on the acridine ring. The formation of the acid chloride appeared to have been straightforward, but the reaction of the acid chloride with 2,6-dibromophenol had not proceeded well. 4-Dimethylaminopyridine (DMAP) is a frequently used catalyst when the esterification reaction involves sterically hindered alcohols or phenols.[5] Owing to the electron-donating effect, DMAP possesses greater nucleophilicity than pyridine. It could attack the carbonyl carbon atom, displace Cl^- anion and form the reactive intermediate more quickly and effectively. Furthermore it acts as a good leaving group during the attack of a phenol with the reactive intermediate. Synthesis of **20** was therefore performed by using DMAP as a catalyst and stirring 1,3-dimethylacridine-9-carboxylic acid chloride (**20e**) and 2,6-dibromophenol in pyridine at room temperature for 23 h, as illustrated in **figure 4.2**. The resulting mixture was evaporated under vacuum to remove pyridine. The product was separated by chromatography (silica column), eluted with DCM. The first component, with Rf = 0.57 (TLC in DCM) was obtained in 11% yield and proved to be the desired product.

In the ^{1}HNMR spectrum of **20**, there were 3 doublets, 2 singlets and 3 triplets or apparent triplets in the aromatic region, and 2 singlets in the aliphatic region, integrating in a ratio of 1:1:1:1:2:1:1:1:3:3. The signal integrating for 2 protons was a doublet. In the ^{13}CNMR spectrum, a signal at 164.2 ppm was attributed to the carbonyl group and 2 peaks at 24.5 and 22.2 ppm were attributed to the 2 methyl groups. Another 17 aromatic peaks attributed to 17 different aromatic carbons were found. The IR spectrum showed an absorption at 1760 cm^{-1} for the carbonyl group. The CI-mass spectrum gave a pseudo molecular ion peak at m/z 486 ($[M + H]^+$, 13%).

The successful synthesis of compound **20** having been achieved, attention was next turned to the synthesis of compound **21**.

4.2.2 Synthesis of compound 21

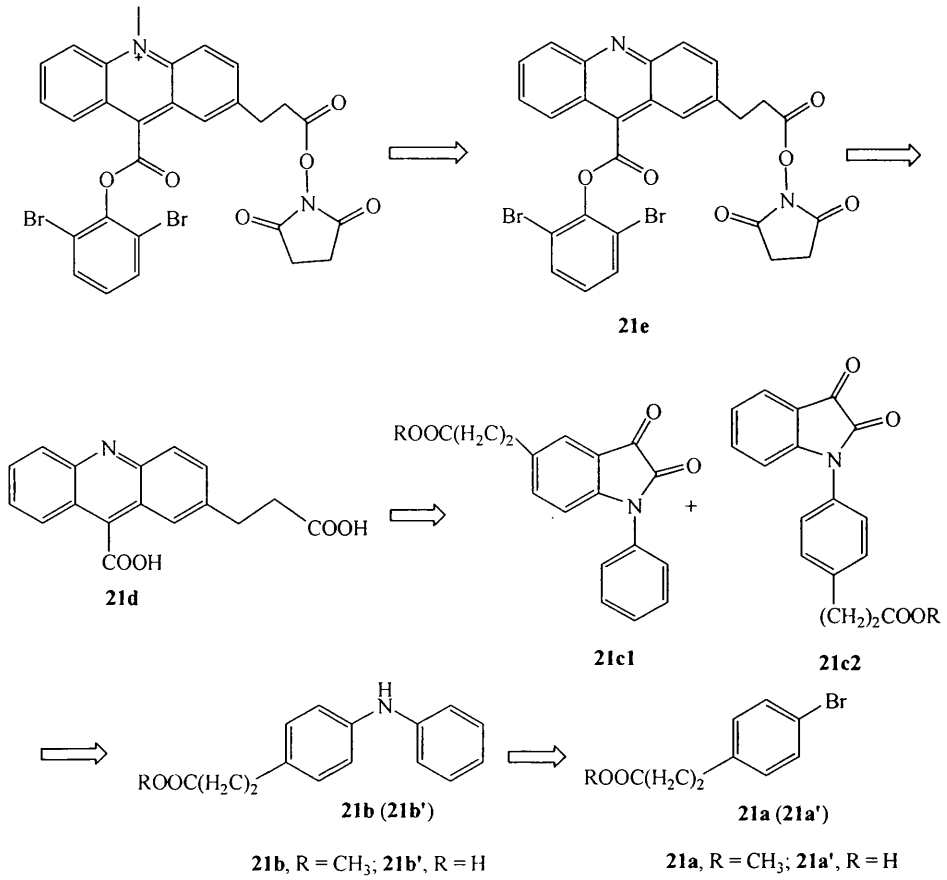


Figure 4.8, the retrosynthetic route for **compound 21**

Compound 21 would be synthesized by the route shown in **figure 4.8**. **Compound 21b** and **compound 21d** would be the important intermediates. The synthesis of **compound 21b** or **21b'** would involve a cross-coupling reaction. Conversion of **21b** or **21b'** to **21c** followed by conversion to **21d** would accomplish the desired substituted acridine ring. According to the literature,[1, 2] the carbon-nitrogen cross coupling reaction could be carried out by using CuI as a catalyst, in the presence of K_2CO_3 at high temperature as shown in **section 4.2.2.1.** However, in the recent literature,[6, 7] similar cross-coupling reactions were catalyzed by a palladium complex at lower temperature with a higher yield. Because the catalyst CuI is cheap and easily available, the coupling reaction was be first attempted by using CuI as a catalyst.

4.2.2.1 Synthesis of *N*-phenyl-4-(2-carbonylethyl)aniline catalysed by CuI (21b')

The synthesis of **compound 21b'** (**figure 4.8**) was attempted by a cross coupling reaction of 3-(4-bromophenyl)propionic acid and formanilide,[1] catalysed by CuI in

the presence of K_2CO_3 at 200 °C for 24 h. The resulting mixture showed 3 spots in addition to the starting materials on a TLC plate. After tedious column chromatography (silica, several hundred times of the sample weight), the product was obtained in a 12% yield. Then, according to the literature,[2] synthesis of *N*-phenyl-4-(2-carbonylethyl)aniline (**21b'**) was conducted by heating 3-(4-bromophenyl)propionic acid and formanilide at reflux in nitrobenzene and catalysed by CuI in the presence of K_2CO_3 powder for 2 days. The resulting mixture was stripped of solvent under vacuum and then refluxed in concentrated HCl and acetic acid (1:1) for 3 h. After cooling, the mixture was extracted with EtOEt. The extract was dried over $MgSO_4$, filtered and evaporated to give a dark green oil, which solidified on standing. This was subjected to chromatography on a silica column. The product was obtained in only 11% yield. The IR spectrum gave absorptions consistent with what F. McCapra had reported, 3401 cm for υ_{N-H}.[2] However, the ^{1}HNMR spectrum showed that the integral of protons in the aromatic region was 1.5 times that in the aliphatic region for the 2 CH_2 groups (the ideal ratio should be 9:4). This suggested the presence of impurities, possibly the starting material, 3-(4-bromophenyl)propionic acid. This coupling reaction differs from that used for synthesis of **20a** only in that the starting material, 3-(4-bromophenyl)propionic acid has a functional group, the carboxylic acid. 3-(4-Bromophenyl)propionic acid was then protected by conversion to an ester, before the synthesis of **21b** was attempted under similar coupling conditions. However, a similar result was obtained. So the coupling reaction for synthesis of **21b** would be catalyzed by Pd[DPPF]Cl_2 instead of CuI, as described in **section 4.2.2.2**.

4.2.2.2 Synthesis of *N*-phenyl-4-(2-methoxycarbonylethyl)aniline (21b) catalysed by Pd[DPPF]Cl_2

Palladium-catalyzed cross-coupling reactions (C-C or C-N coupling) of aryl halides are now common synthetic procedures. Indeed, (DPPF)$PdCl_2$ has proved to be an effective catalyst for the amination of aryl bromides and iodides with both aryl and alkyl primary amines. The efficient reaction of aryl iodides, as well as aryl bromides, extends the scope of amination reactions to a wide range of electrophiles. Aryl halides with either electron-donating or electron-withdrawing substituents, as well as

ones bearing *ortho* substituents, react with anilines within only 3 h.[6-8] Before the cross-coupling reaction, 3-(4-bromophenyl)propionic acid was first esterified, as illustrated in **figure 4.9**.

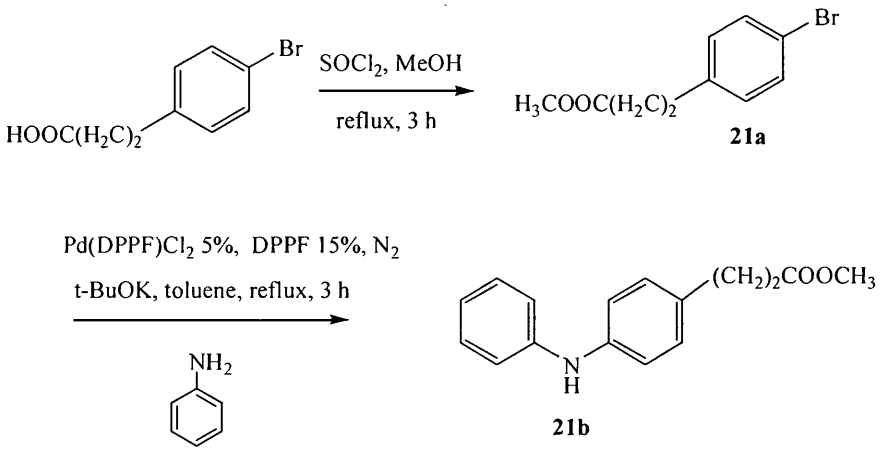


Figure 4.9, synthesis of *N*-phenyl-4-(2-methoxycarbonylethyl)aniline

The 3-(4-bromophenyl)propionic acid was refluxed in $SOCl_2$ for 3 h. The excess $SOCl_2$ was evaporated under vacuum. Pyridine and methanol were added and the mixture was stirred overnight. Afterwards, the excess methanol and pyridine were evaporated to leave a pale yellow oil, which was then purified by column chromatography on a silica column, eluted with DCM, to give an oil (**21a**) in 87% yield.[9-10] In the ^{1}HNMR spectrum, there were 2 peaks in the aromatic region, and 3 peaks in the aliphatic region, integrating in a ratio of 2:2:3:2:2. A singlet at 3.69 ppm was attributed to the methoxy group and signals at 2.92 and 2.63 ppm were attributed to the 2 CH_2 groups. In the ^{13}CNMR spectrum, there were 1 signal at 173.4 ppm for the carbonyl group, another 4 signals in the aromatic region for the 4 different aromatic carbons, and another 3 signals in the aliphatic region. The EI/CI mass spectrum showed pseudo molecular ions at m/z 262, 260 ($[M+NH_4]^+$, for ^{79}Br & ^{81}Br isotope peaks 100% respectively) and 242, 244 ($[M]^+$, 6%), corroborating the successful synthesis of **21a**.

The cross-coupling reaction was performed by refluxing methyl 3-(4-bromophenyl)propionoate (**21a**), aniline, DPPF, $(DPPF)PdCl_2$ and sodium t-butoxide (100:120:15:5:140 approximately) in redistilled toluene under argon for 3 h.[6] The volatiles were removed from the resulting mixture under vacuum. The residue was loaded onto a silica column for chromatography, eluted with DCM. The

fractions with Rf = 0.84 were evaporated to leave a yellow solid, m.p 185-186 °C, which proved to be a side product. ^{1}HNMR ($CDCl_3$, δ, ppm), 7.27 (s), 4.15 (s), 3.88 (s), integrating in a ratio of 4:1:1. It possibly resulted from decomposition of the catalyst. The fractions with Rf = 0.24 were pooled and evaporated to afford the desired product, **21b**.

In the ^{1}HNMR spectrum of **21b**, there were 4 signals in the aromatic region (signals for two sets of protons overlapped accidentally) and 4 other signals, integrating in a ratio of 2:2:4:1:1:3:2:2. A broad singlet at 5.61 ppm was attributed to the proton linked with the N atom; a singlet at 3.49 ppm was attributed to the methoxy group; 2 triplets at 2.72 and 2.43 ppm were attributed to the 2 CH_2 groups. In the ^{13}CNMR spectrum, a peak at 173.9 ppm was attributed to the carbonyl group; another 8 peaks in the aromatic region and 3 peaks in the aliphatic region were found. The IR spectrum gave a strong absorptions at 3383 (N-H) and 1723 cm^{-1} (C=O). In the EI/CI mass spectrum, the ion peaks at m/z 255 ($[M]^+$), 273 ($[M+NH_4]^+$ and 256 ($[M+H]^+$) further proved the structure of **compound 21b**.

4.2.2.3 Synthesis of 5-(2-methoxycarbonylethyl)-*N*-phenylisatin (21c1) and *N*-(4-(2-methoxycarbonylethyl)phenyl)isatin (21c2)

The syntheses of of 5-(2-methoxycarbonylethyl)-*N*-phenylisatin (**21c1**) and *N*-(4-(2-methoxycarboxylethyl)phenyl)isatin (**21c2**) were performed by a procedure similar to that used for **compound 20c** in **section 4.2.1.3**. The retrosynthesis is illustrated in **figure 4.8**. The crude product was subjected to chromatography, eluted with DCM. The 1st fraction proved to be a mixture of **compound 21c1** and **21c2**. TLC developed in several solvent systems showed no evidence of significant separation of the isomers (**compound 21c1** and **21c2**). However, after column chromatography, the ratio of the isomers was different in different eluted fractions, becoming 3:1 in the last fraction. It was expected the isomer with the lower Rf value should be **compound 21c2** and it was hoped to get one pure isomer by efficient crystallization. However, the amount of the mixture was not enough for an efficient crystallisation. Because both of the isomers would give the same product in the next stage, no further effort was made for the separation.

4.2.2.4 Synthesis of 2-(2-carbonylethyl)acridine-9-carboxylic acid (21d)

The mixture of 2 isatin isomers, 5-(2-methoxycarbonylethyl)-*N*-phenylisatin (**21c1**) and *N*-(4-(2-methoxycarbonylethyl)phenyl)isatin (**21c2**), was refluxed in NaOH aqueous solution (10%) for 20 h (**figure 4.8**). The resulting mixture was cooled to room temperature and acidified with concentrated HCl to pH 2. A copious amount of yellow solid precipitated. The precipitate was filtered, washed with distilled H_2O, DCM and methanol, and then dried under vacuum to give a yellow solid in 84% yield. The product did not melt even up to 290 °C, but at that point, the compound turned black. In the ^{1}HNMR spectrum, there were 3 doublets, 1 multiplet (an apparent triplet overlapped with a doublet), a singlet and an apparent triplet in the aromatic region and 2 triplets in the aliphatic region, integrating in a ratio of 1:1:1:2:1:1:2:2. In the ^{13}CNMR spectrum, peaks at 174.4 and 169.1 ppm were attributed to the 2 carboxylic groups; there were another 11 peaks in the aromatic region for 13 different aromatic carbons (2 pairs of them overlapped); 2 signals at 35.4 and 31.5 ppm were attributed to the 2 CH_2 groups. The mass spectra showed m/z at 296 ($[M+H]^+$) in CI mode and 294 ($[M-H]^-$) in ES^- mode, corroborating the structure of the product.

