Tetrahedron Letters 61 (2020) 152332

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Chemoselective reaction of bifunctional carboxysulfonic acid systems: Preparation of useful intermediates for chemiluminescent, fluorescent and UV absorbing bifunctional linkers

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ARTICLE INFO

Article history:
Received 13 May 2020
Revised 30 July 2020
Accepted 2 August 2020
Available online 16 August 2020

Keywords:
Chemiluminescent energy transfer
Protein bioconjugation
Heterobifunctional crosslinker
Sulfonyl chloride-fluoride exchange

ABSTRACT

Heterobifunctional compounds are of considerable interest in convergent synthesis strategies as well as in the labeling/tagging of biological molecules. Herein is described a synthetic strategy to functionalize sulfonic acids in the presence of carboxylic acids without the need for protection/deprotection steps. Bifunctional carboxysulfonic acids are transformed under mild conditions to the corresponding carboxysulfonyl fluorides which are reacted with the amine of choice to provide sulfonamides. The carboxylic acid remains free and is available for subsequent activation and further chemical elaboration. The generality of this approach is demonstrated herein, and an example of utility is outlined in which carboxysulfonyl-containing chemiluminescent reagents are transformed into unique red-shifted chemiluminescent reagents.

Introduction

Heterobifunctional molecules containing chemically compatible functional groups are extremely useful for the development of cross-linking systems that can be employed in the study of biological molecules and small molecule interactions [1,2]. Heterobifunctional cores possessing UV absorbing or fluorescent chromophores are of special utility as they provide traceable probes for attachment to biological moieties [3,4] as well as for studying small molecule interactions through FRET measurements and other techniques [5]. We recently described the synthesis and utility of a fluorescent cross-linker **1** derived from an unsymmetrical rhodamine dye **2** possessing carboxylic and sulfonic acid functional groups [6,7] (Fig. 1). Differentiation of the carboxysulfonic acid was achieved by first converting the carboxylic acid of **2** to a pentafluorophenyl ester followed by introduction of linking arm **A** via an amide linkage. This allowed for the sequential installation of the linking arms **A** and **B** through carboxamide followed by sulfonamide bond formations [7].

During the course of our research it became necessary to functionalize the sulfonic acid of a bifunctional carboxysulfonic acid while preserving the carboxylic acid for later derivatization. We sought an approach to achieve this end without the need for a protection/deprotection sequence. By avoiding protection/deprotection, we hoped to reduce unnecessary yield loss and material waste, allowing a faster, greener, and more cost-effective process. For example, selective activation of the sulfonic acid in the presence of the carboxylic acid would be adventitious for our bifunctional chemiluminescent acridinium labeling reagent **3** [8,9], CPSP (carboxypropyl sulfopropyl acridinium) (Fig. 2).

Chemiluminescent reagents are used extensively for the detection and quantification of analytes in immunoassays, forensic science and entertainment [10,11]. Abbott Laboratories utilizes CPSP (**3**) as the immunoassay detection reagent for assays performed on the Architect™ and Alinity™ line of automated immunoassay analyzers [11–13]. When treated with alkaline hydrogen peroxide, acridinium chemiluminescence of **3** is reported to proceed with loss of the sulfonamide **4**, resulting in the strained dioxetanone **5**. Decomposition of the dioxetanone affords the acridone **6** in the excited state which emits a single photon at ~440 nm (blue light) when it returns to the ground state **7** [8,11] (Scheme 1).

