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Synthesis and Properties of New Luminescent 10-Carboxymethylacridinium Derivatives

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Abstract *: A novel method was developed to synthesize acridinium esters having a binding functional group at N-10. An acridine ester having steric hindrance is synthesized quickly, with excellent yields, using dimethylaminopyridine as a catalyst. Alkylation of the N-10 on the acridine ring proceeds using the trifluoromethanesulfonic acid ester under mild conditions. Deprotection of the benzyl ester using acid thus enables simple, high-yield synthesis for acridinium compounds with a binding functional group at N-10.* Copyright © 1996 Elsevier Science Ltd

Recently, assays using chemiluminescent compounds as markers are attracting interest because of their high sensitivity and wide dynamic range.[1,2,3] Acridinium esters, in particular, are used in chemiluminescence immunoassay (CLIA) because of their high luminescence quantum yield.[4] The poor stability of conventionally used 4'(2"-carboxyethyl)phenyl 10-methylacridinium-9-carboxylate (CPMA) [5] in an aqueous solution has made it unsuitable for use in CLIA.[6] A number of derivatives have been synthesized to achieve greater stability and luminescence. However, synthetic routes to such compounds are complex because antibody binding functional groups are introduced on the phenyl group.[6,7,8]

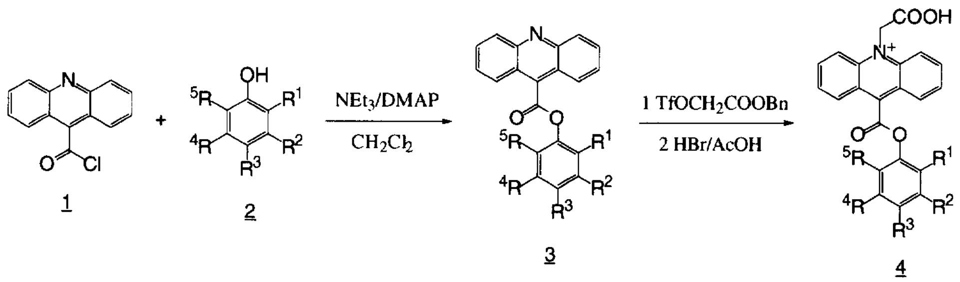
This report describes a simple way of synthesizing an acridinium ester with an antibody binding functional group at N-10 on the acridine ring. The synthesis of acridine esters having steric hindrance near the ester was investigated. Alkylation reactions for N-10 using a trifluoromethanesulfonate ester were also studied and the effects of the substitutional group on luminescence and stability are reported.

Esters with steric hindrance are difficult to hydrolyze. Improving acridinium ester stability conceivably should enable substitution using a methyl or similar group near the ester. Synthesizing an acridinium ester from acridine-9-carbonylchloride and phenol, however, requires severe reaction conditions for steric hindrance caused by the hydrogen atom at the peri position on the acridine ring. When a substitutional group is introduced at the ortho position of phenol, this tendency is markedly reinforced.[7] A common problem inherent in these reactions is that esterification with phenols having steric hindrance is time-consuming and, moreover, the yields are low. This problem was solved by employment of dimethylaminopyridine as a catalyst[9] (Scheme 1).[10] Using the catalyst, acridine ester 3 is quickly obtained in good yield from the corresponding phenol derivative 2 (Table 1). An acridine ester having strong steric hindrance because of methyl groups substituted at positions 1 and 8 on the acridine ring and both ortho positions on the phenyl ring was also obtained in 71% yield.

Alkylation of the nitrogen atom in 2-methylquinoline[11] or on the acridine ring[4] is quite difficult due to steric hindrance. When a methyl group is introduced at the nitrogen atom on the acridine ring, a mixture of acridine ester and iodomethane must usually be heated or be stirred in a sealed tube at 100°C for 7 days.[4] To introduce a binding functional group at N-10 on the acridine ring, benzyl iodoacetate was used as the alkylating agent in the absence of a solvent. But the yield was only 5%, even after heating the mixture for 72 hours at 120°C. Purification of the acridinum ester obtained by the above method was very difficult because benzyl iodoacetate, used in great excess, became polymerized at the high temperature. A study of alkylating agents showed that benzyloxycarbonylmethyl trifluoromethanesulfonate[12] enables the corresponding acridinium ester to be produced under mild conditions and in high yield (Scheme 1).[13] The trifluoromethanesulfonate ester is an extremely powerful alkylating agent.[14] The alkylation reaction using this ester was about 80% completed at about 6 hours, and almost 100% completed by 10 hours. A study of the reaction molar ratio of the alkylating agents with acridine showed that the reaction proceeded smoothly at a molar ratio of 1.1. The reaction proceeded at room temperature in all cases. Because alkylation reactions proceeded at a molar ratio of 1.1 and under mild conditions, the products were sufficiently pure, and purification was not needed. Benzyloxycarbonylmethyl trifluoromethanesulfonate is very stable and solidifies at less than 5°C. This ester can still be used without additional treatment if stored at -20°C or below for up to 3 years. The above suggests that trifluoromethanesulfonate esters could be extremely useful as reagents for alkylating nitrogen atoms on nitrogen-containing heterocyclic compounds.