4.2.2.5 Synthesis of 2,6-dibromophenyl 2-(2-succinimidyloxycarbonylethyl)acridine-9-carboxylate (21e)

Compound 21e was synthesized according to McCapra.[2] 2-(2-Carbonylethyl)-acridine-9-carboxylic acid (**21d**) was refluxed in $SOCl_2$ for 3 h. The excess $SOCl_2$ was evaporated under vacuum. To the residue, pyridine and *N*-hydroxysuccinimide were added. After the mixture was stirred for 4 h under argon, it was cooled in an ice bath, *p*-toluenesulfonyl chloride was added and the mixture was stirred for 15 min. Then the ice bath was removed, 2,6-dibromophenol was added and the mixture was stirred for a further 2.5 h at room temperature. The resulting mixture was evaporated to remove pyridine. The residue was washed with diluted HCl (0.1 mol L^{-1}) and then dried under vacuum. The crude product was purified by chromatography, eluted with DCM, and the fractions with Rf = 0.22 were pooled and evaporated under vacuum to give a pale yellow powder (49.1 mg, 22%), which proved to be succinimidyl *p*-toluenesulfonate, m.p. 145-146 °C. Its structure is shown in **figure 4.10**. ^{1}HNMR,

(chloroform-d, δ, ppm), 7.85 (2H, d, J = 8 Hz), 7.33 (2H, d, J = 8 Hz), 2.74 (4H, s), 2.41 (3H, s). MS (m/z), EI: 269 ($[M^+]$, 2%), 155 (56%), 91.3 (100%); CI: 287 ($[M+NH_4]^+$, 100%). The fractions with Rf = 0.11 were pooled and evaporated under vacuum to give a pale yellow solid, proved to be the desired product (33 mg, 6% yield). The mechanism for the esterification with *p*-toluenesulfonic acid chloride was shown in **figure 4.11**. A slight modification was carried out to the procedure in order to improve the yield to 29%. Firstly, *N*-hydroxysuccinimide (NHS) was added at an earlier stage, *i.e.* during refluxing of the **21d** and $SOCl_2$, to drive the NHS esterification fully.[9] Secondly, the reaction time for the esterification with 2,6-dibromophenol was prolonged to 24 h, as illustrated in **figure 4.12**.

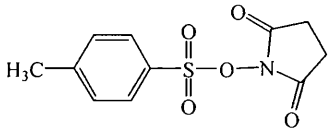


Figure 4.10, a side product, succinimidyl *p*-toluenesulfonate

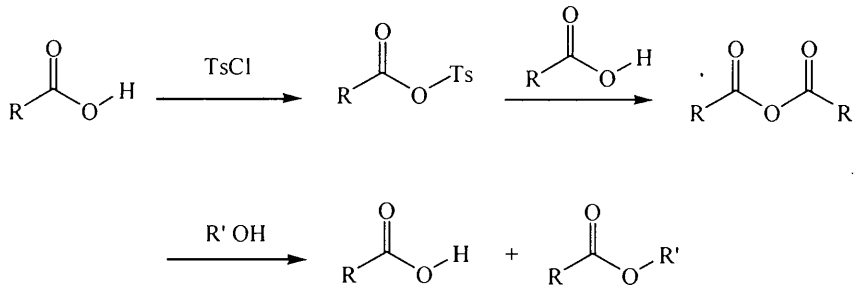


Figure 4.11, mechanism of the esterification with *p*-toluenesulfonyl chloride.[1]

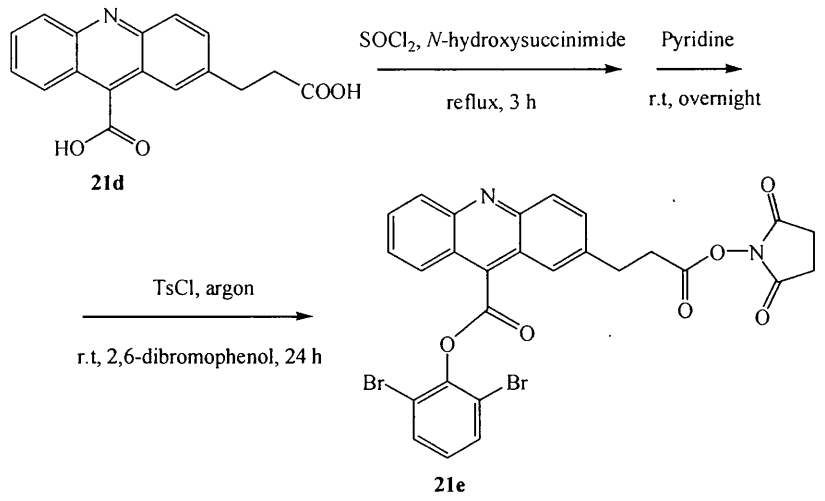


Figure 4.12, synthesis of 2,6-dibromophenyl 2-(2-succinimidyloxycarbonylethyl)acridine-9-carboxylate (**21e**)

In the ^{1}HNMR spectrum of **21e**, the proton integrals in the aromatic and aliphatic regions were in a ratio of 10: 8. Two triplets at 3.37 and 3.20 ppm were attributed to the 2 CH_2 groups, and a singlet at 2.97 ppm integrating for 4 protons was attributed to the 2 CH_2 groups of the succinimidyl moiety. In the ^{13}CNMR spectrum, 3 signals for the 4 CH_2 groups (2 of them are identical) in the aliphatic region, and another 12 peaks in the aromatic region, for 17 different aromatic carbons (5 pairs of them presumably overlapping), were found; peaks at 169.3 (a pair of them overlapped presumably) and 163.7 ppm were attributed to the 3 different carbonyl groups. The EI/CI mass spectrum gave ion peaks at m/z 626 ($[M]^+$) and 627 ($[M+H]^+$), 512 and 260 (**figure 4.13**).

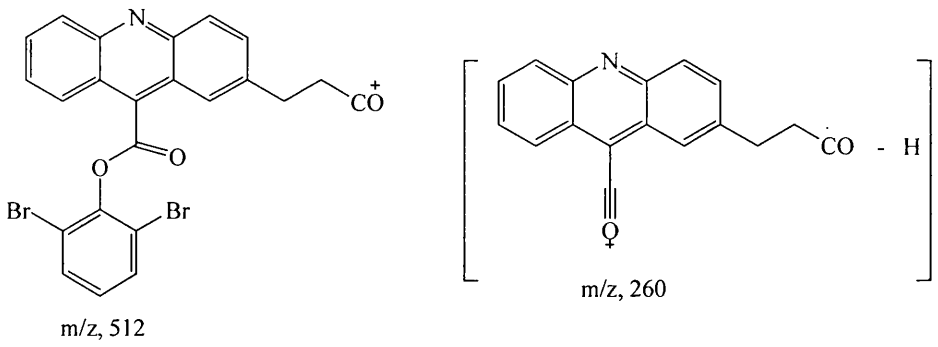
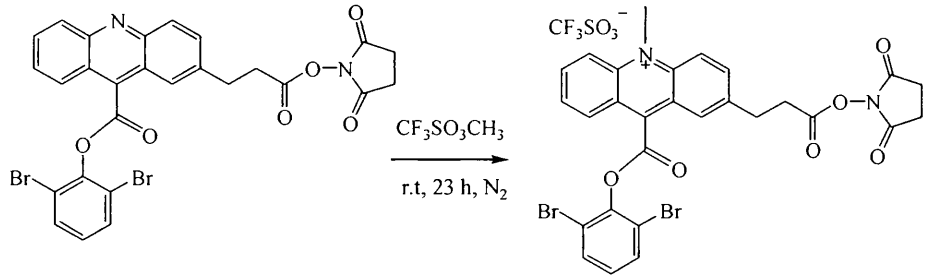


Figure 4.13, MS fragments for **compound 21e**

4.2.2.6 Synthesis of 9-(2,6-dibromophenoxycarbonyl)-2-(2-succinimidyloxycarbonylethyl)-10-methylacridinium trifluoromethanesulfonate (21)

Compound 21 was synthesized in a synthetic pathway similar to those used for **compounds 2, 7, 12, 16,** and **18,** by stirring 2,6-dibromophenyl 2-(2-succinimidyloxycarbonylethyl)acridine-9-carboxylate (**21e**) and methyl triflate in dry DCM under argon (**figure 4.14**). After the mixture was stirred at room temperature for 3 h, the expected precipitate had not appeared, therefore, the mixture was stirred for a further 20 h, but there was still no precipitate. The resulting mixture was evaporated to give a red brown residue. The residue was re-dissolved in DCM and the product was precipitated with EtOEt in a way similar to that used for purifying LiAE. But TLC showed that the starting material was not removed completely. Possibly, this is due to the difference of the solubility between **21** and **21e** not being so great as those of other corresponding pairs, *e.g.* **2/1a, 7/7a, 12/12a, 16/16e** and **18/18a**. Therefore, the crude product was subjected to chromatography on a silica column, eluted with DCM+CH_3CN (4:1, and then 3:1). The fractions with Rf = 0.19 (developed in DCM+CH_3CN, 3:1) were combined and evaporated to give a brown yellow oil, which solidified on standing, in 54% yield.



21

Figure 4.14, synthesis of 9-(2,6-dibromophenoxycarbonyl)-2-(2-succinimidyloxycarbonylethyl)-10-methylacridinium trifluoromethanesulfonate (**21**)

In the ^{1}HNMR spectrum of **21**, there were 7 peaks in the aromatic region for 9 different protons (2 pairs of them overlapped accidentally), and 4 peaks in the aliphatic region, integrating in a ratio of 2:2:1:1:1:2:1:3:2:2:4. A singlet at 5.08 ppm was attributed to the methyl group, 2 triplets were attributed to the 2 different CH_2 groups and a singlet at 3.07 ppm was attributed to the 4 protons of the succinimidyl moiety. In the ^{13}CNMR spectrum, there were peaks at 170.2, 169.0 and 161.8 ppm, accounting for the 3 carbonyl groups, and another 17 peaks in the aromatic region accounting for the 17 different aromatic carbons. A signal at 40.4 ppm was attributed to the methyl group; signals at 33.4 and 30.3 ppm were attributed to the 2

different CH_2 groups, and a peak at 26.3 ppm was attributed to the 2 identical CH_2 groups of the succinimidyl moiety. The ES mass spectrum showed cations at m/z 641 ($[M\text{-}CF_3SO_3]^+$) and 544, and an anion at m/z 149 ($[CF_3SO_3]^-$), corroborating the structure of the compound **21** (**figure 4.15**).

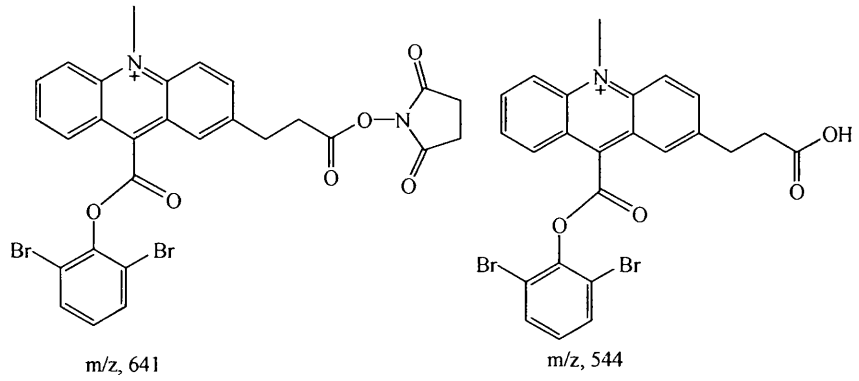


Figure 4.15, MS fragments for **compound 21**

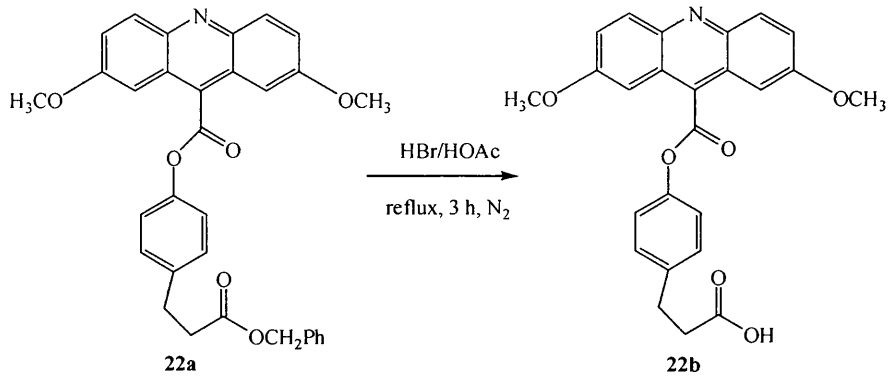
4.2.3 Synthesis of 9-(4-(2-succinimidyloxycarbonylethyl)phenoxycarbonyl)-2,7-dimethoxy-10-methylacridinium trifluoromethansulfonate (22)[11, 12]

The structure of **compound 22'**, synthesized before by Li in the Smith group, is shown in **figure 4.1**.[12] Its precursor (**compound 22a**) shown in **figure 4.16**, available generously from Dr. Li, is an important intermediate for the synthesis of **22**. Selective hydrolysis of the benzyl group of **compound 22a** would give the corresponding carboxylic acid (**22b**). **Compound 22b** would be esterified with *N*-hydroxysuccinimide to afford **compound 22c**, which would be methylated to provide the final product **22**.

4.2.3.1 Synthesis of 4-(2-carbonylethyl)phenyl 2,7-dimethoxyacridine-9-carboxylate (22b)

Compound 22b was synthesized by selectively hydrolysing 4-(2-benzyloxycarbonylethyl)phenyl 2,7-dimethoxyacridine-9-carboxylate (**22a**) in HBr+HOAc (1:4) at 100 °C for 3 h, as illustrated in **figure 4.16**. The resulting mixture after cooling, was poured into water and extracted with MeOH (20%) + $CHCl_3$. The organic extracts were dried over $MgSO_4$, filtered, and evaporated on a rotary evaporator to leave a yellow solid, which was taken up in hot DCM. Hexane

was then added until the solution became cloudy. The mixture was left to stand overnight at room temperature. The precipitate that formed was collected by filtration and dried under vacuum to yield the desired product.



22a 22b

Figure 4.16, Synthesis of 4-(2-carbonylethyl)phenyl 2,7-dimethoxyacridine-9-carboxylate **(22b)**

In the ^{1}HNMR spectrum of **22b**, there were 5 peaks in the aromatic region and 3 peaks in the aliphatic region, integrating in a ratio of 2:2:2:2:2:6:2:2. A singlet at 3.78 ppm was attributed to the 2 methoxy groups, and 2 triplets at 2.69 and 2.38 ppm were attributed to the 2 CH_2 groups. Due to its poor solubility in DMSO, the signals of the ^{13}CNMR spectrum were too weak to be clear. The IR spectrum showed absorptions at 1745 (C=O of ester bond) and 1704 cm^{-1} (C=O of carboxylic acid). The mass spectrum gave a pseudo molecular ion peak at m/z 432 ($[M+H]^+$).

