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Fluorogenic Diels–Alder reactions of novel phencyclone derivatives

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ABSTRACT

With the aim of obtaining fluorogenic Diels–Alder reactions, novel phencyclone derivatives were synthesized. In a short time and under convenient reaction conditions, the formation of Diels–Alder adducts was observed by color-change and up to 33-fold enhancement of fluorescence. The modification of thymidine and deoxycytidine derivatives with a maleimide side chain and their usage in fluorogenic Diels–Alder reaction is also reported.

Fluorogenic reactions, in which covalent bonds are formed by click chemistry, have gained great interest in recent years. Especially, azide–alkyne cycloadditions, thiol–ene, and Diels–Alder reactions are the most commonly studied bioorthogonal reaction types[1] used for that purpose. Fluorogenic Cu(I) catalyzed Huisgen 1,3-dipolar cycloadditions take place between alkynes and azides of two non-fluorescent molecules to produce highly fluorescent triazole adducts.[2] The main disadvantage of this method is the need to copper(I) usage, which is toxic and not suitable for in vivo biological applications.[3] Recently, Jewett et al. synthesized a cyclooctyne-fused coumarin derivative, coumBARAC, which is able to undergo Cu-free fluorogenic click reactions.[4]

The thiol–ene click reaction is another example of a fluorogenic covalent bond formation reaction. It mainly depends on the maleimide group, which is attached to the dye structure, directly or with a spacer. The maleimide group acts as an acceptor and it quenches the fluorescence of the dye (donor) by a PET (photoinduced electron transfer) mechanism. The Michael addition between the RSH groups and the C=C-bond of maleimide inhibits the quenching effect of maleimide moiety and restores the fluorescence.[5] A new structural example of this type, based on chromenoquinoline, has been published recently, in which ICT (intramolecular charge-transfer) was given as a reason for the fluorescence quenching effect of maleimide.[6]

Diels–Alder reactions have got great attention in organic synthesis due to their highly efficient, versatile, and selective character[7] and can be considered as a kind of 'click-type' reaction due to these properties. It has been used several times in fluorogenic or fluorescent quenching detections by carbon–carbon bond formation for catalyst screening,[8] detection of carbon–carbon bond formation,[9] studies of fluorescent molecular thermometers,[10] and monitoring of reaction progresses.[11] Moreover, carbon–heteroatom bond formation based fluorogenic inverse electron demand Diels–Alder reactions have also found a lot of interest in chemical, biological, and materials science applications.[12]

Here, we describe the synthesis of new derivatives of phencyclone which have the ability to exhibit fluorescence enhancement by Diels–Alder reaction with maleimide derivatives. To the best of our knowledge, this is the first example of a fluorogenic Diels–Alder reaction, where the diene part contains the fluorogenic group and the reaction between diene and dienophile leads to conjugation to provide 'turn-on' fluorescence at room temperature.

Phencyclone systems seemed to be good candidates for the 'conjugation on—fluorescence on' idea. Phencyclone is well known as an effective diene component for Diels–Alder reactions[13] and N-substituted maleimides are excellent dienophiles reacting with phencyclone.[14] After checking the fluorescence of the Diels–Alder adduct of phencyclone and *N*-phenylmaleimide,[15] however, we did not observe an apparent fluorescence. Therefore we have modified phencyclone with electron-donating –OR groups and have prepared compounds **1a** and **1b** in 34% and in 51% yields, respectively, according to conventional routes (Scheme 1)[16] by Knoevenagel condensation of **2a** and **2b**.

The straightforward [4+2] Diels–Alder cycloaddition between N-substituted maleimides **3a–d** and phencyclone derivatives **1a–b** furnished the adducts, **4a–f**, in high yields (Scheme 2). With relatively smaller –R1 groups of maleimides, the yields are relatively higher; nevertheless, **4d** and **4f** could be isolated in 77% and 79% yields, respectively. The Diels–Alder addition takes place approximately in 30 min at a 1:1 molar ratio of both substances, however,