Since CPSP possesses the same chemical functionalities as **2**, we envisioned it too could be useful as a heterobifunctional, albeit, chemiluminescent probe (Fig. 3). A chemoselective reaction of the sulfonyl group would allow for selective introduction of the linking functionalities (e.g. linking functional group B) in **8**, as well as potential solubilizing and/or groups for reducing non-specific binding (e.g. PEG chains and zwitterionic chains) and moieties to

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https://doi.org/10.1016/j.tetlet.2020.152332
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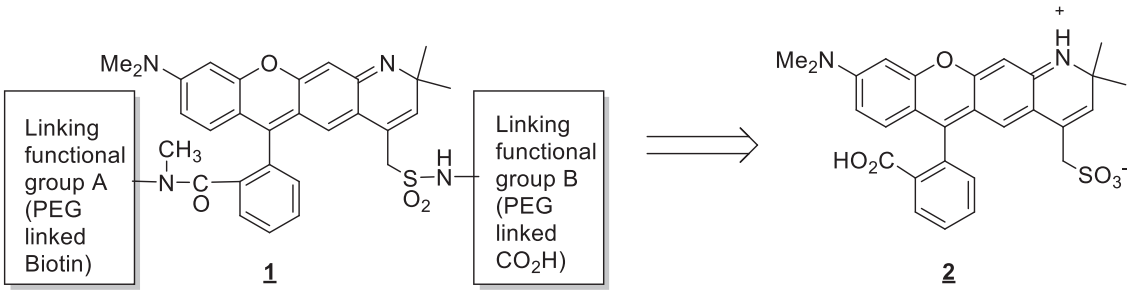


Fig. 1. Retrosynthesis of rhodamine heterobifunctional linking probe from an unsymmetrical bifunctional rhodamine **2** [7].

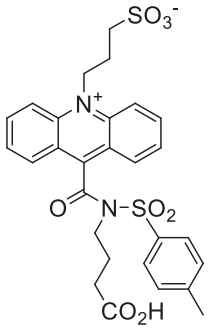
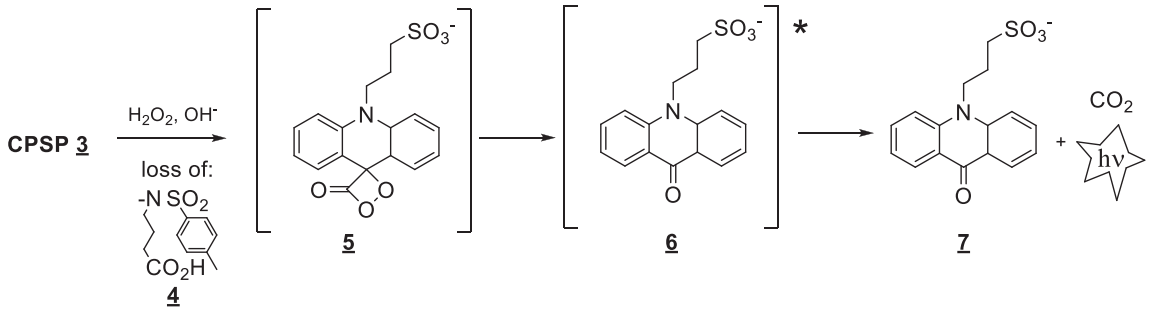


Fig. 2. Structure of chemiluminescent reagent CPSP **3** (carboxypropyl sulfopropyl acridinium) used in Abbott's automated immunoassay analyzers.

potentially modulate the chemiluminescent emission signal intensity whilst leaving the carboxyl free as a point of attachment for bioconjugation or further chemical modifications (e.g. linking functional group A) [14]. Acridinium **3** can also function as a cleavable heterobifunctional cross-linker when treated with alkaline hydrogen peroxide [15]. Also, exemplified later in this text, addition of a fluorophore covalently bound to the sulfonic acid moiety (**9**) allows for the modification of chemiluminescent emission wavelength through an energy transfer process [14,16].

Chemoselective activation of the carboxylic acid can easily be achieved via mixed anhydride or carbodiimide strategies without the need for sulfonic acid protection [17,18]. For example, carboxylic pentafluorophenyl and NHS esters are commonly prepared from dyes containing sulfonic acid functionality [18]. However, selective sulfonic acid activation to a sulfonyl chloride in the presence of a carboxylic acid requires protection of the carboxylic acid,



Scheme 1. Proposed mechanism for the hydrogen peroxide induced chemiluminescent reaction of CPSP **3** [8,11].