4.2.3.2 Synthesis of 4-(2-succinimidylyoxycarbonylethyl)phenyl 2,7-dimethoxyacridine-9-carboxylate (22c)

Compound 22c was synthesized by using 4-(2-carbonylethyl)phenyl 2,7-dimethoxyacridine-9-carboxylate (**22b**) and *N*-hydroxysuccinimide. **Compound 22b** was heated in $SOCl_2$ at 65 °C for 3 h. The excess $SOCl_2$ was evaporated under vacuum, and pyridine was then added, followed by *N*-hydroxysuccinimide. The mixture was stirred overnight at room temperature. The synthesis is illustrated in **figure 4.17**. The resulting mixture was stripped of pyridine under vacuum to leave a brown residue. DCM was added to take up the product. The extract was loaded onto a silica column for chromatography, eluted with DCM + EtOAc (30%). The fractions

(Rf = 0.25) were pooled and evaporated to give a bright yellow solid. However, the ^{1}HNMR spectrum showed impurities, which had the same Rf value as the product **22c**, possibly resulting from hydrolysis of the phenyl ester bond during the process of heating **22b** with thionyl chloride. The reaction was slightly modified, by heating **22b** in thionyl chloride for a shorter time (0.5 h) in the first stage. A pure product was achieved in 33% yield.

In the ^{1}HNMR spectrum of **22c**, there were 2 signals in the aromatic region for 5 different aromatic protons (4 of them overlapped accidentally), and 4 signals in the aliphatic region, integrating in a ratio of 2:8:6:2:2:4. A singlet at 3.95 ppm was attributed to the 2 methoxy groups and 2 triplets at 3.13 and 2.98 ppm were attributed to the 2 CH_2 groups linked with the phenoxy ring. A singlet at 2.83 ppm was attributed to the 2 identical CH_2 groups of the succinimidyl moiety. In the ^{13}CNMR spectrum, there were 3 peaks at 169.5, 168.2 and 167.0 ppm attributed to the 3 carbonyl groups and another 11 signals in the aromatic region for the 11 different aromatic carbons. A signal at 56.0 ppm was attributed to the 2 methoxy carbons, peaks at 32.9 and 30.3 ppm were attributed to the 2 different CH_2 groups linked with the phenoxy ring, and a peak at 26.0 ppm was attributed to the 2 identical CH_2 groups of the succinimidyl moiety. The EI-CI mass spectrum showed ions at m/z 528 ($[M]^+$), 414 and 266 (**figure 4.18**); the accurate molecular mass was found at 528.1525, against 528.1527 by calculation.

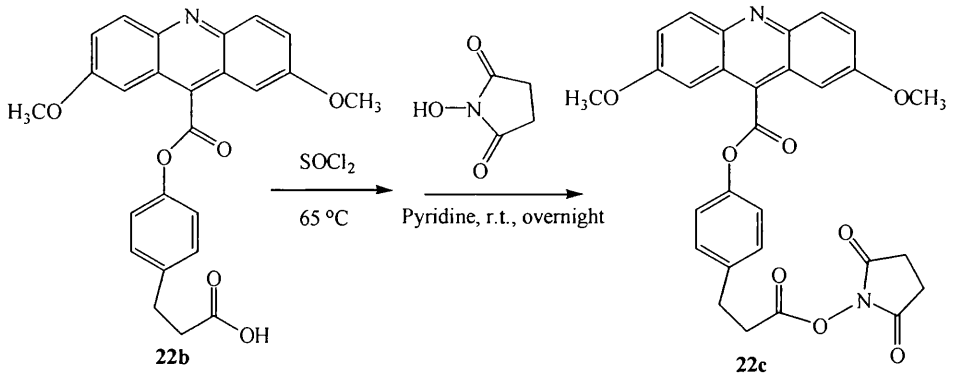
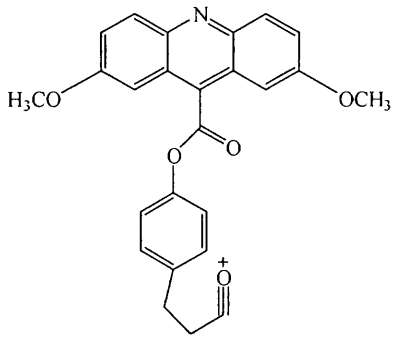


Figure 4.17, synthesis of 4-(2-succinimidyloxycarbonylethyl)phenyl 2,7-dimethoxyacridine-9-carboxylate (**22c**)



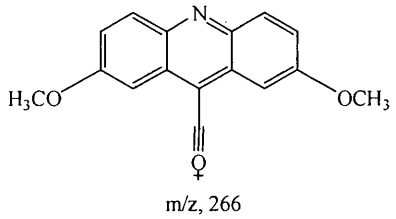


Figure 4.18, MS fragments for **compound 22c**

4.2.3.3 Synthesis of 9-(4-(2-succinimidyloxycarbonylethyl)phenoxycarbonyl)-2,7-dimethoxy-10-methylacridinium trifluoromethansulfonate (22)

Compound 22 was synthesized by stirring 4-(2-succinimidyloxycarbonylethyl)phenyl 2,7-dimethoxyacridine-9-carboxylate (**22c**) and methyl triflate in dry DCM, as illustrated in **figure 4.19**. The starting materials were stirred at room temperature overnight in dry DCM under argon. The excess triflate and solvent were evaporated under vacuum. The crude product was purified by chromatography over a silica column, eluted with DCM+CH_3CN (3:1), the fractions with Rf = 0.16 being pooled and evaporated under vacuum gave an orange-yellow solid. In the ^{1}HNMR spectrum, there were 2 doublets and 1 multiplet (3 signals overlapped accidentally) in the aromatic region, and 5 signals in the aliphatic region, integrating in a ratio of 2:2:6:3:6:2:2:4. A singlet at 5.02 ppm was attributed to the methyl group next to the N atom of the acridinium ring, a singlet at 4.06 ppm was attributed to the 2 methoxy groups, 2 triplets at 3.02 and 2.92 ppm were attributed to the 2 different CH_2 groups and a singlet at 2.76 ppm was attributed to the 2 identical CH_2 groups of the succinimidyl moiety. In the ^{13}CNMR spectrum, there were 3 lines at 170.0, 168.6 and 164.2 ppm attributed to the 3 carbonyl groups, and another 11 lines attributed to the 11 different aromatic carbons. A peak at 56.6 ppm was attributed to the 2 methoxy groups; a signal at 40.5 ppm was attributed to the *N*-methyl group; 2 peaks at 32.4 and 29.3 ppm were attributed to the 2 different CH_2 groups; and a peak at 25.8 ppm was attributed to the 2 identical CH_2 groups on the NHS moiety. The mass spectrum showed a cation at m/z 543 ($[M\text{-}CF_3SO_3]^+$) and an anion at m/z 149 ($[CF_3SO_3]^-$), corroborating the successful synthesis of **22**.

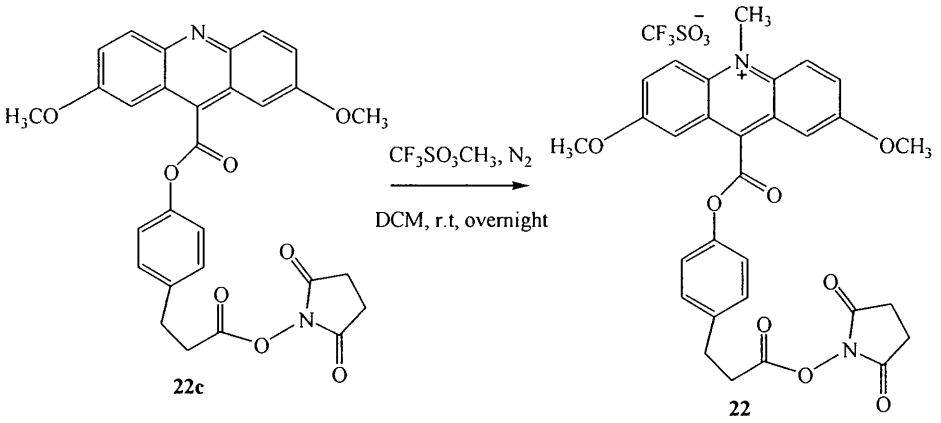
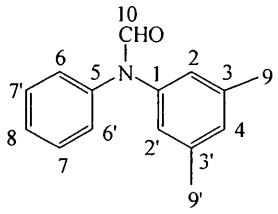


Figure 4.19, Synthesis of 9-(4-(2-succinimidyloxycarbonylethyl)phenoxycarbonyl)-2,7-dimethoxy-10-methylacridinium trifluoromethansulfonate (**22**)

4.3 Experimental details and characterization

4.3.1.1 Synthesis of *N*-(3,5-dimethylphenyl)formanilide (20a)

In a round bottom flask (25 ml), equipped with an air condenser and a stirrer bar, formanilide (3.274 g, 27.1 mmol), 5-bromo-*m*-xylene (7.028 g, 38.2 mmol), potassium carbonate (3.724 g, 27.0 mmol) and copper iodide (5.163 g, 27.2 mmol) were mixed and heated at reflux for 18 h. The resulting mixture was extracted with DCM (90 ml). The extract was dried over $MgSO_4$, filtered, and evaporated on a rotary evaporator to give a yellow oil. The oil (9.142 g) was subjected to chromatography over a silica column, eluted with hexane firstly, then DCM. The fractions (Rf = 0.73 in DCM) were pooled and evaporated to give a white solid (1.126g, 20 % yield), m.p. 144 °C, which proved to be a side product *N,N*-(bis(3,5-dimethylphenyl)aniline. The fractions with Rf = 0.30 were combined and evaporated to provide a pale yellow oil (4.118 g, 67% yield), which solidified on standing, m.p. 54-55 °C, and which proved to be the desired product.



^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 8.59, 8.55, (1H, H_{10} splitting into 2 singlets, because of restricted rotation about the N-CO bond), 7.32 (2H, H_7, $H_{7'}$, m), 7.16-7.25 (2H, H_8, H_6 or $H_{6'}$, m), 7.08 (1H, H_6 or $H_{6'}$, d, J = 7 Hz), 6.85 (2H, H_2, $H_{2'}$, s), 6.71 (1H, H_4, s), 2.23 (6H, H_9, $H_{9'}$, s).

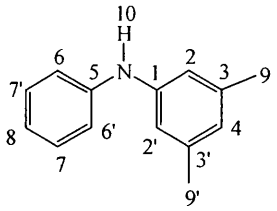
^{13}CNMR (400 Hz, chloroform-d, δ, ppm), 162.0 (C_{10}), 142.4 and 142.0, (C_5, splitting into 2 peaks, because of restricted rotation about the N-CO bond), 140.2 and 139.7 (C_1, splitting into 2 lines), 139.7 and 139.4 (C_3/$C_{3'}$, splitting into 2 lines), 129.8 (C_7/$C_{7'}$), 129.3 (C_4), 126.8 (C_8), 124.3 (C_6/$C_{6'}$), 123.5 (C_2/$C_{2'}$), 21.7 (C_9/$C_{9'}$).

IR (υ, cm^{-1}), 1682 (C=O).

MS (m/z), EI: 225 (M^+, 100%), 197 ($[M-CHO+H]^+$, 79%), 77 (49%); CI: 243 ($[M+NH_4]^+$, 100%), 226, ($[M+H]^+$, 86%).

4.3.1.2 Synthesis of *N*-(3,5-dimethylphenyl)aniline (20b)

In a round bottom flask (50 ml), equipped with a condenser and a stirrer bar, **compound 20a** (1.95 g, 8.67 mmol) was gently refluxed in ethanol (15 ml) with KOH (1.95g, 34.8 mmol) for 20 h. Then the solvent was removed on a rotary evaporator. To the residue, DCM (100 ml) was added. The mixture was filtered, and the filtrate was concentrated on a rotary evaporator, and then loaded onto a silica column for chromatography, eluted with DCM. The fractions with Rf = 0.78 were combined and evaporated to give a brown solid (1.168g, 68% yield), m.p. 51-52 °C.



^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 7.20 (2H, H_7, $H_{7'}$, dd, J = 8, 7 Hz,), 7.02 (d, H_6, $H_{6'}$, d, J = 8 Hz), 6.87 (1H, H_8, t, J = 7 Hz), 6.67 (2H, H_2, $H_{2'}$, s), 6.54 (1H, H_4, s), 2.20 (6H, H_9, $H_{9'}$, s), H_{10} was not found, presumably because it was very broad.

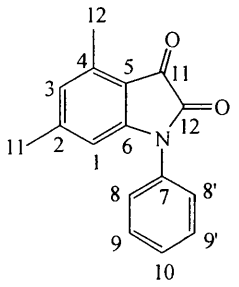
^{13}CNMR (400 Hz, chloroform-d, δ, ppm), 142.8 (C_5), 139.5 (C_1, C_3/$C_{3'}$), 129.7 (C_7/$C_{7'}$), 123.8 (C_4), 122.0 (C_8), 118.7 (C_6/$C_{6'}$), 116.4 (C_2/$C_{2'}$), 21.8 (C_9/$C_{9'}$).

IR (υ, cm^{-1}), 3382 (N-H).

MS (m/z), EI: 197 (M^+, 54%), 77 (43%); CI: 198 ($[M+H]^+$, 100%).

4.3.1.3 Synthesis of 4,6-dimethyl-*N*-(phenyl)isatin (20c)

To oxalyl chloride (1.162g, 8.20 mmol) in DCM (5 ml) in a round bottom flask (25 ml), equipped with a stirrer, *N*-(3,5-dimethylphenyl)aniline (**20b**) (1.168g, 5.93 mmol) in DCM (6.8 ml) was added slowly. The flask was equipped with a condenser and refluxed for 0.5 h. The resulting mixture was allowed to cool, and the excess oxalyl chloride and DCM were evaporated on a rotary evaporator. To the brown residue, DCM (10 ml) was added again, followed by $AlCl_3$ (2.77 g, 20.8 mmol) in portions carefully. The mixture was refluxed for a further 1 h. Then after cooling and removal of the solvent from the resulting mixture under vacuum, any remaining reactive species were destroyed with ice and dilute HCl (1 mol L^{-1}, 10 ml) carefully. The mixture was extracted with DCM (20 ml × 3). The extract was dried over $MgSO_4$, filtered and evaporated. The crude product was purified by column chromatography (silica), eluted with DCM. The fractions with Rf = 0.61 were combined and evaporated to afford an orange solid (0.991 g, 67% yield), m.p. 157-158 °C.



^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 7.39 (2H, H_9, $H_{9'}$, apparent t, J = 8 Hz,), 7.29 (1H, H_{10}, t, J = 8 Hz), 7.23 (2H, H_8, $H_{8'}$, d, J = 8 Hz), 6.58 (1H, H_1, s), 6.32 (1H, H_3, s), 2.43 (3H, H_{11} or H_{12}, s), 2.16 (3H, H_{11} or H_{12}, s).

