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Safe and easy route for the synthesis of 1,3-dimethyl-1,2,3-triazolium salt and investigation of its anticancer activities

Jaya P. Shrestha, Cheng-Wei Tom Chang *

Department of Chemistry and Biochemistry, Utah State University, 0300 Old Main Hill, Logan, UT 84322-0300, USA

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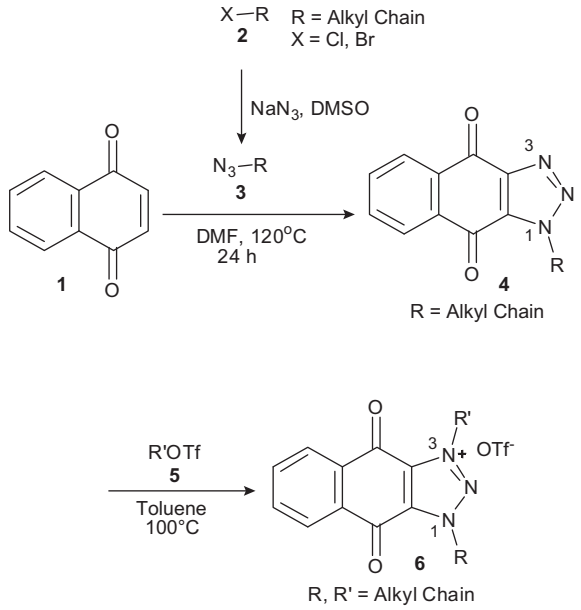
ABSTRACT

We have developed a new safe and easy route for the synthesis of 1,3-dimethyl-1,2,3-triazolium derivatives. We have reported the synthesis of 4,9-dioxo-1,3-dimethylnaphtho[2,3-*d*][1,2,3]triazol-3-ium chloride from methylation of 1-methyl-1*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-dione. The synthesis of 1-methyl-1*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-dione is inefficient as a significant amount of by-product is formed that is difficult to separate and also unsafe as it requires the use of hazardous methylazide as a starting material. It is, however, important to develop an improved method for the synthesis of 4,9-dioxo-1,3-dimethylnaphtho[2,3-*d*][1,2,3]triazol-3-ium salt due to its significant anticancer activities. Herein, we report a safe and convenient route for the synthesis of this compound, which lead to more detailed exploration of its profound anticancer activities. The improved method can be applicable for the synthesis of other 1,3-dimethyl-1,2,3-triazolium salts of interest without the use of potentially explosive methylazide. The compound synthesized in this new method shows significant anticancer activities against melanoma, colon cancer, non-small cell lung cancer and central nervous system (CNS) cancer with GI50 values ranging from low μM to nM.

1,3-Dipolar cycloaddition of azides, for example in the case of 'Click' chemistry, has attracted great interest due to its potential applications in various areas.[1] This reaction is typically carried out by reacting alkynes with azides leading to the formation of 1,2,3-triazoles.[2] In a similar fashion, we have reported the synthesis of 1-alkyl-1*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-diones, **4** via a tandem cycloaddition/oxidation of 1,4-naphthoquinone, **1** and alkyl azides, **3** (Scheme 1).[3,4] Further alkylation of the cycloaddition/oxidation adducts using alkyl triflate, **5** forms the 4,9-dioxo-1,3-dialkylnaphtho[2,3-*d*][1,2,3]triazol-3-ium salts, **6** which can be viewed as cationic anthraquinone analogs.[5,6] Several of the cationic anthraquinone analogs exert interesting biological activities ranging from antibacterial, antifungal to anticancer. With the exception of methylazide and ethylazide, the alkyl azides employed for this synthesis were prepared from a nucleophilic substitution of sodium azide and the corresponding alkyl halides, **2**. Since small organic azides are known to be explosive, we used methyl and ethyl 1-bromoacetate as the surrogate of methyl or ethyl halides.[4] This method is, however, inefficient which offers only modest yields and hard to scale-up.

In an attempt to resolve this problem, we have previously developed an alternative one-pot approach for the synthesis of the same cationic compounds by generating alkylazides in situ



Scheme 1. Two-step synthesis of compound **4**.