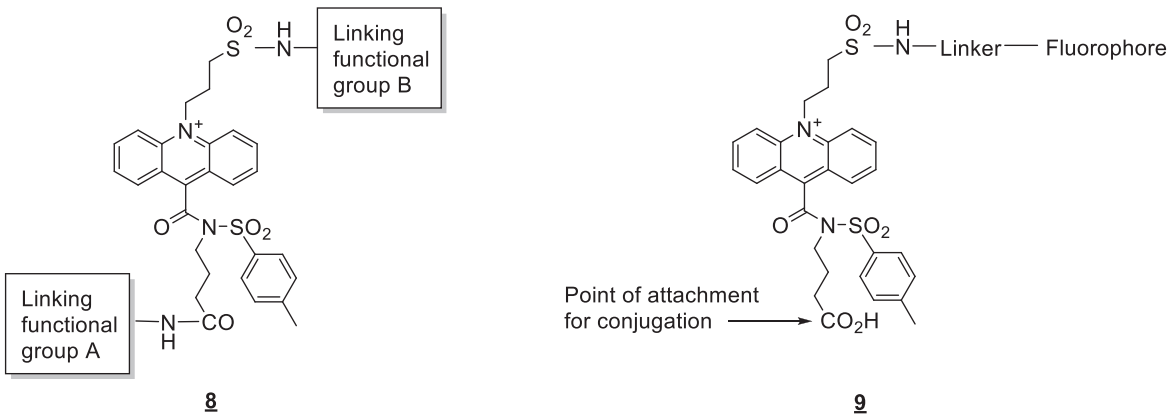
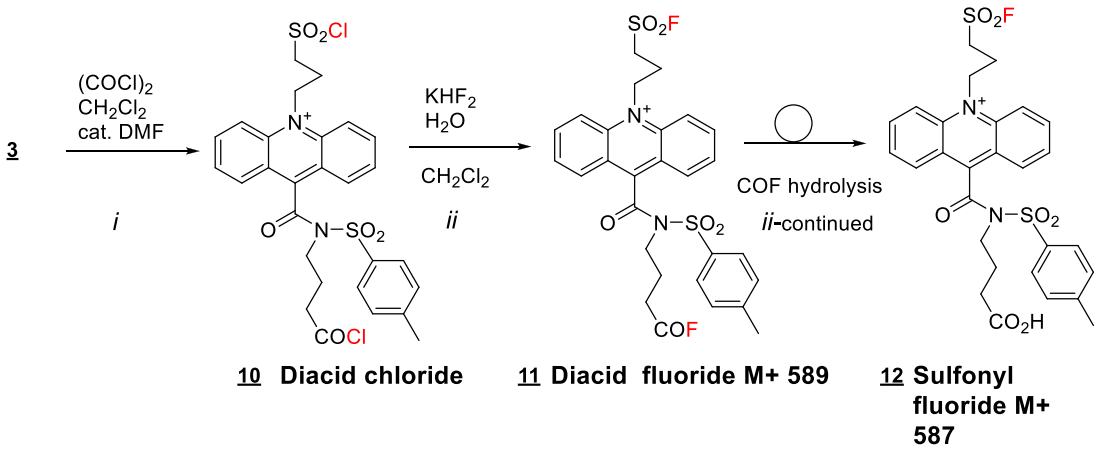


Fig. 3. Structures of potentially useful bifunctional acridinium probes derived from CPSP **3**.

usually as an ester since reagents such as oxalyl chloride or thionyl chloride, typically employed, react simultaneously with the carboxylic acid [19,20]. To solve the carboxysulfonic acid chemoselectivity problem, we employed a modification of the methods described by Sharpless et al. for producing sulfonyl fluorides from sulfonyl chlorides [21]. Sulfonyl fluorides, while less reactive toward amine nucleophiles (and water) than the corresponding sulfonyl chloride [22,23], do react with primary and secondary amines allowing easy installation of linking functionalities [21,24]. We envisioned converting CPSP **3** to the mixed diacid chloride **10**, followed by conversion to the mixed diacid fluoride **11** utilizing the Sharpless method (Scheme 2). Since the relative rate of hydrolysis of sulfonyl fluorides is slow compared to carboxylic acid fluorides [22,23], selective hydrolysis of the carboxylic acid fluoride in **11** while preserving the carboxysulfonyl fluoride in **12** seemed viable. This route would provide a simple, one pot conversion of a carboxysulfonic acid to a carboxysulfonyl fluoride without the need for protection/deprotection of the carboxylic acid.