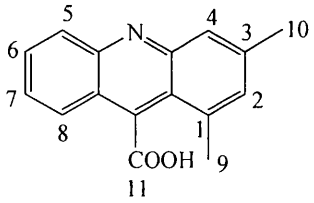
^{13}CNMR (400 Hz, chloroform-d, δ, ppm), 182.9 (C_{11}), 158.4 (C_{12}), 152.5 (C_2), 150.2 (C_6), 141.9 (C_7), 133.6 (C_4), 130.3 (C_9/$C_{9'}$), 129.1 (C_3), 127.5 (C_{10}), 126.7 (C_8/$C_{8'}$), 114.1 (C_5), 109.8 (C_1), 23.2 (C_{11}), 18.6 (C_{12}).

IR (υ, cm^{-1}), 1746 (Ar-C=O), 1721 (-NC=O).

MS (m/z), EI: 251 (M^+, 19%), 223 ($[M-2CH_3+2H]^+$, 78%), 77 (100%); CI: 269 ($[M+NH_4]^+$, 100%), 238 (17%).

4.3.1.4 Synthesis of 1,3-dimethylacridine-9-carboxylic acid (20d)

To a round bottom flask (25 ml), equipped with a stirrer and a condenser, 4,6-dimethyl- *N*-(phenyl)isatin (**20c**) (0.739 g, 2.94 mmol) and KOH (1.414 g, 25.2 mmol) in H_2O (13 ml) were added. The mixture was refluxed for 39 h. After the mixture was cooled, it was acidified with concentrated HCl to pH = 2. The yellow precipitate was filtered, re-dissolved in K_2CO_3 (10%) aqueous solution, and then re-precipitated with concentrated HCl carefully. The precipitate was collected by filtration, washed with DCM (10 ml) and methanol (10 ml), and pumped overnight. The product (0.419 g) was obtained in 57% yield, m.p. > 280 °C.



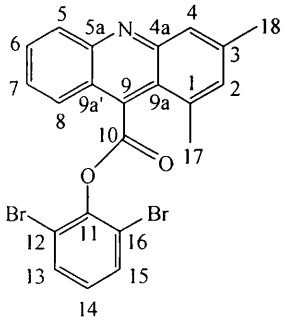
^{1}HNMR (400 Hz, DMSO-d_6, δ, ppm), 8.24 (1H, H_8, d, J = 8 Hz), 8.08-7.97 (2H, H_5, H_6, m), 7.93 (1H, H_4, s), 7.78 (1H, H_7, apparent t, J = 8 Hz), 7.49 (1H, H_2, s), 2.84 (3H, H_9 or H_{10}, s), 2.57 (3H, H_9 or H_{10}, s). H_{11} was not found, presumably because the chemical shift was more than 11.5 ppm or because the peak was too broad.

IR (υ, cm^{-1}), 1714 (C=O).

MS (m/z), EI: 251 (M^+, 11%), 207 ($[M-COOH]^+$ or $[M-2CH_3-OH+3H]^+$, 27%), 44 (100%); CI: 252 ($[M+H]^+$, 17%), 210 (100%). MS/ACC, 252.1019 ($[M+H]^+$) for theoretical mass of $C_{16}H_{14}NO_2$; 252.1022 found.

4.3.1.5 Synthesis of 2,6-dibromophenyl 1,3-dimethylacridine-9-carboxylate (20e)

1,3-Dimethylacridine-9-carboxylic acid (**20d**) (226 mg, 0.900 mmol) was refluxed in thionyl chloride (15 ml) in a round bottom flask (25 ml) for 23 h. Excess thionyl chloride was removed on a rotary evaporator to leave a brown solid. Then pyridine (10 ml), DMAP (87 mg, 0.707 mmol) and 2,6-dibromophenol (208 mg, 0.826 mmol) were added respectively. The flask was flushed with N_2 and the mixture was stirred at room temperature overnight. The resulting mixture was stripped of pyridine under vacuum. The residue was taken in DCM for chromatography over a silica column, eluted with DCM, The fractions with Rf = 0.57 were pooled and evaporated to give a yellow solid (44 mg, 11% yield), which turned purple later.



^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 8.62 (1H, H_8, broad d, J = 9 Hz), 8.08 (1H, H_5, broad d, J = 9 Hz), 7.78 (1H, H_4, s), 7.63 (1H, H_6, ddd, J = 9, 7, 1 Hz), 7.51 (2H, d, H_{13}, H_{15}, J = 8 Hz), 7.43 (1H, H_7, ddd, J = 9, 7, 1 Hz), 7.13 (1H, H_2, s), 6.92 (1H, H_{14}, t, J = 8 Hz), 2.67 (3H, H_{17} or H_{18}, s), 2.38 (3H, H_{17} or H_{18}, s).

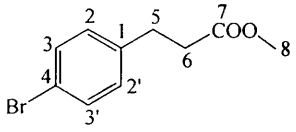
^{13}CNMR (400 Hz, chloroform-d, δ, ppm), 164.2 (C_{10}), 150.6 (C_{11}), 148.2 (C_{5a}), 146.7 (C_{4a}), 140.7 (C_3), 136.2 (C_9), 134.5 (C_1), 133.9 (C_{13}/C_{15}), 133.5 (C_{14}), 130.6 (C_2), 129.9 (C_5), 129.0 (C_6), 127.6 (C_7), 127.1 (C_4), 126.6 (C_8), 123.1 ($C_{9a'}$), 121.6 (C_{9a}), 118.1 (C_{12}/C_{16}), 24.5 (C_{18}), 22.2 (C_{17}).

IR (υ, cm^{-1}), 1760 (C=O).

MS (m/z), EI: 485 ($[M]^+$, 1%), 234 (100%), 206 (69%); CI: 486 ($[M+H]^+$, 13%), 236 (30%), 208 (100%).

4.3.2.1 Synthesis of methyl 3-(4-bromophenyl)propionoate (21a)

In a round bottom flask (100 ml), equipped with a condenser and a $CaCl_2$ drying tube, 3-(4-bromophenyl)propionic acid (1.130g, 4.93 mmol) was refluxed in $SOCl_2$ (10 ml) for 2 h. The resulting mixture was evaporated to leave an oil, to which, methanol (3 ml) and pyridine (10 ml) were added and the whole mixture was then stirred overnight at room temperature. The resulting mixture was evaporated on a rotary evaporator to leave a pale yellow oil, which was chromatographed on a silica column, eluted with DCM. The fractions with Rf = 0.83 were pooled and evaporated to give a pale yellow oil (1.045g) in 87% yield, (**lit.** [10], b.p. 154-155 °C /16mmHg).



^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 7.42 (2H, H_3, $H_{3'}$, d, J = 8 Hz), 7.10 (2H, H_2, $H_{2'}$, d, J = 8 Hz), 3.69 (3H, H_8, s), 2.92 (2H, H_5, t, J = 8 Hz), 2.63 (2H, H_6, t, J = 8 Hz).

^{13}CNMR (400 Hz, chloroform-d, δ, ppm), 173.4 (C_7), 139.8 (C_1), 132.0 ($C_3/C_{3'}$), 130.5 ($C_2/C_{2'}$), 120.5 (C_4), 52.1 (C_8), 35.8 (C_6), 30.7 (C_5).

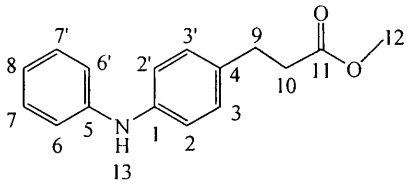
IR (υ, cm^{-1}), 1733 cm^{-1}.

MS (m/z), EI: 244, 242 ($[M]^+$), 6%), 184, 182 (100%), 171, 169 (52%); CI: 262, 260 ($[M+NH_4]^+$, 100%), 182 (27%).

4.3.2.2 Synthesis of 4-(2-methoxycarbonylethyl)-*N*-phenylaniline (21b)

To a two-neck round bottom flask (100 ml), methyl 3-(4-bromophenyl)propionoate (**21a**) (2.181g, 8.97 mmol), aniline (1.074g, 11.54 mmol), DPPF (0.756g, 1.36

mmol), (DPPF)$PdCl_2$ (0.367g, 0.449 mmol) and sodium t-butoxide (1.237g, 12.9 mmol) were added. The flask was equipped with a condenser and a stirrer bar, and flushed with nitrogen. Then, re-distilled toluene (20 ml) was introduced with a syringe. The mixture was heated at 100 °C for 3 h. After the reaction was cooled to room temperature, the volatiles were removed over a rotary evaporator. The residue was loaded onto a silica column for chromatography, eluted with DCM. The fractions with Rf = 0.24 were pooled and evaporated to give a pale yellow oil (1.33 g, 58% yield).



^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 7.07 (2H, H_7, $H_{7'}$, apparent t, J = 8 Hz), 6.92 (2H, H_3, $H_{3'}$, d, J = 8 Hz), 6.89-6.78 (4H, H_2, $H_{2'}$, H_6, $H_{6'}$, m), 6.72 (1H, H_8, t, J = 8 Hz), 5.61 (1H, H_{13}, broad s), 3.49 (3H, H_{12}, s), 2.72 (2H, H_9, t, J = 8 Hz), 2.43 (2H, H_{10}, t, J = 8 Hz).

^{13}CNMR (400 Hz, chloroform-d, δ, ppm), 173.9 (C_{11}), 143.9 (C_5), 141.7 (C_1), 133.7 (C_4), 129.7 (C_7/$C_{7'}$), 129.6 (C_3/$C_{3'}$), 121.1 (C_8), 118.8 (C_6/$C_{6'}$), 117.7 (C_2/$C_{2'}$), 52.0 (C_{12}), 36.3 (C_{10}), 30.7 (C_9).

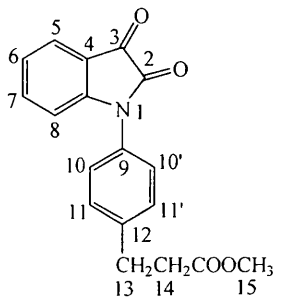
IR (υ, cm^{-1}), 3383 (N-H), 1723 (C=O).

MS (m/z), EI: 255 ($[M]^+$, 23%), 240 (7%), 195 (13%), 182 (100%), 167 (18%); CI: 273 ($[M+NH_4]^+$, 15%), 256 ($[M+H]^+$, 100%), 182 (16%).

4.3.2.3 Synthesis of 5-(2-methoxycarbonylethyl)-*N*-phenylisatin (21c1) and *N*-(4-(2-methoxycarbonylethyl)phenyl)isatin (21c2)

4-(2-Methoxycarbonylethyl)-*N*-phenylaniline (1.30g, 5.21 mmol) in DCM (6 ml) was added to stirred oxalyl chloride (1.017g, 8.01 mmol) in DCM (9 ml) slowly. The flask (50 ml) was equipped with a condenser and a $CaCl_2$ drying tube. The mixture was refluxed for 30 min and then the excess $(COCl)_2$ and solvent were removed. The

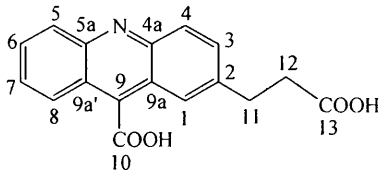
black brown residue was dissolved DCM (12 ml), and then anhydrous $AlCl_3$ (2.2g, 16.5 mmol) was added in portions carefully. The mixture was refluxed for 50 min. The solvent was removed under vacuum, and then remaining reactive material was destroyed with HCl (1 mol L^{-1}, 10 ml). The mixture was filtered, and the filtrate was extracted with EtOEt (5×15 ml). The extract was combined with the precipitate and the solvent was removed under vacuum. The crude product was purified by chromatography over a silica column, eluted with DCM. The fractions with Rf = 0.17 were pooled and evaporated to leave a red oil (solidifying on standing, 978 mg, 61% yield). The ^{1}HNMR spectrum showed that it was a mixture of two isomers, both of them giving the same product in the next stage. The mixture was characterized with MS and IR. MS (m/z), EI: 309 ($[M]^+$, 22%) and 207 (100%); CI: 327 ($[M+NH_4]^+$, 100%), 313 ($[M+NH_4-CH_3+H]^+$, 48%), 296 (46%). IR (υ, cm^{-1}), 1732, 1705, 1669 (C=O). Compound **21c2** could be characterized by its ^{1}HNMR spectrum.



^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 7.62 (1H, H_8, dd, J = 8, 1 Hz), 7.47 (1H, H_7, apparent td, J = 8, 1 Hz), 7.32 (2H, H_{10}, $H_{10'}$, d, J = 8 Hz), 7.27 (2H, H_{11}, $H_{11'}$, d, J = 8 Hz), 7.10 (1H, H_6, apparent td, J = 8, 1 Hz), 6.82 (1H, H_5, dd, J = 8, 1 Hz), 3.63 (3H, H_{15}, s), 2.96 (2H, H_{13}, t, J = 8 Hz), 2.62 (2H, H_{14}, t, J = 8 Hz).

4.3.2.4 Synthesis of 2-(carbonylethyl)acridine-9-carboxylic acid (21d)

In a round bottom flask (50 ml), the mixture of 2 isatin isomers **21c1** and **21c2** (779.7 mg, 2.52 mmol) was refluxed in NaOH aqueous solution (10%, 15 ml) for 20 h. The resulting mixture was cooled and acidified with concentrated HCl to pH 2.0. A copious quantity of yellow compound precipitated, and was collected by filtration, washed with H_2O (30 ml), methanol (20 ml) and DCM (30 ml), and then evaporated under vacuum. A yellow solid (629 mg, 84% yield) was obtained, m.p. > 290 °C.



^{1}HNMR (400 Hz, DMSO-d_6, δ, ppm), 8.21 (1H, H_8, broad d, J = 8 Hz), 8.17 (1H, H_5, broad d, J = 9 Hz), 8.06 (1H, H_4, d, J = 8 Hz), 7.91 (1H, H_6, ddd, J = 9, 7, 1 Hz), 7.86 (1H, H_3, d, J = 8Hz), 7.85 (1H, H_1, s), 7.72 (1H, H_7, ddd, J = 8, 7, 1 Hz), 3.10 (2H, H_{11}, t, J = 8 Hz), 2.70 (2H, H_{12}, t, J = 8 Hz); H_{10} and H_{13} were not found, presumably because the chemical shifts were more than 11.5 ppm or because the peaks were too broad.

^{13}CNMR (400 Hz, DMSO-d_6, δ, ppm), 174.4 (C_{13}), 169.1 (C_{10}), 148.2 (C_{5a}), 147.9 (C_{4a}), 141.1 (C_2, C_9), 133.7 (C_3), 131.5 (C_5), 129.9 (C_4, C_6), 128.2 (C_7), 126.1 (C_1), 123.7 (C_8), 122.0 ($C_{9a'}$), 121.8 (C_{9a}), 35.4 (C_{12}), 31.5 (C_{11}).