* Corresponding author.
E-mail address: tom.chang@usu.edu (C.-W.T. Chang).

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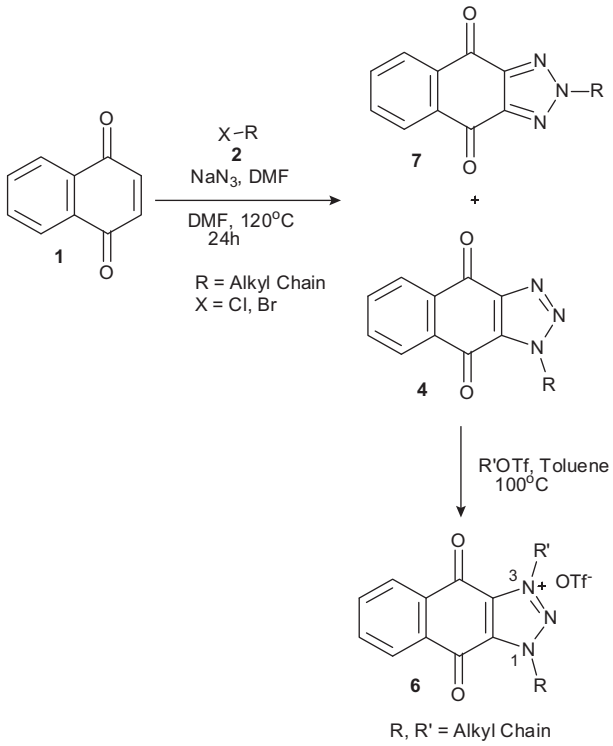
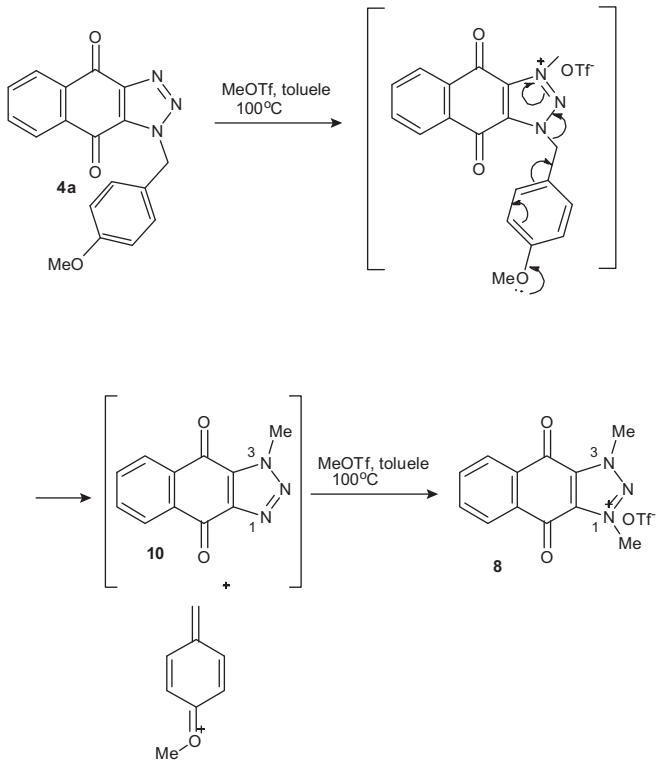
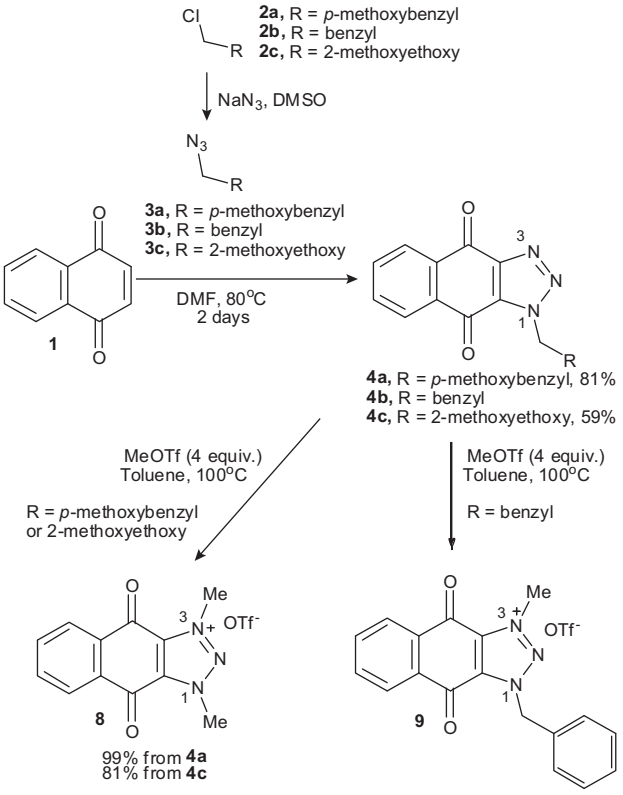