Synthetic observations [25]

The transformation of bifunctional CPSP **3** to the sulfonyl fluoride **12** was monitored using the following methods. The diacid chloride **10** was produced by reaction of CPSP (**3**) with oxalyl chloride with a catalytic amount of DMF in CH_2Cl_2 [26,27]. The formation of **10** was verified by reacting a small aliquot of the reaction mixture with piperidine and observing diamide formation by UPLC-MS analysis [28]. After isolation, the diacid-chloride was



Scheme 2. Synthetic route employed for the preparation of CPSP sulfonyl fluoride **12** from CPSP **3**. Reagents: *i*) oxalyl chloride/DCM/catalytic DMF, volatile components removed *in vacuo* and product used without purification/characterization; *ii*) Saturated aqueous KHF_2/DCM. *ii-continued*) acyl fluoride hydrolysis occurs under the reaction conditions.

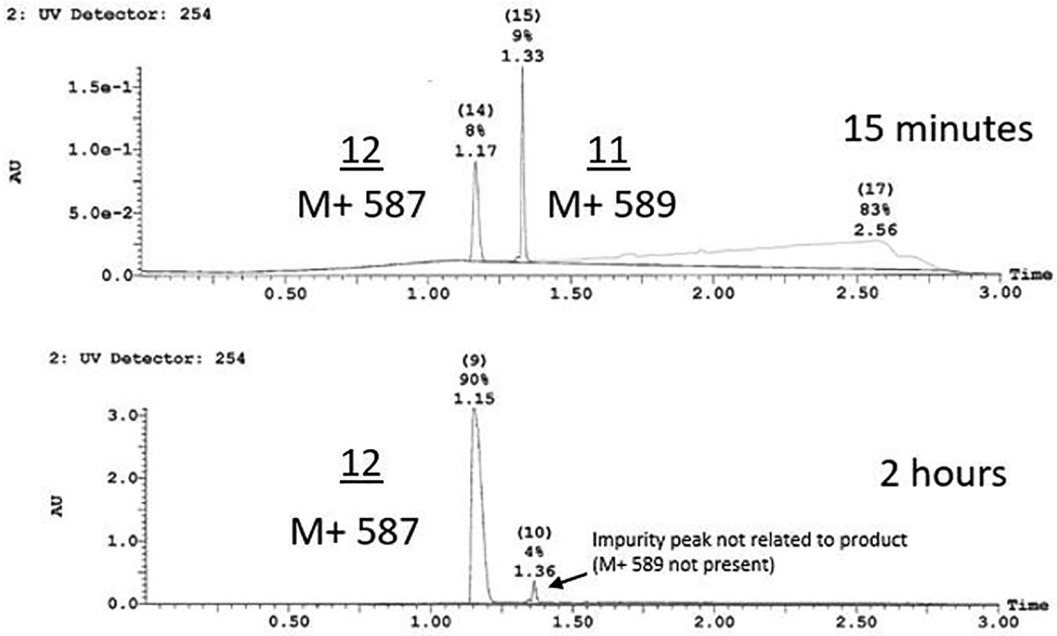
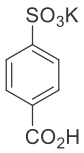
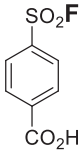
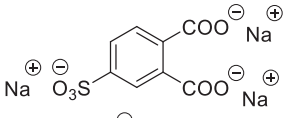
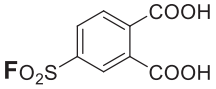
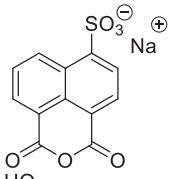
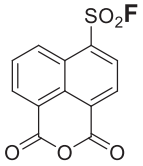
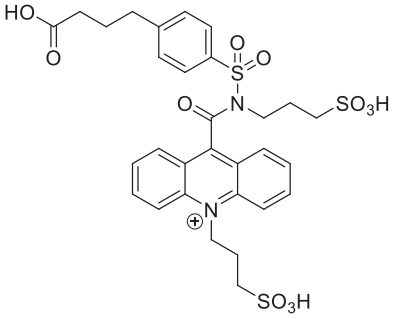
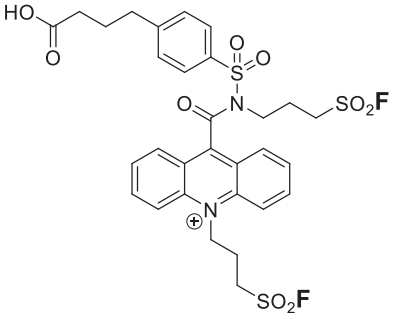
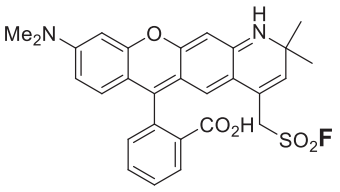