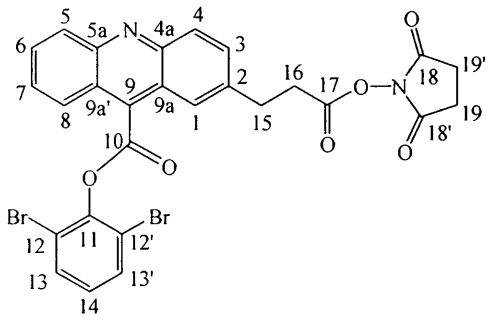
IR (υ, cm^{-1}), 1709 cm^{-1}.

MS (m/z), CI: 296 ($[M+H]^+$, 100%), 252 (62%), 208 (39%); ES^-: 294 ($[M-H]^-$, 100%), 240 (96%).

4.3.2.5 Synthesis of 2,6-dibromophenyl 2-(2-succinimidyloxycarbonylethyl)-acridine-9-carboxylate (21e)

In a round bottom flask (25 ml), equipped with a stirrer bar, a condenser and a $CaCl_2$ drying tube, 2-(2-carbonylethyl)acridine-9-carboxylic acid (250 mg, 0.84 mmol) was refluxed in $SOCl_2$ (6 ml) with *N*-hydroxysuccinimide (184 mg, 1.60 mmol) for 3 h. The excess $SOCl_2$ was evaporated under vacuum. To the residue, pyridine (7 ml) was added. After the mixture was stirred overnight under argon, it was cooled in an ice bath. *p*-Tolenesulfonyl chloride (279 mg, 1.47 mmol) was added, the mixture was stirred for 15 min, and then the ice bath was removed. 2,6-Dibromophenol (230 mg, 0.92 mmol) was added and the mixture was stirred for a further 1 day. The resulting mixture was evaporated under vaccum. The residue was washed with HCl (0.1 mol L^{-1}, 8 ml) and then dried under vacuum to provide the crude product, which was

purified by chromatography, eluted with DCM. The fractions with Rf = 0.11 were pooled and evaporated under vacuum to give a pale yellow solid, m.p. 207-208 °C, (154 mg, 29% yield).



^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 8.28-8.17 (3H, H_1, H_5, H_8, m), 8.19 (1H, H_4, d, J = 9 Hz), 7.78 (1H, H_6, ddd, J = 9, 7, 1 Hz), 7.75 (1H, H_3, d, J = 9, 1 Hz), 7.63 (1H, H_7, ddd, J = 9, 7, 1 Hz), 7.46 (2H, H_{13}, $H_{13'}$, d, J = 8 Hz), 6.92 (1H, H_{14}, t, J = 8 Hz), 3.37 (2H, H_{15}, t, J = 8 Hz), 3.20 (2H, H_{16}, t, J = 8 Hz), 2.97 (4H, H_{19}, $H_{19'}$, s).

^{13}CNMR (400 Hz, chloroform-d, δ, ppm), 169.3 (C_{17}, C_{18}/$C_{18'}$), 163.7 (C_{10}), 148.5 (C_{5a}), 148.1 (C_{4a}), 140.7 (C_{11}), 133.1 (C_2, C_9), 132.8 (C_3, C_{13}/$C_{13'}$), 131.0 (C_{14}), 130.4 (C_4, C_5), 128.7 ($C_{9a'}$, C_{9a}), 128.6 (C_6), 125.2 (C_7), 123.2 (C_1, C_8), 118.2 (C_{12}/$C_{12'}$), 34.5 (C_{16}), 31.3 (C_{15}), 26.3 (C_{19}/$C_{19'}$).

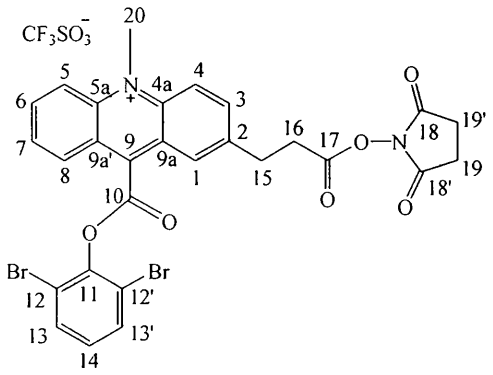
IR (υ, cm^{-1}), 1739 cm^{-1}.

MS (m/z), EI: 626 ($[M]^+$, 3%), 512 (9%), 347 (5%), 333 (11%), 260 (100%), 251.9 (49%); CI: 627 ($[M+H]^+$, 17%), 583 (23%), 486 (100%).

4.3.2.6 Synthesis of 9-(2,6-dibromophenoxycarbonyl)-2-(2-succinimidyloxy-carbonylethyl)-10-methylacridinium trifluoromethanesulfonate (21)

To a round bottom flask (5 ml), 2,6-dibromophenyl 2-(2-succinimidyloxycarbonylethyl)acridine-9-carboxylate (**21e**) (47 mg, 0.075 mmol) was added. The flask was flushed with argon, and then dry DCM (3 ml) and methyl triflate (110 μl, 0.97 mmol) were introduced with syringe, respectively. The mixture was stirred at room temperature for 23 h. The resulting mixture was evaporated to

give a red brown residue, which was loaded onto a silica column for chromatography, eluted with DCM + CH_3CN (4:1, and then 3:1), the fractions (Rf = 0.19 with solvent DCM + CH_3CN, 3:1) were combined and evaporated to give a brown yellow oil, which solidified on standing, m.p. 86-87 °C (32 mg, 54% yield).



^{1}HNMR (400 Hz, acetone-d_6, δ, ppm), 8.98-8.90 (2H, H_5, H_8, m), 8.59-8.53 (2H, H_1, H_4, m), 8.49 (1H, H_6 or H_7, apparent t, J = 8 Hz), 8.42 (1H, H_3, d, J = 8 Hz), 8.14 (1H, H_6 or H_7, apparent t, J = 8 Hz), 7.52 (2H, H_{13}, $H_{13'}$, d, J = 8 Hz), 7.03 (1H, H_{14}, t, J = 8 Hz), 5.08 (3H, H_{20}, s), 3.44 (2H, H_{15}, t, J = 7 Hz), 3.33 (2H, H_{16}, t, J = 7 Hz), 3.07 (4H, H_{19}, $H_{19'}$, s).

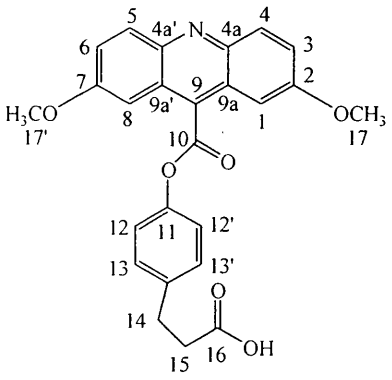
^{13}CNMR (400 Hz, acetone-d_6, δ, ppm), 170.2 (C_{18}/$C_{18'}$), 169.0 (C_{17}), 161.8 (C_{10}), 146.5 (C_{11}), 143.7 (C_9), 143.1 (C_{5a}), 142.4 (C_{4a}), 142.2 (C_{13}/$C_{13'}$), 142.0 (C_2), 139.9 (C_5), 133.0 (C_{14}), 130.7, 129.4, 127.2, 125.5 (C_4, C_6, C_7, C_8), 124.1, 124.0 (C_{9a}, $C_{9a'}$), 120.4, 120.3 (C_1, C_3), 117.8 (C_{12}/$C_{12'}$), 40.4 (C_{20}), 33.4 (C_{16}), 30.3 (C_{15}), 26.3 (C_{19}/$C_{19'}$).

IR (υ, cm^{-1}), 1738 cm^{-1}.

MS (m/z), ES^+: 641 ($[M\text{-}CF_3SO_3]^+$, 100%), 558 (76%), 544 (78%), 530 (48%), 420 (29%), 344 (48%), 248 (31%), 209 (42%), 196 (49%); ES^-: 149 ($[CF_3SO_3]^-$, 100%), 80 (58%). Acc-MS: calculated mass: 638.9761 ($[M\text{-}CF_3SO_3]^+$); measured mass: 638.9758.

4.3.3.1 Synthesis of 4-(2-carbonylethyl)phenyl 2,7-dimethoxyacridine-9-carboxylate (22b)

To a solution of HBr (48%) + HOAc (1:4) (10 ml), 4-(2-benzyloxycarbonylethyl)phenyl 2,7-dimethoxyacridine-9-carboxylate (**22a**) (0.517 mg, 0.991 mmol) was added, and the mixture was heated at 100 °C for 3 h. After the resulting mixture was cooled, it was poured into water (100 ml) and extracted with MeOH (20%) + $CHCl_3$ (5 × 100 ml). The organic extracts were combined, dried over $MgSO_4$, filtered and evaporated on a rotary evaporator. The residue was dissolved in hot DCM, and then hexane was added until the solution became cloudy. The mixture was left to stand overnight at room temperature. The solid that formed was collected by filtration and pumped overnight to yield the desired product (378 mg, 88%), m.p. 308-309 °C.



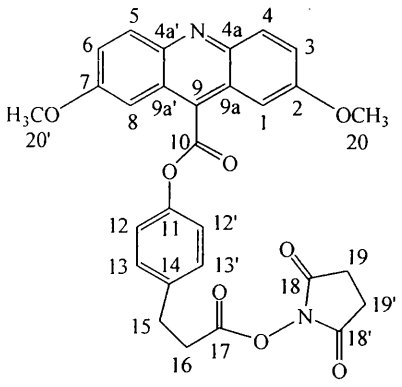
^{1}HNMR (400 Hz, DMSO-d_6, δ, ppm), 7.95 (2H, H_4, H_5, d, J = 9 Hz), 7.38 (2H, H_3, H_6, d, J = 9 Hz), 7.28 (2H, H_{13}, $H_{13'}$, d, J = 8 Hz), 7.22 (2H, H_{12}, $H_{12'}$, d, J = 8 Hz), 7.15 (2H, H_1, H_8, s), 3.78 (6H, H_{17}, $H_{17'}$, s), 2.68 (2H, H_{14}, t, J = 7 Hz), 2.38 (2H, H_{15}, t, J = 7 Hz). H_{16} was not found, presumably because the chemical shift was more than 11.5 ppm or because the peak was too broad.

IR (υ, cm^{-1}), 1745 (C=O of ester bond), 1704 (C=O of carboxylic acid) and a broad band for OH/NH group at 2600-3600 cm^{-1}.

MS (m/z), CI: 432 ($[M+H]^+$, 29%), 240 (89%).

4.3.3.2 Synthesis of 4-(2-succinimidyloxycarbonylethyl)phenyl 2,7-dimethoxyacridine-9-carboxylate (22c)

In a round bottom flask (25 ml), equipped with a stirrer and a condenser, 4-(2-carbonylethyl)phenyl 2,7-dimethoxyacridine-9-carboxylate (**22b**) (286 mg) was heated in $SOCl_2$ at 65 °C for 0.5 h. The resulting mixture was evaporated under vacuum to remove $SOCl_2$, then pyridine (5 ml) and *N*-hydroxysuccinimide (119 mg, 1.03 mmol) were added and the mixture was stirred overnight at room temperature. The resulting mixture was evaporated under vacuum to leave a brown residue, which was taken up in DCM and loaded onto a silica column for chromatography, eluted with DCM + EtOAc (30%). The fractions with Rf = 0.25 were pooled and evaporated to give a bright yellow solid (130 mg, 33% yield), m.p. 193-194 °C.



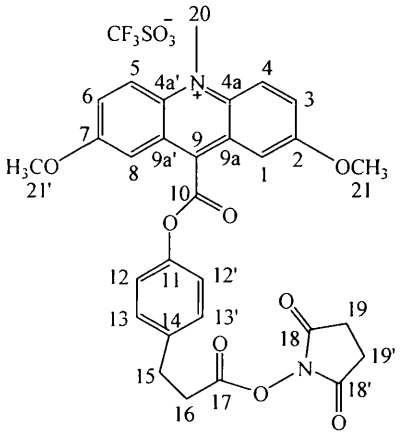
^{1}HNMR (400 Hz, chloroform-d, δ, ppm), 8.12 (2H, H_4, H_5, d, J = 9 Hz), 7.45-7.33 (8H, H_3, H_6, H_1, H_8, H_{12}, $H_{12'}$, H_{13}, $H_{13'}$, m), 3.95 (6H, H_{20}, $H_{20'}$, s), 3.13 (2H, H_{15}, t, J = 7 Hz), 2.98 (2H, H_{16}, t, J = 7 Hz), 2.83 (4H, H_{19}, $H_{19'}$, s).

^{13}CNMR (400 Hz, chloroform-d, δ, ppm), 169.5 (C_{18}/$C_{18'}$), 168.2 (C_{17}), 167.0 (C_{10}), 159.0 (C_2/C_7), 149.8 (C_{11}), 144.4 (C_{4a}/$C_{4a'}$), 138.0 (C_{14}), 132.0 (C_4/C_5), 130.5 (C_9 or C_{9a}/$C_{9a'}$), 130.2 (C_{13}/$C_{13'}$), 130.1 (C_9 or C_{9a}/$C_{9a'}$), 124.6 (C_3/C_6), 122.0 (C_{12}/$C_{12'}$), 100.4 (C_1/C_8), 56.0 (C_{20}/$C_{20'}$), 32.9 (C_{16}), 30.3 (C_{15}), 26.0 (C_{19}/$C_{19'}$).
IR (υ, cm^{-1}), 1731 (C=O).

MS (m/z), EI: 528 ($[M]^+$, 1%), 414 (2%), 266 (78%); CI: 432 (2%), 240 (100%); MS/ Acc: measured 528.1525; calculated for $C_{29}H_{24}N_2O_8$, 528.1527.

4.3.3.3 Synthesis of 9-(4-(2-succinimidyloxycarbonylethyl)phenoxycarbonyl)-2,7-dimethoxy-10-methylacridinium trifluoromethanesulfonate (22)

To a round bottom flask (5 ml), 4-(2-succinimidyloxycarbonylethyl)phenyl 2,7-dimethoxyacridine-9-carboxylate (**22c**) (51.9 mg, 0.095 mmol) was added. The flask was flushed with N_2, and then dry DCM (2.5 ml) and methyl triflate (200 µl, 1.77 mmol) were introduced with syringe respectively. The mixture was stirred overnight, and then the volatiles were evaporated under vacuum. The crude product was purified by chromatography over a silica column, eluted with DCM+CH_3CN (3:1). The fractions with Rf = 0.16 were pooled and evaporated under vacuum to give an orange solid (31.2 mg, 46% yield), m.p. 94-96 °C.



^{1}HNMR (400 Hz, acetone-d_6, δ, ppm), 8.81 (2H, H_4, H_5, d, J = 9 Hz), 7.98 (2H, H_{13}, $H_{13'}$, d, J = 8 Hz), 7.55-7.40 (6H, H_{12}, $H_{12'}$, H_3, H_6, H_1, H_8, m), 5.02 (3H, H_{20}, s), 4.06 (6H, H_{21}, $H_{21'}$, s), 3.02 (2H, H_{15}, t, J = 7 Hz), 2.92 (2H, H_{16}, t, J = 7 Hz), 2.76 (4H, H_{19}, $H_{19'}$, s).