Because acridinium esters are readily reduced under the reducted deprotection of the benzyl ester to form low-luminescence acridane esters, substituted phenyl 10-benzyloxycarbonylmethylacridinium-9-carboxylate trifluoromethanesulfonate derivatives were debenzylized using an acid under mild conditions.[15]

The chemiluminescence intensity of the acridinium esters produced by the above method is high (Table 1) compared with that of CPMA. (The chemiluminescence intensity of CPMA is 0.3×10^{20} counts/mol.) The first step in the chemiluminescence reaction of the acridinium ester is believed to be the nucleophilic attack at C-9 on the acridine ring of the hydrogen peroxide anion. When the electron density of the acridine ring decreases, luminescence is believed to increase.[16,17] Luminescence, in fact, increases for compounds in which a phenol group is substituted for an electron-attractive group. In the acridinium esters synthesized in this experiment, a carboxyl group is substituted at N-10. These compounds show higher chemiluminescence intensity because of its electron attraction.



Scheme 1

Table 1 Synthesis of acridinium ester derivatives and their chemiluminescence intensity and stability

R^1	R^2	R^3	R^4	R^5	RT^a	Yield ^{b}(%)	Yield ^{c}(%)	CL^d	Stability(%)
Me	H	H	H	H	0.5	86	85	4.6	70
Ph	H	H	H	H	0.5	85	89	2.5	76
Me	H	H	H	Cl	1.0	85	68	2.8	32
Cl	H	H	H	Cl	1.0	85	71	3.2	58
Me	H	F	H	H	1.0	80	96	4.3	47
Me	H	H	H	NO_2	1.0	83	32	3.2	58
OMe	H	H	H	F	0.5	88	99	4.4	32
Me	H	NO_2	H	Me	1.0	81	44	1.0	80
Me	NO_2	H	H	Me	1.0	81	78	1.0	73
Me	H	COMe	H	Me	2.0	82	86	2.7	88
Me	COMe	H	H	Me	2.0	83	66	3.1	83
Me	H	Cl	H	Me	2.0	82	83	2.3	90
Me	H	Br	H	Me	2.0	81	81	1.1	90
Me	H	Cl	H	Cl	2.0	80	46	0.5	79
Cl	H	Cl	H	Cl	1.0	81	90	1.2	47
Me	H	COMe	-(CH=CH)-$_2$		1.0	90	70	2.4	69
Cl	H	H	-(CH=CH)-$_2$		1.0	80	94	1.9	66

a: reaction time of esterification (hours), b: isolate yield of acridine esters **3**, c: isolate yield of acridinium esters **4**, d: chemiluminescence intensity ($\times 10^{20}$counts/mol) was measured by Bertold LB 953 for 2s after injection of a hydrogen peroxide/sodium hydroxide solution.

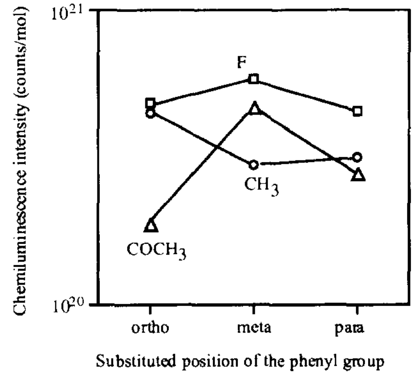


Fig. 1 Chemiluminescence intensity of substituted phenyl acridiniumu-9-carboxylate

The substituted position and type of functional group to be introduced in the phenol group were also investigated from the aspect of luminescence (Fig. 1). Luminescence was found to be higher when a methyl group -- an electron-donating group -- was substituted at the ortho and para positions, and when an acetyl group or fluorine -- an electron-attractive group -- was substituted at the meta position.

The stability of the synthesized acridinium esters was very high compared with that of CPMA.[18] (The stability of CPMA is 2%.) Even though the acridinium ester we synthesized was substituted by an electron-donating methyl group, it had excellent luminescence and stability. This is believed to be due to the fact that the acridine ring does not assume a completely planar structure because a bulky carboxymethylene group has been substituted at N-10. As a result, stability is improved with the methyl group projecting in the empty direction. Steric hindrance of the acridinium esters increased due to the above.

Because acridinium esters with a substitutional group at N-10 emit light when bound to an antibody, luminescence is expected to increase due to the protein's hydrophobicity. Such esters can also be used in a homogeneous chemiluminescence energy transfer assay system[19] wherein the energy of the excited state product (the donor) is not directly emitted as light. This acceptor is capable of losing this energy as electromagnetic radiation with a wavelength differing from the wavelength of the donor chemiluminescence. This phenomenon has been known.[20] Since the wavelength of the emitted light is specific for the acceptor or the donor-acceptor complex, the acceptor which might be an antibody labeled with a fluorogenic compound or a donor-complexed substrate (e.g., antigen) can be determined without the necessity of separating the bound complex.

As described above, our investigation of synthesis conditions for acridinium esters having a binding functional group at N-10 and having excellent luminescence, stability, and usefulness has enabled us to establish high-yield esterification and alkylation reactions that proceed under mild conditions.

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