Fig. 4. Reaction progress monitored by UPLC-MS. Top: 15 min reaction time; Bottom: 2 h reaction time.

re-dissolved in dry CH_2Cl_2 and the solution was treated with an aqueous saturated solution of KHF_2 [21]. The biphasic mixture was stirred vigorously for 15 min and a mixture of the bisfluoride **11** and monofluoride **12** was observed by UPLC-MS (Fig. 4). This result indicated that the diacid fluoride **11** was produced with concomitant hydrolysis of the carboxylic acid fluoride as expected. Also, although no acid chlorides were observed by UPLC-MS analysis indicating a fast fluoride-chloride exchange reaction, it is possible that direct carboxylic acid chloride hydrolysis may also be occurring simultaneously. After 2 h of vigorous stirring, complete hydrolysis to the carboxysulfonyl fluoride **12** was observed.

Table 1
Reaction conditions: **A**) mixed acid chloride from oxalyl chloride/DCM/cat. DMF. Evaporation followed by DCM/saturated aqueous potassium bifluoride; **B**) mixed acid chloride from oxalyl chloride/DCM/cat. DMF. Evaporation followed by saturated aqueous potassium bifluoride (no DCM); All isolated yields.

Substrate		Reaction Conditions	Sulfonyl Fluoride Product		% Yield
3	see text	**A**	**12**	see text	100
13		**B**	**17**		100
14		**B**	**18**		60
15		**A**	**19**		100
16		**A**	**20**		100
2	see text	**A**	**21**		96

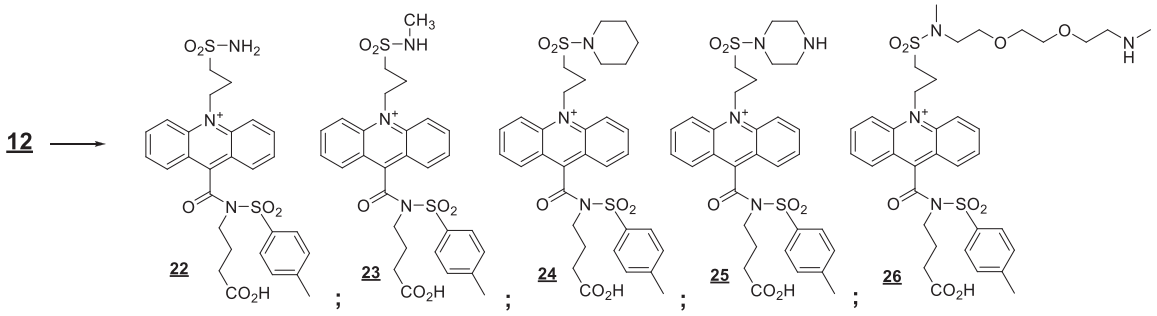


Fig. 5. Products of sulfonyl fluoride **12** reacting with various amines (10-fold molar excess). Reaction solvent: **22** H_2O, **23** THF, **24**, **25**, **26** DCM; All yields (isolated) exceeded 94% from **12**.