^{13}CNMR (400 Hz, acetone-d_6, δ, ppm), 170.0 (C_{18}/$C_{18'}$), 168.6 (C_{17}), 164.2 (C_{10}), 159.8 (C_2/C_7), 149.1 (C_{11}), 142.0 (C_9), 139.5 (C_{14}), 137.9 (C_{4a}/$C_{4a'}$), 132.3 (C_4/C_5), 130.7 (C_{13}/$C_{13'}$), 125.7 (C_{9a}/$C_{9a'}$), 122.1 (C_{12}/$C_{12'}$), 121.9 (C_1/C_8), 102.5 (C_3/C_6), 56.6 (C_{21}/$C_{21'}$), 40.5 (C_{20}), 32.4 (C_{16}), 29.3 (C_{15}), 25.8 (C_{19}/$C_{19'}$).

IR (υ, cm^{-1}), 1732 (C=O).
MS (m/z), ES^+: 543 ($[M\text{-}CF_3SO_3]^+$, 100%), 446 (10%), 253 (6%); ES^-: 149 ($[CF_3SO_3]^-$, 100%). Acc-MS: calculated mass: 543.1762 ($[M\text{-}CF_3SO_3]^+$); measured mass: 543.1756.

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Chapter 5

Chemiluminescence tests

There are 22 target compounds in total that have been synthesized. Except for **compounds 3** (LiAE) and **20**, their chemiluminescent kinetics and storage stabilities have been tested. **Compound 3**, was expected to have the same chemiluminescent properties as **compound 4**; therefore, only **compound 4** was tested. **Compound 20** is not in an acridinium format, the kinetics of its chemiluminescence would be so slow as to prevent it being tested properly; therefore, its chemiluminescence was not intended to be tested.

5.1 Chemiluminescence measurements and initiating reagents[1, 2]

Samples were tested in a Luminometer (Ciba-Corning Magic Lite Analyzer). The procedure used was as follows.

The previously prepared **sample solution** (10 μl) was introduced to a test tube manually and the test tube was placed in the luminometer. The light triggering solution (**reagent 1**) was first injected to the test tube, followed by the light triggering solution (**reagent 2**), automatically. Firstly, the kinetics curve (light output *versus* time) was plotted by the machine automatically, and then the light output (efficiency) of each sample solution was measured for a suitable time (15s). The light output was expressed in relative light units (RLU).

Reagent 1 and **Reagent 2** solutions were kindly provided by MLT (Molecular Light Technology Research Ltd, Cardiff).

Reagent 1 (in 1 liter): nitric acid (6.3 ml, 70%), hydrogen peroxide (16.5 ml, 30%), and distilled water (977 ml).

Reagent 2 (in 1 liter): sodium hydroxide (10 g) and cetyltrimethylammonium chloride (7.5 ml, 25%) and distilled water (983 ml).

Sample solutions

The stock solutions of acridinium esters were prepared by dissolving the pure sample (1 mg or so, weighed accurately) in acetonitrile (1 ml). The stock solutions were firstly diluted with acetonitrile to 1×10^{-2} mg/ml, and further dilutions were made

with acetonitrile or fixed phosphate pH buffers to yield ready-to use solutions (*ca.* 1 ng/ml).

5.2 Results and discussion

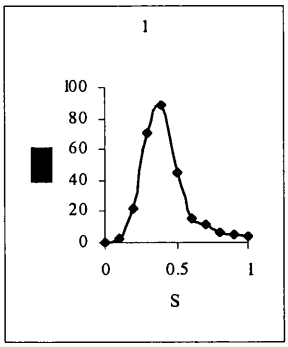
5.2.1 The kinetics

The kinetics curves (chemiluminescence *versus* time, Relative Light Units against seconds) were plotted by the machine automatically, but the length of the chemiluminescent time was controlled manually *via* the control panel. The results are displayed in **figure 5.1**. The compound number is shown in the top of each picture. The structures of **compounds 1, 2, 3, 4 ,5 and 6** are referred to in **figure 3.2** and **table 5.1**; **compounds 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18** and **19** are referred to in **figure 3.2** and **table 3.1**; **compounds 21** and **22** are referred to in **figure 4.1**.

Table 5.1, the specific compounds, numbering **1, 2, 4, 5** and **6**, synthesized in **chapter 2**.[a]

Compound number	Type a or b	n	R_1	R_2	R_3
1	a	11	Br	H	Br
2	a	0	Br	H	Br
4	b	3	Br	H	Br
5	b	5	Br	H	Br
6	b	10	Br	H	Br

a: The substituents shown are referred to in **figure 3.1** ($Y = CF_3SO_3$).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0
0.5
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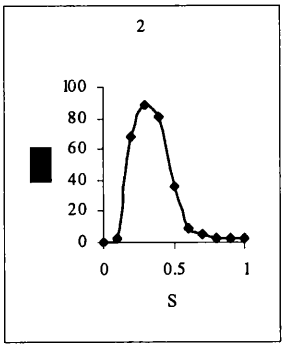
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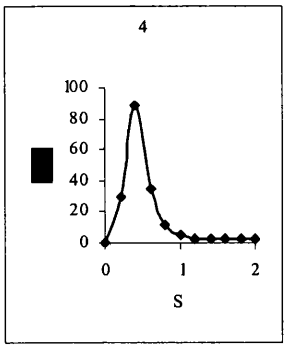
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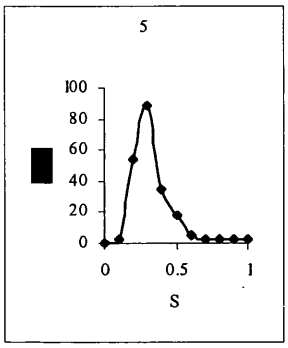
0
1
2
S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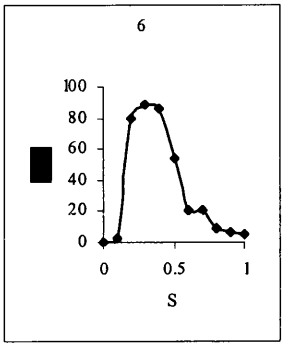
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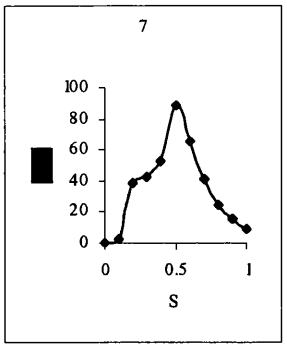
S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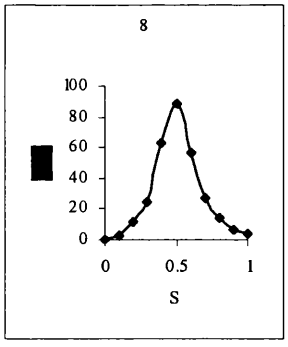
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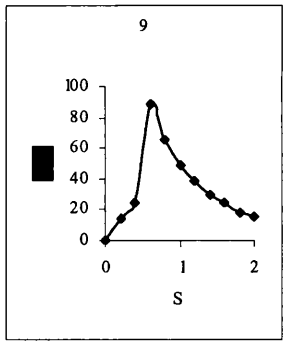
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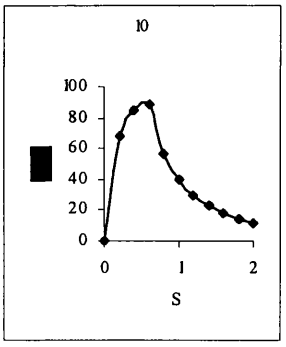
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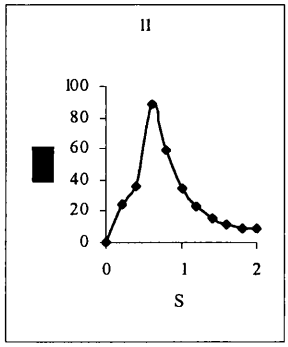
S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2
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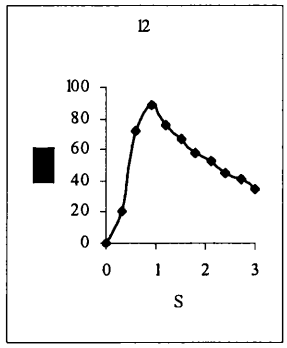
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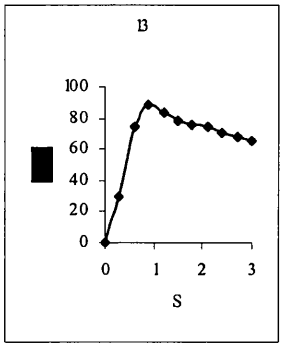
2
3
S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20
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0
1
2
3
S

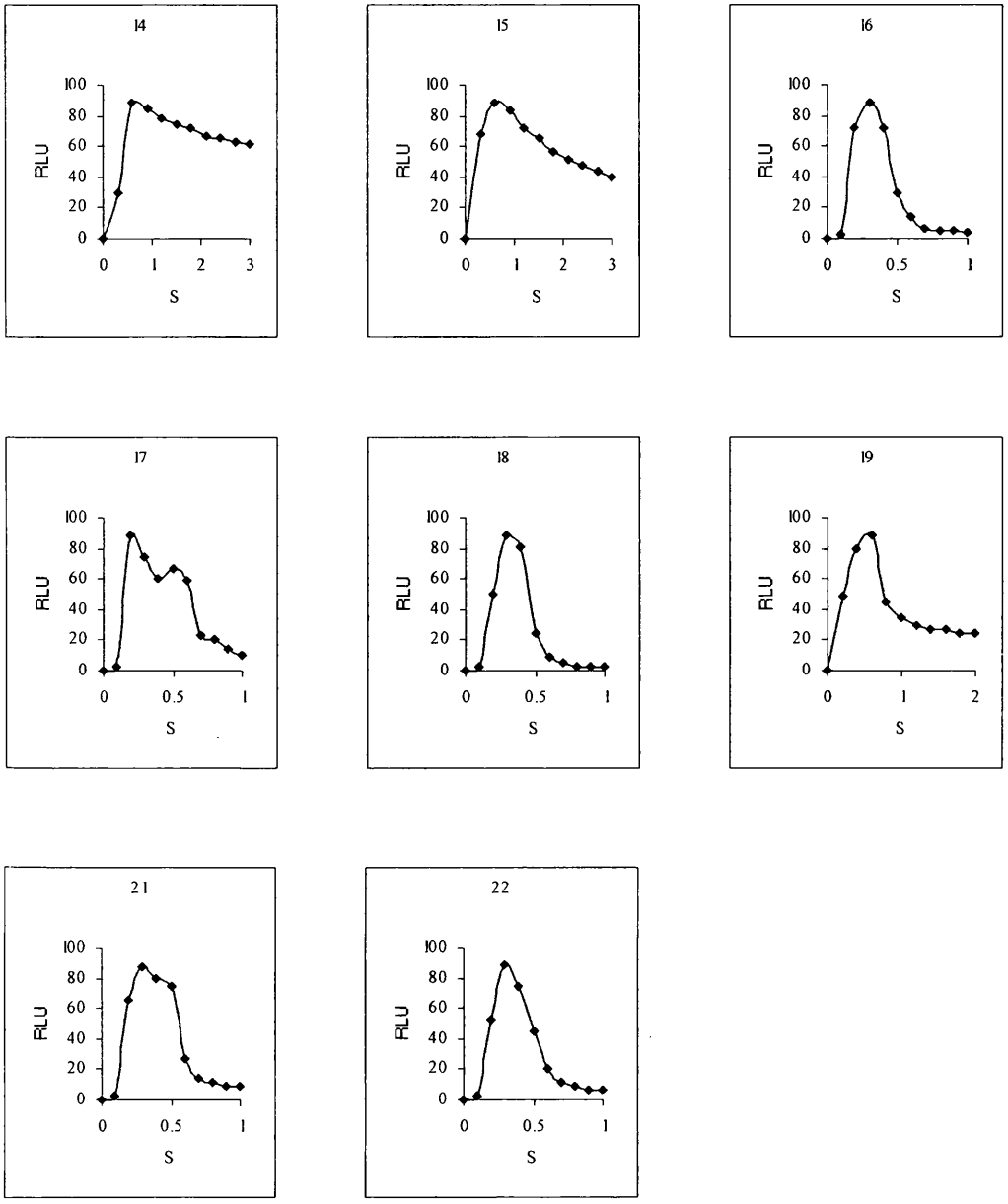


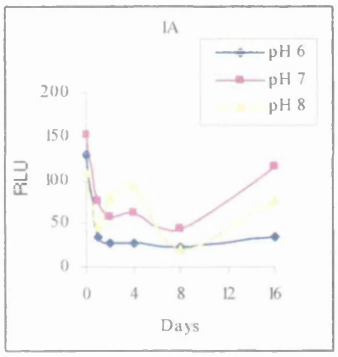
Figure 5.1, chemiluminescent kinetics of the novel AEs.

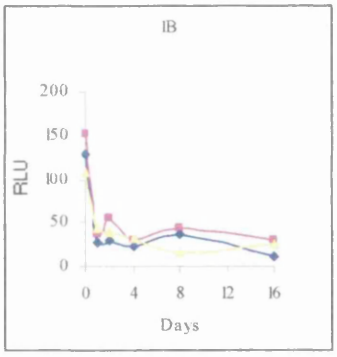
The electronic natures of substituents on the leaving group affect the chemiluminescent kinetics, due to the ease or difficulty of expelling the leaving group during the chemiluminescent process, as shown in the mechanism of chemiluminescence (**figure 1.7**).[3] AEs having electron-withdrawing groups on the phenoxy moiety should react to provide chemiluminescence quickly. By contrast, AEs having electron-donating groups on the phenoxy moiety should glow slowly. However, substituents on the acridinium moiety would be expected to have relatively

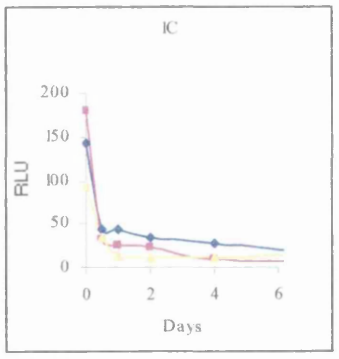
little effect. From the pictures in **figure 5.1**, it is clear that the nature of the alkyl substituent at the 10 position does not affect the kinetics; and that neither the 2-succinimidyloxycarbonylethyl group substituted at the side of the acridinium ring in compound **21**, nor the 2,7-dimethoxy groups substituted at the side of the acridinium ring in compound **22**, have significant effects on chemiluminescent kinetics. However, substituents on the leaving group do indeed affect the kinetics. AEs with a 2,6-dibromophenoxy leaving group (**compounds 1, 2, 4, 5, 6** and **21**), a 2,6-bis(trifluoromethyl)phenoxy leaving group (**compounds 16** and **17**), a 2,6-dinitrophenoxy leaving group (**compounds 18** and **19**), or an unsubstituented phenoxy group (**compounds 7, 8, 9, 10, 11** and **22**), all showed similar kinetics because they all flashed quickly and the differences in their kinetics were too small to be significant. For AEs with a 2,5-dimethylphenoxy leaving group, (**compounds 12, 13, 14** and **15**), the chemiluminescent kinetics were significantly slower. Therefore, AEs with electron-donating substituents on the leaving group, together with those AEs with electron withdrawing substituents or unsubstitued AEs, could be used to establish a multi-analysis pattern based on different chemiluminescent kinetics, but a combination of AEs with electron-withdrawing groups and ones with unsubstituted AEs will not be suitable for such a propose.