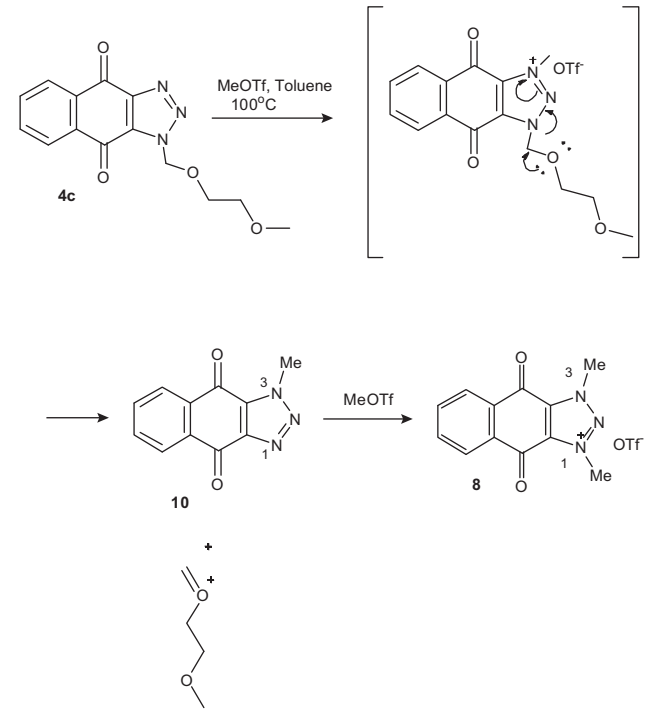
Scheme 2. One-pot synthesis of compound **4**.



Scheme 4. Proposed mechanism for the formation of compound **8** from compound **4a**.



Scheme 3. Synthesis of compound **8**.



Scheme 5. Proposed mechanism for the formation of compound **8** from compound **4c**.

(Scheme 2).[4,5] This method has the shortcoming of generating by-product, **7**, which is difficult to be separated from the desired product, **4**. Also from this study, a particular cationic anthraquinone analog, 4,9-dioxo-1,3-dimethylnaphtho[2,3-*d*][1,2,3]triazol-3-ium (R=R′=Me, **8**) was noted to manifest strong anticancer activities, especially against melanoma, colon cancer, non-small cell lung

Table 1
Anticancer activities of **8**[a, b]

Melanoma	GI_{50}	Colon cancer	GI_{50}	Non-small cell lung cancers	GI_{50}	CNS cancer	GI_{50}
LOX IMVI (Malignant)	0.50	COLO 205(Adenocarcinoma)	1.01	A549/ATCC(Adenocarcinoma)	0.32	SF-268(Astrocytoma)	0.95
MALME-3M (Malignant)	0.15	HCC-2998(Carcinoma)	1.24	EKVX (Adenocarcinoma)	1.21	SF-295(Gliolastoma-Multiforme)	1.05
M14	0.68	HCT-116(Carcinoma)	0.68	HOP-62 (Adenocarcinoma)	1.54	SF-539	1.35
MDA-MB-435 (Adenocarcinoma)	0.20	HCT-15 (Adenocarcinoma)	1.61	NCI-H226 (Squamous)	0.51	SNB-19(Gliblastoma)	1.59
SK-MEL-2 (Malignant)	1.57	HT29(Adenocarcinoma)	1.14	NCI-H23(Adenocarcinoma)	0.62	SNB-75 (Astrocytoma)	1.68
SK-MEL-28 (Malignant)	1.61	KM12(Adenocarcinoma)	0.79	NCI-H322M(Carcinoma)	0.28	U251 (Gliblastoma)	1.27
SK-MEL-5 (Malignant)	0.38	SW-620(Adenocaracinoma)	1.15	NCI-H460 (Large cell carcinoma)	0.30		
UACC-257	1.79			NCI-H522(Adenocarcinoma)	1.41		