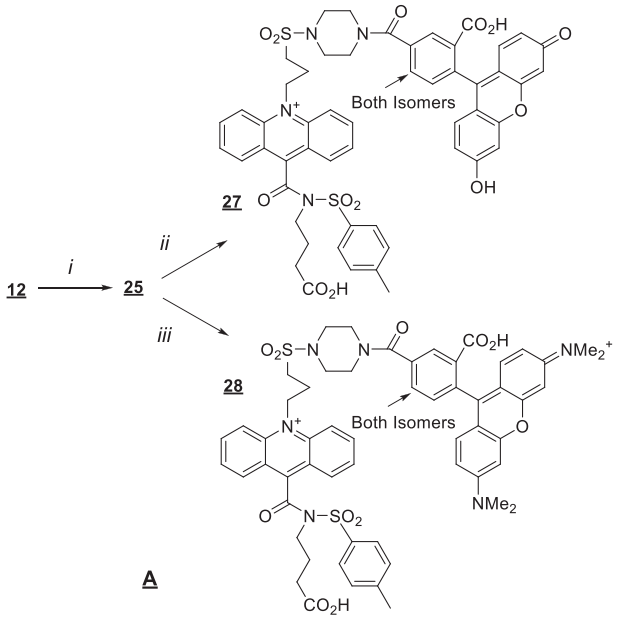
Carboxysulfonic acid general reaction

We explored the scope of this transformation utilizing commercially-available carboxysulfonic acids (**13**, **14**, **15**), SPSP (sulfopropyl sulfopropyl acridinium) **16** [9] and a bifunctional rhodamine **2** [29] as substrates (Table 1).

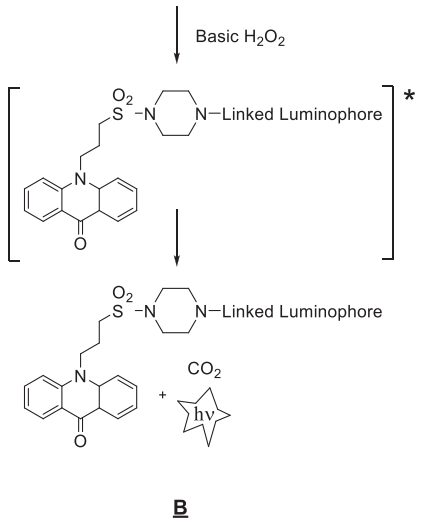
Example of utility

As shown in Fig. 5, CPSP sulfonyl fluoride **12** reacts smoothly with ammonia or primary amines producing primary or secondary sulfonamides (**22**, **23**) in aqueous or organic solutions. Its reaction with secondary amines, especially when using hindered amines, is sluggish [14,24] but excellent yields of tertiary sulfonamides (**24**, **25**, **26**) were obtained when a 10-fold molar excess of the secondary amine was employed. We also observed that the use of excess diamine mitigated disulfonamide formation during the reaction. Utilizing a diamine as the reactant produces a carboxysulfonamido-amine (examples **25** and **26**) and such compounds may be further elaborated for use as bifunctional linkers in a similar fashion to that of the previously described rhodamine **2** [6,7].

Our research interests in red-shifted chemiluminescent emission focused on attaching fluorophores to a chemiluminescent acridinium scaffold to evaluate chemiluminescence energy transfer. The most facile route was to covalently couple the luminophore via the sulfonic acid [14,16]. The sulfonyl fluoride **12** was treated with a 10-fold molar excess of piperazine and the resulting sulfonamide **25** was reacted with the NHS-ester of 5(6)-carboxyfluorescein isomer mixture or 5(6)-carboxytetramethyl-rhodamine isomer mixture (TAMRA) to afford the acridinium linked fluorescein **27** and rhodamine **28**, both as 5(6)-regioisomeric mixtures (Scheme 3A). These constructs allowed for the systematic study of the energy transfer between the luminophore and the excited state acridone intermediate formed upon triggering the acridinium constructs with alkaline hydrogen peroxide (Scheme 3B).