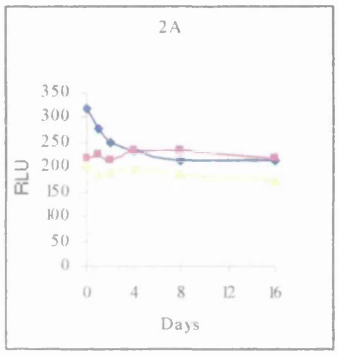
5.2.2 Storage stability

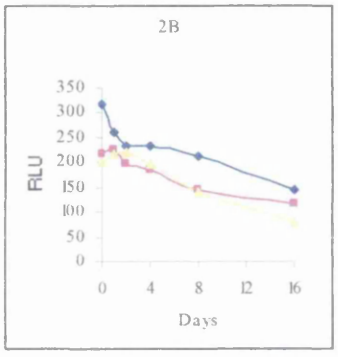
The stock solutions of the acridinium esters were first diluted to 1x 10^{-4} mg/ml with acetonitrile. Further dilution was carried out with buffers of pH = 6, 7 and 8, to yield the ready-to-use solutions (*ca.* 1 ng/ml). Each of the sample solutions was divided into three portions, and incubated at 8 °C, 25 °C and 37 °C, respectively. The values of measured relative light units for the 1 ng/ml ready to use samples were adjusted to their corresponding RLU values calculated for a concentration of 1 nmol L^{-1}. Sample solutions incubated at 37 °C were monitored over 1 week; and those at 8 °C and 25 °C were monitored over 2 weeks. In each case, the total emission (in RLUs) over 15 seconds was measured at intervals. The results are shown in **figure 5.2**.