[a] Unit: μM.
[b] The anticancer activities were tested through the Developmental Therapeutic Program (DTP) of National Cancer Institute (NCI) (Ref. 11).

cancer and central nervous system (CNS) cancer. Thus, there is an imminent need for an efficient and safer synthetic approach for the synthesis of this particular compound.

Alkyl azides that have the ratio of the sum of carbon and oxygen atoms to nitrogen atoms lower than three are considered too reactive or explosive and cannot be safely isolated.[7–9] Therefore, 1,2,3-triazole substituted with shorter alkyl chains, such as methyl or ethyl, cannot be safely synthesized by using methylazide or ethylazide. In an effort to expand the library of cationic anthraquinone analogs, we were surprised to find out that, upon methylation of a triazole adduct, 1-*p*-methoxybenzyl-1*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-dione, **4a**, an unexpected product, **8** was obtained in good yield (Scheme 3). However, same methylation of 1-benzyl-1*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-dione, **4b**,[4] yielded the expected product, **9** rather than compound **8**. Therefore, we proposed that the electron-donating effect of *p*-methoxy renders the *p*-methoxybenzyl group a good leaving group during methylation (Scheme 4). The resulted 1-methyl-1*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-dione, **10**,[4] can then be further methylated in the presence of excess MeOTf to form compound **8**. Although no attempt has been made to isolate compound **10** during this process, we have previously demonstrated that compound **8** can be synthesized from methylation of compound **10** using MeOTf.[5]

The relationship between electron-donating effect and leaving group capability was re-exemplified in another compound **4c** containing a 2-methoxyethoxymethyl group at the N-1 position (Scheme 5). Upon methylation using methyl triflate, compound **8** was also obtained in good yield. This reaction proceeded, presumably, via similar mechanism and intermediate. In both syntheses, the initial cycloaddition/oxidation starts by using *p*-methoxybenzyl azide or 2-methoxyethoxymethyl azide rather than hazardous methylazide and, thus, is superior in providing compound **8**. In fact, both of these syntheses can provide 1–2 g of compound **8** in one batch. When necessary, the counterion, triflate can be exchanged into chloride using Dowex 1X (Cl^-) resin.

Following the success in safe and scale-up synthesis of compound **8**, more detailed investigation on the anticancer activities of **8** was conducted through the Developmental Therapeutic Program (DTP) of National Cancer Institute (NCI). Compound **8** is particularly active against melanoma, colon cancer, non-small cell lung cancer and central nervous system (CNS) cancer with GI_{50} (50% growth inhibition) values ranging from low μM to nM (Table 1).[10] Continuing investigation of the anticancer activity of compound 8 is currently being carried out also by NCI.

In summary, we have successfully developed a convenient and safe route for the large scale synthesis of 4,9-dioxo-1,3-dimethylnaphtho[2,3-*d*][1,2,3]triazol-3-ium triflate with significant anticancer activities. This new method is essential for providing sufficient amount of material for ongoing anticancer investigations in vivo which could lead to the development of new chemotherapeutic agents. This protocol can also be applicable for the synthesis of other 1,3-dimethyl-1,2,3-triazolium salts of various applications without the use of potentially explosive methylazide. Finally, this discovery can also augment the application of 'Click' chemistry that involves the use of small molecules of organic azides.

Acknowledgments

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Supplementary data

Supplementary data (Experimental procedures and spectroscopic information for the synthesized compounds.) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.08.078.

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