Scheme 3A. Preparation of CPSP-luminophore constructs **27** and **28**. Reagents: *i*) **12**, 10x excess piperazine/DCM, 100% yield; *ii*) NHS-5(6)-carboxyfluorescein/DMF/DIEA, 15.6% yield; *iii*) NHS-5(6)-carboxytetramethylrhodamine/DMF/DIEA, 25% yield.



Scheme 3B. Treatment of CPSP-linked-luminophore with alkaline hydrogen peroxide produces an excited state acridone-linked-luminophore through the pathway outlined in Scheme 1.

For the initial fluorescein and rhodamine linked constructs, we were delighted to observe only fluorescein and rhodamine emission (525 and 575 nm respectively) upon treating the acridinium-fluorophore constructs with alkaline hydrogen peroxide. In fact, >95% shifted emission was observed for both compounds with minimal emission corresponding to the excited state acridone [25].

The still images of the chemiluminescent activation and respective emission spectra depict the impact on energy transfer from excited acridone to the luminophore (Fig. 6).

Discussion

Heterobifunctional molecules containing chemically compatible functional groups are extremely useful for the development of cross-linking systems that can be employed in the study of biological molecules and small molecule interactions. Heterobifunctional moieties containing a UV absorbing or fluorescent chromophore are of special utility as they provide traceable probes for attachment to biological molecules. They are also useful to study small molecule interactions through FRET measurements and other techniques. We developed a new synthetic strategy to discretely activate a sulfonic acid in the presence of a carboxylic acid in molecules containing both functional groups and we demonstrated the generality of the reaction on multiple substrates. This simple chemoselective process allows for functionalization of the sulfonic acid with linkers for further cross-linking, modification of the molecule's physical properties (solubility, non-specific binding interaction, etc.), and in the case of a chromophoric species, its photophysical properties. In the latter case, we demonstrated this by attaching a fluorophore to the chemiluminescent reagent, CPSP (**3**). When the CPSP-fluorophore construct is chemically triggered with alkaline hydrogen peroxide, we observe >95% energy transfer to the fluorophore from the intermediate excited state acridone. This is of value as it may allow for the design of systems to measure multiple analytes, especially in the realm of immunoassay and other analytical methods [14]. We are currently investigating the mechanism of the energy transfer process of these acridinium-fluorophore constructs and some preliminary results have been reported here and comprehensive results will be reported in a future publication.