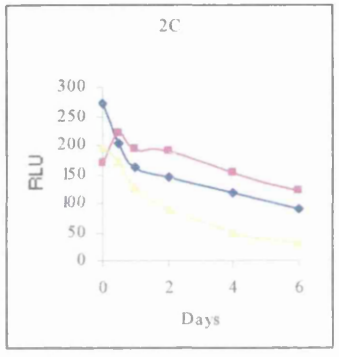

1A
pH 6
pH 7
pH 8
RLU
Days

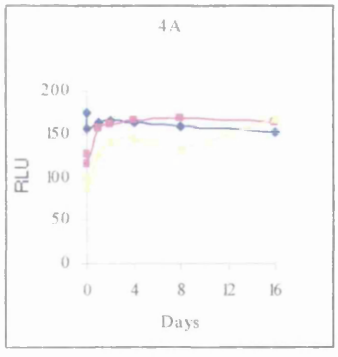

1B
RLU
Days

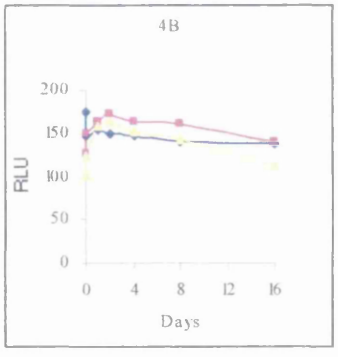

1C
RLU
Days

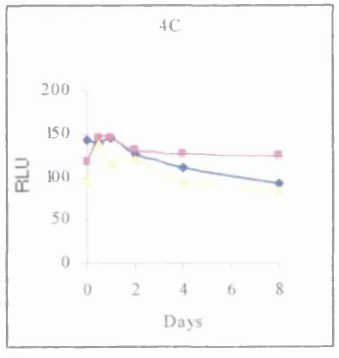

2A
RLU
Days

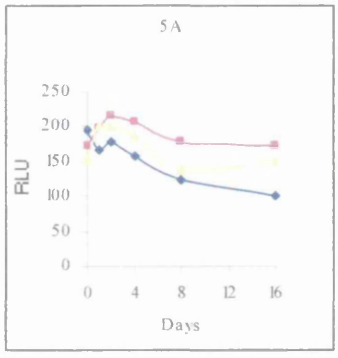

2B
RLU
Days

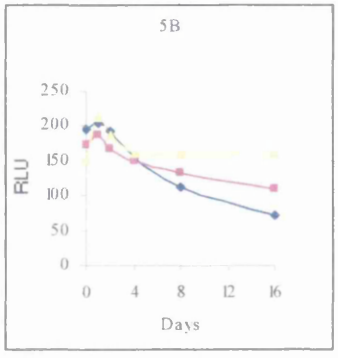

2C
RLU
Days

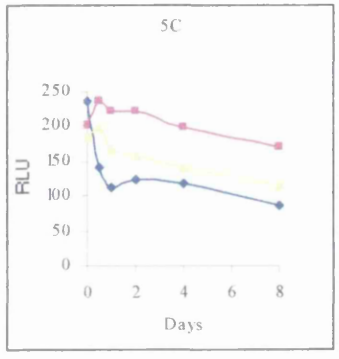

4A
RLU
Days

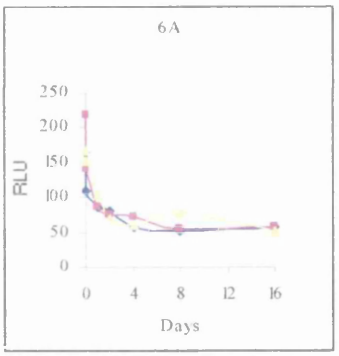

4B
RLU
Days

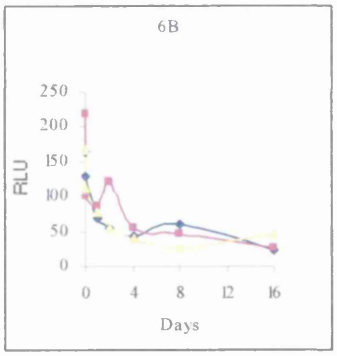

4C
RLU
Days


5A
RLU
Days


5B
RLU
Days


5C
RLU
Days


6A
RLU
250
200
150
100
50
0
0 4 8 12 16
Days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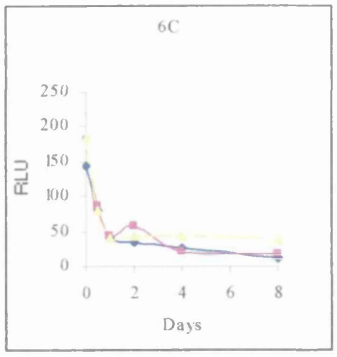
Days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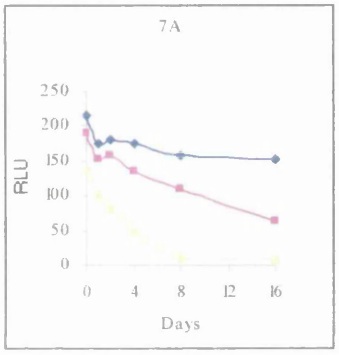
Days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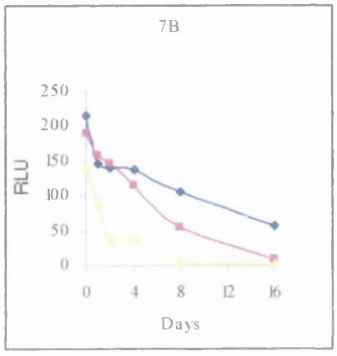
Days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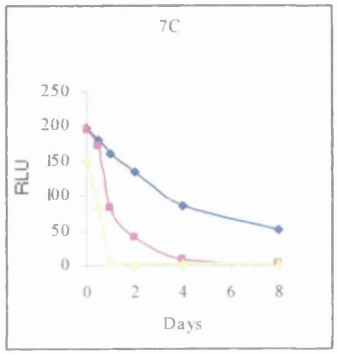
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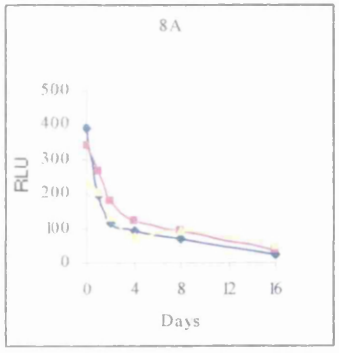
Days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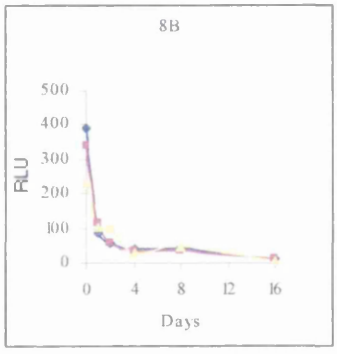
Days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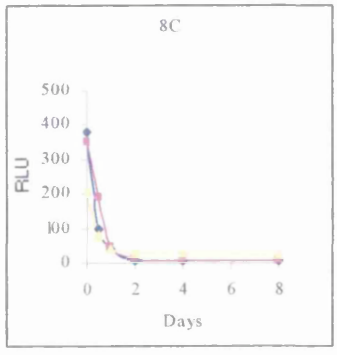
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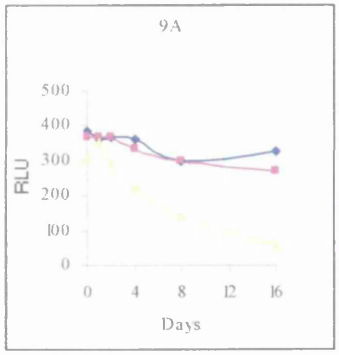
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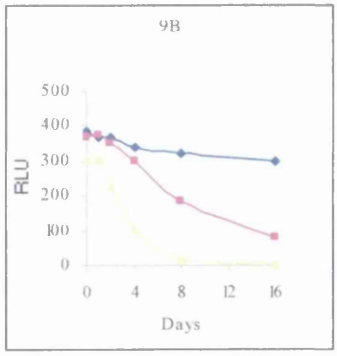
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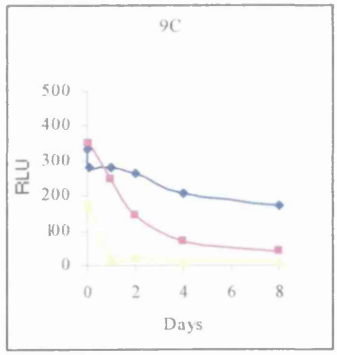
Days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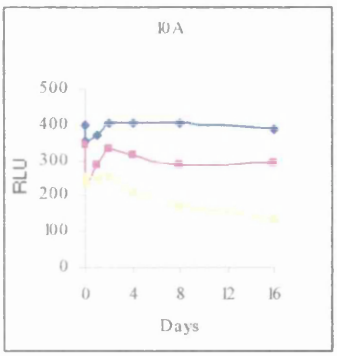
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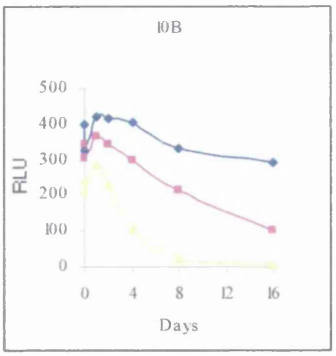
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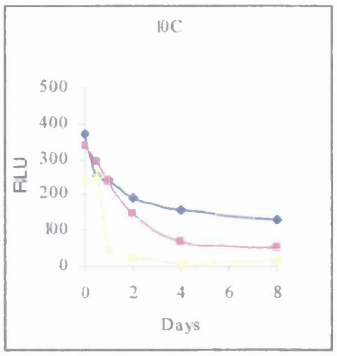
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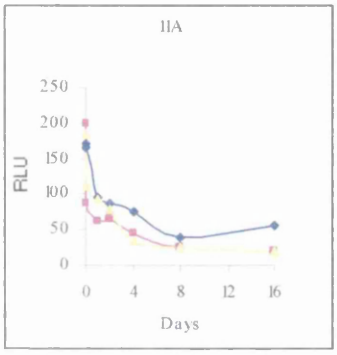
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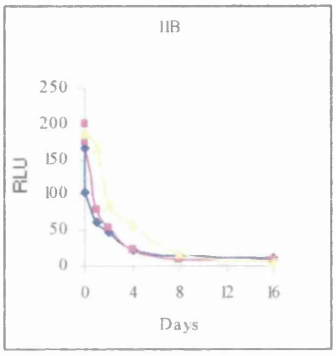
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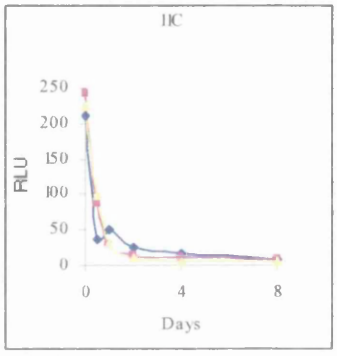
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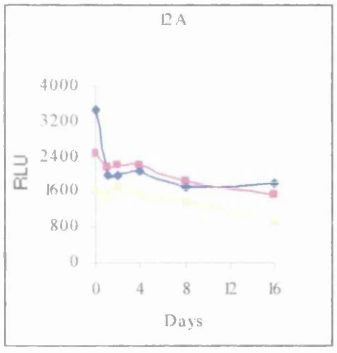
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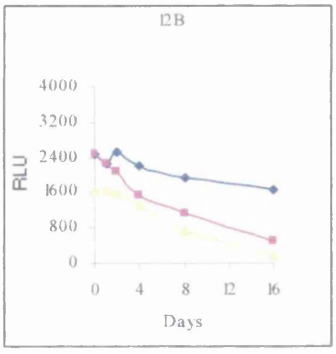
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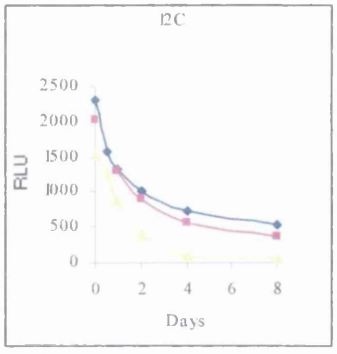
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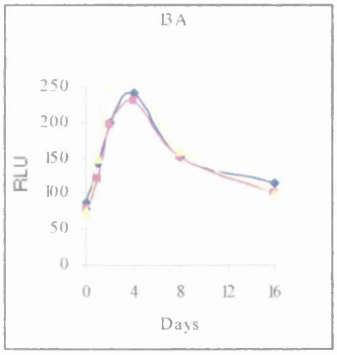
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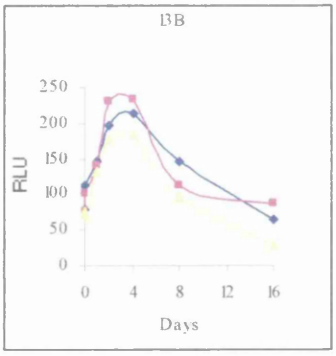
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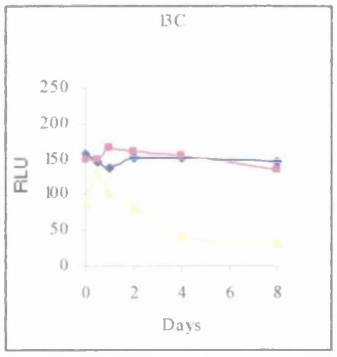
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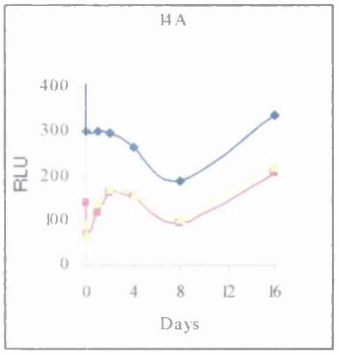
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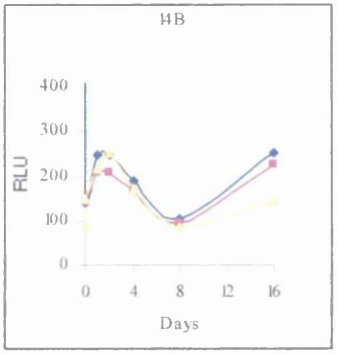
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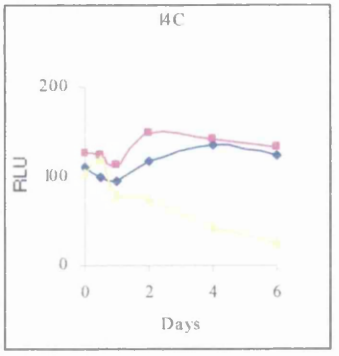
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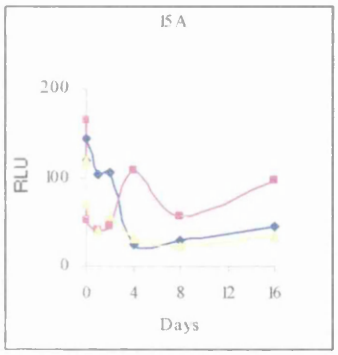
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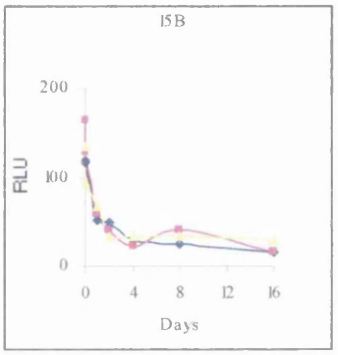
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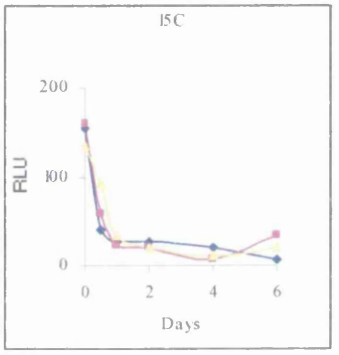
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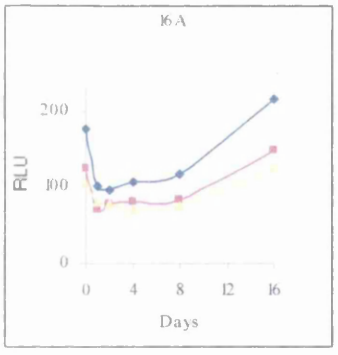
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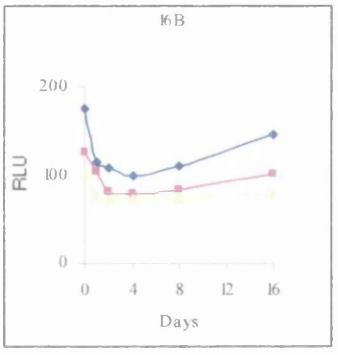
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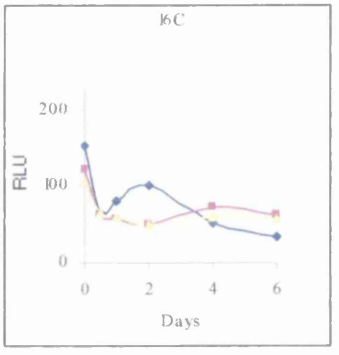
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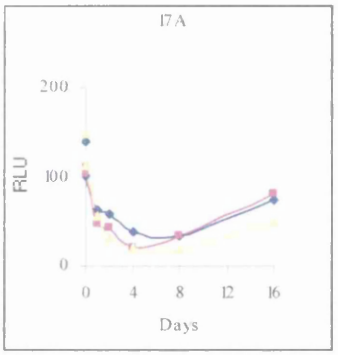
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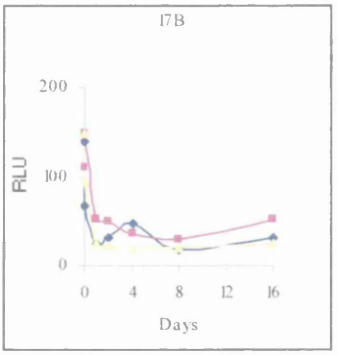
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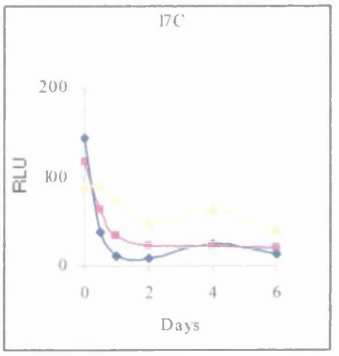
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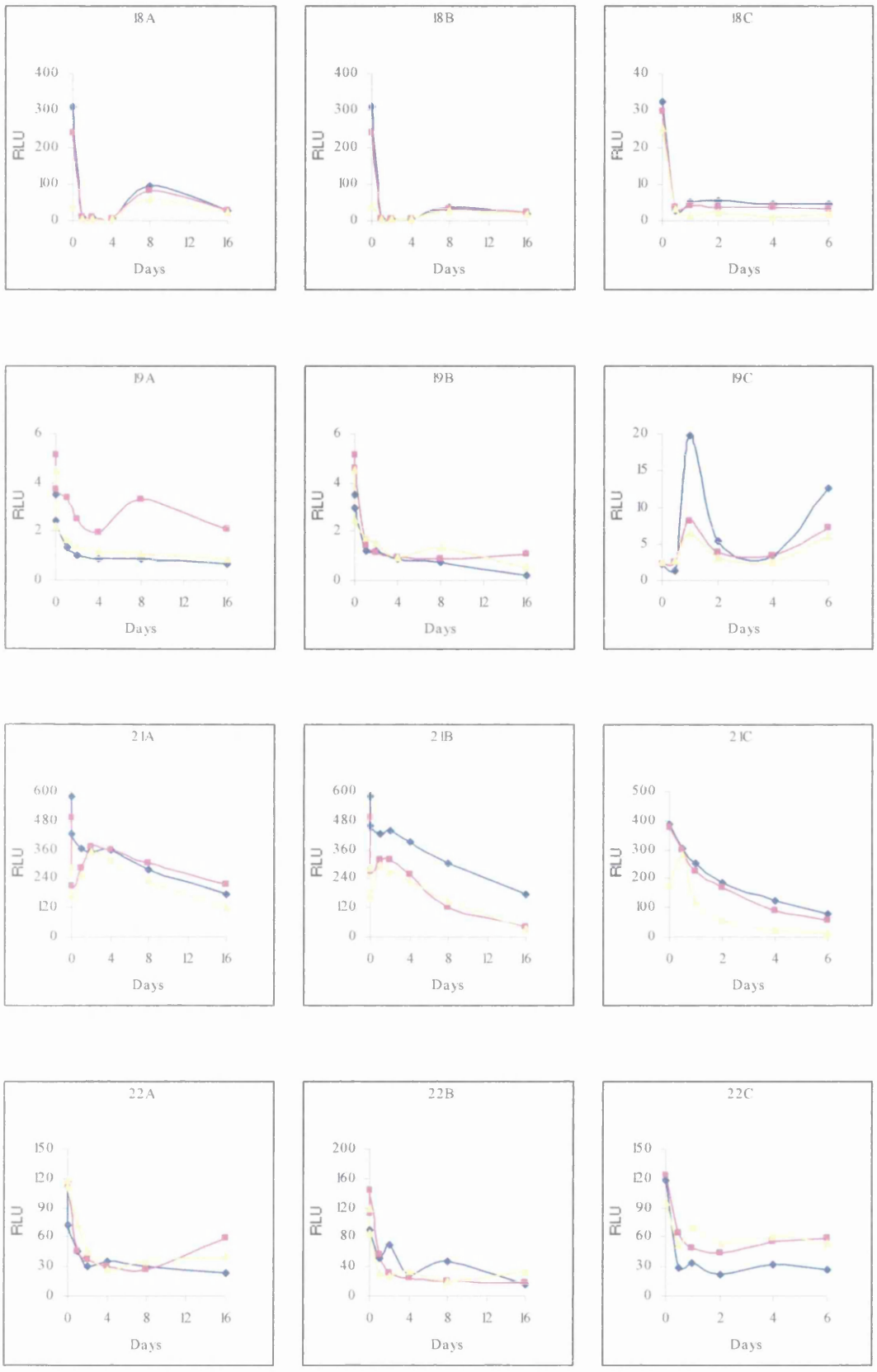

Figure 5.2. storage stabilities for AEs **1, 2, 4, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 21** and **22** stored in pH 6 (blue curves), 7 (red curves) and 8 (yellow curves) buffer solutions at different temperatures (A: at 8 °C, B: 24 °C, C: 37 °C).

The stabilities of acridinium esters could be tested by monitoring chemiluminescent decay during storage, because hydrolysis of the acridinium ester group would cause decrease in chemiluminescence. Generally, The AEs stored at 8 °C (plots labelled **A**) showed stronger intensities of chemiluminescence than the same AEs stored at higher temperatures, 24 °C (plots labelled **B**) or 37 °C (plots labelled **C**) over the same period. AEs stored at 37 °C showed the weakest chemiluminescence, and after 1 week or so at this temperature, the chemiluminescence for most AEs diminished by more than 90% per cent, so the samples were monitored for only 1 week. The results shows that AEs stored at 8 °C are more stable than at 24 °C or 37 °C, which is in accord with expectation.

By comparing the *N*-methylated AEs (**compounds 2** and **7**) with the *N*-dodecylated AEs (**compounds 1** and **8**), the methylated ones showed reasonable plots in line with expectations, *i.e*: as the length of the storage time increased, the intensity of chemiluminescence decreased slowly, due to hydrolysis of the sample during storage. For the dodecylated AEs, the chemiluminescence decreased sharply within 1 day even at 8 °C at pH 6, 7 and 8, as shown for **compound 1** and **compound 8** in **figure 5.2**. No previous report has been found for the chemiluminescent properties of AEs with a log-chain alkyl group attached to the nitrogen atom. The reason why AEs possessing a long alkyl chain linked to the N atom are so unstable is not clear. Possibly the pseudo-base for a compound with a bulkier substituent at the nitrogen atom is favoured (**figure 5.3**). The leaving group may be more easily expelled due to steric expulsion at C9 position in a pseudo-base than that in an acridinium salt. It may also have something to do with the different solubilities of the compounds, which might cause them to disperse differently in the aqueous media. For example, the long alkyl chains encourage micelle formation, with the polar acridinium ester portions of the molecules being on the outside of the micelle, where they are exposed to the aqueous medium.

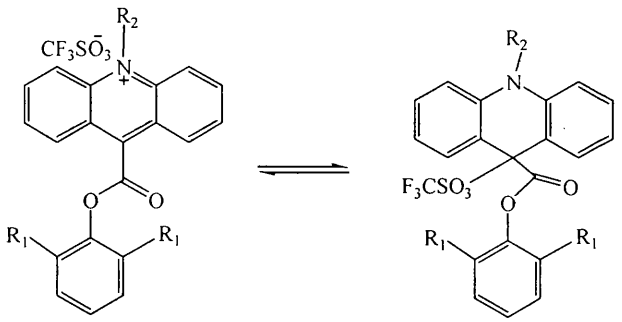

Figure 5.3, the equilibrium of acridinim salt and pseudobase

For AEs having a linker group with a 10 CH_2 spacer (*i.e.* **compounds 6, 11, 15, 17** and **19**), the chemiluminescence also decreased abruptly within 1 day during storage. This is a similar case to the dodecylated AEs. The poor solubility in water, leading to aggregation of micelles of AE, probably account also for the fluctuation of chemiluminescence with duration of storage. 9-(2,5-Dimethylphenoxycarbonyl)AEs (**compounds 13, 14** and **15**) also showed fluctuations of chemiluminescence during storage, perhaps for the same reason.

Generally, AEs stored in pH 6 and 7 buffer solutions showed relatively good stabilities. This is consistent with what Dr. Chen reported. The peudobase is favoured in a basic buffer solution while an acridinium salt is favoured in an acidic buffer solution. Therefore, AEs display better stability in weakly acidic or neutral aqueous solutions than in basic solutions.

It has been reported that the substitution *ortho* to the phenoxy leaving group could provide protection of the compound from hydrolysis, irrespective of the electronic nature of the substituents.[3] This is the case for 9-(2,6-dibromophenoxy)AEs and 9-(2,5-dimethylphenoxy)AEs, but it is not the case for 9-(2,6-dinitrophenoxy)AEs. The ready hydrolysis caused by the strongly electron-withdrawing nature of the nitro groups predominates over any protection against hydrolysis due to *ortho* steric hindrance. The 9-(2,6-dinitrophenoxy)AEs (**compounds 18** and **19**), showed terribly poor stability in pH 6, 7 or 8 buffer solutions, and also poor efficiency in basic buffer

solution (pH = 8). However, the 9-(2,6-bis(trifluoromethyl)phenoxy)AEs (**compounds 16** and **17**), have much better stabilities than the 9-(2,6-dinitrophenoxy)AEs (**18** and **19**), despite the similar electron-withdrawing nature of the substituents. Perhaps the bulkier trifluoromethyl groups provide some protection against hydrolysis because of steric hindrance.

Considering the stability, kinetics and the overall output of chemiluminescence, 9-(2,6-dibromophenoxycarbonyl)-10-(3-succinimidyloxycarbonylpropyl)acridinium triflate (LiAE', **compound 4**) and 9-(2,6-dibromophenoxycarbonyl)-2-(2-succinimidyloxycarbonylethyl)-10-methylacridinium triflate (**compound 21**) showed potentially good properties for application in labelling oligonucleotides and in immunoassays.

References for chapter 5

1. L. Chen. *Ph.D Thesis*, University of wales, Swansea, 2000.

2. Z. Ahmed. *Ph.D Thesis*, University of wales, Swansea, 1996.

3. K. Smith, Z. li, J. Yang, I. Weeks and J.S. Woodhead. *J. Photochem. Photobiol.*, 2000, 132A, 181.