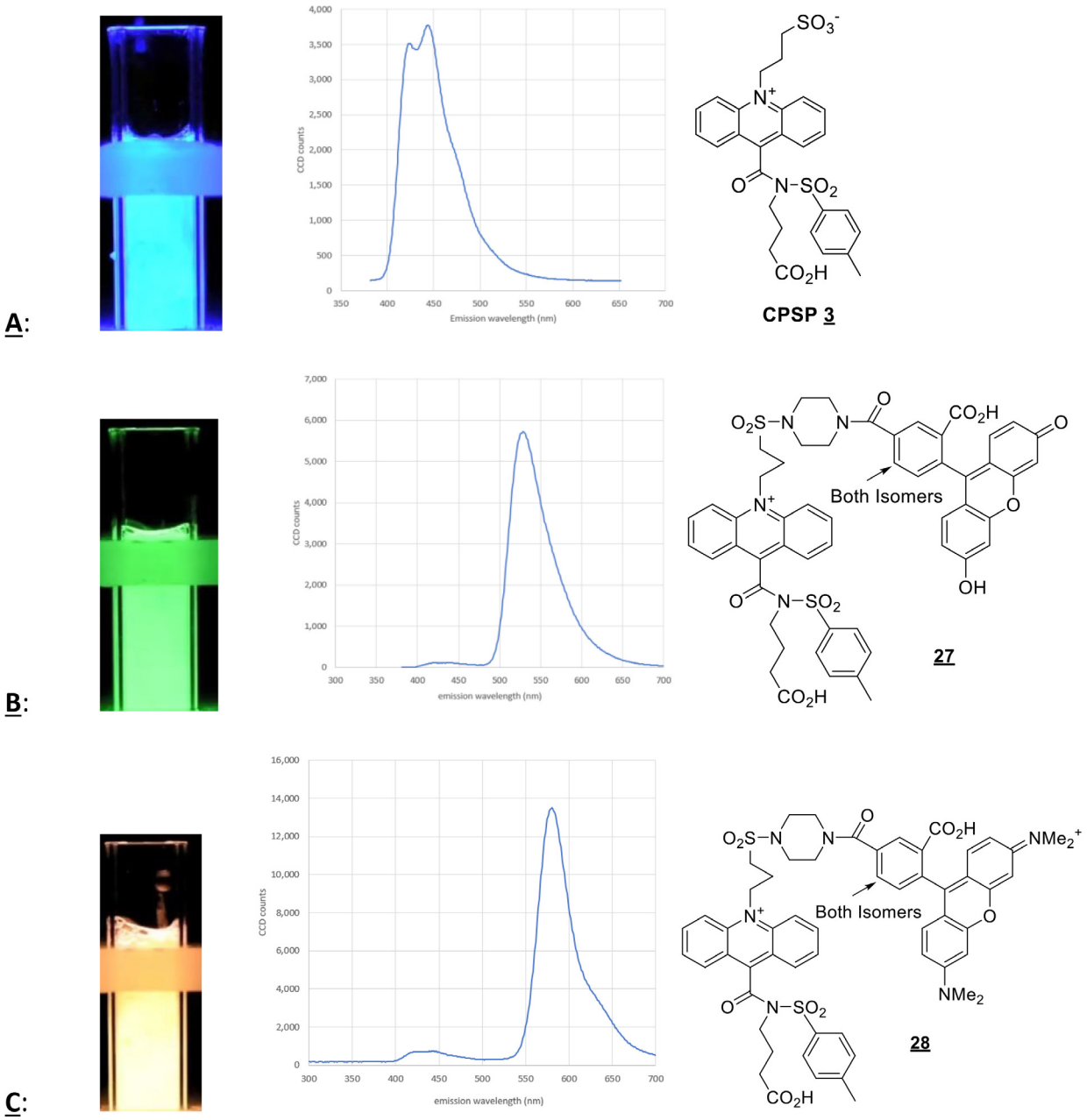


Fig. 6. Chemiluminescent emission flash and their corresponding chemiluminescence emission spectra of constructs: **A**: CPSP **3** (440 nm emission), **B**: CPSP-linked fluorescein **27** (525 nm emission) and **C**: CPSP-linked rhodamine **28** (575 nm emission).

Conclusion

We demonstrated a novel chemoselective approach to prepare carboxysulfonyl fluorides directly from carboxysulfonic acids without the need for protection/deprotection of the carboxylic acid. The generality of this method was demonstrated on several bifunctional substrates. The resulting carboxysulfonyl fluorides allow for chemoselective modifications of the carboxysulfonic acid core. Carboxysulfonyl acridinium, CPSP (**3**), is conveniently modified using this process to incorporate an aminosulfonamide linker which allows the facile introduction of covalently bound fluorophores such as fluorescein and TAMRA to the acridinium core. The simplicity of the chemistry provides an accessible means to modulate the physical and spectroscopic properties of this acridinium. In addition, selective functionalization of carboxysulfonic acids provides a simple method to produce heterobifunctional linkers that are useful for many applications.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors gratefully acknowledge Drs. Brian Bax and Mark Pope (both at Abbott Laboratories) for their many helpful discussions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2020.152332.

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