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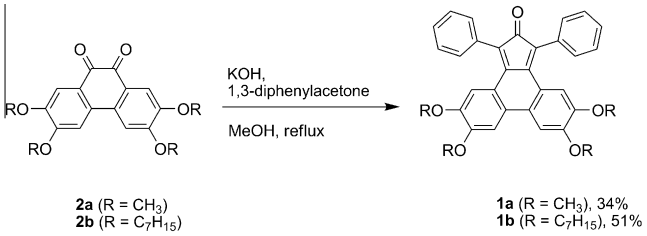
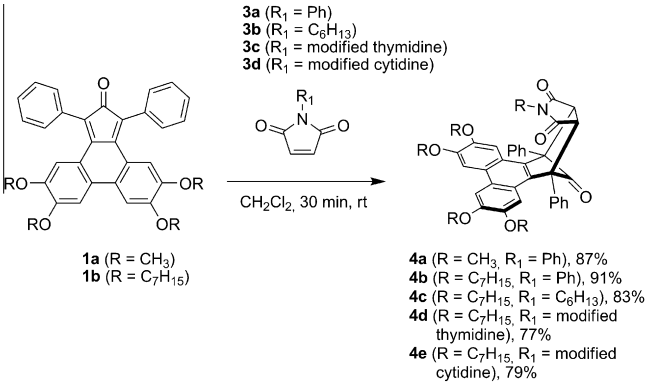
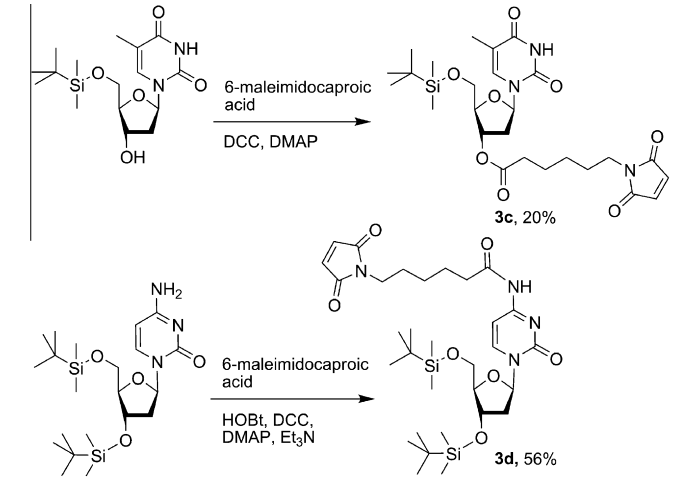


Scheme 1. Synthesis of phencyclone derivatives **1a** and **b**.



Scheme 2. Diels–Alder reaction of **1a** and **b** with N-substituted maleimides **3a–d**.



Scheme 3. Synthesis of maleimide-modified nucleosides, **3c** and **d**.

trials proved that excess of N-substituted maleimides permits the reaction to be completed even faster, approximately between 10 and 15 min (a mixture of diene and maleimide in a molar ratio of 1:1.25 was used). On TLC, the resulting reaction seemed quite clean, however, a short column chromatography was performed to eliminate minor fluorescent side products to avoid interference in fluorescence measurements.

For a first trial some maleimide-modified nucleosides were also used to investigate their fluorogenic Diels–Alder reactions and the fluorescence properties of their adducts. The modification of nucleosides for fluorescent labeling is an important tool in biological and analytical researches.[17] Therefore, modification of nucleosides with a flexible alkyl spacer bearing a maleimide group would be a good choice to use as precursors for this potential bioorthogonal click reaction. For the synthesis of maleimide functionalized nucleosides 6-maleimido-caproic acid was reacted with the 3′-OH group of the

Table 1
Fluorescence quantum efficiencies of Diels–Alder adducts

	R[a]	R_1[b]	Φ_f[c]
4a	CH_3	Ph	0.015
4b	C_7H_{15}	Ph	0.033
4c	C_7H_{15}	C_6H_{13}	0.037
4d	C_7H_{15}	Thymidine deriv.	0.021
4e	C_7H_{15}	Cytidine deriv.	0.020

[a] The substituent of side-chain of phencyclone derivatives.
[b] The R_1 group of maleimide part.
[c] Determined using anthracene (Φ_f = 0.27 in ethanol).[18]

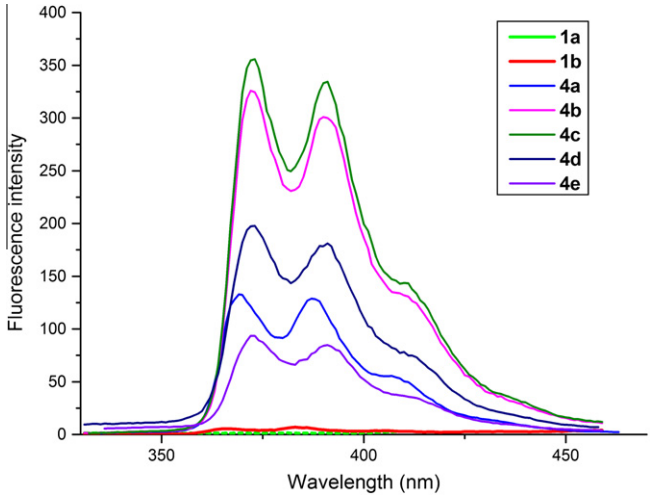


Figure 1. Fluorescence spectra of **1a**,**b** and **4a–e** in dichloromethane at 1.0×10^{-5} M (λ_{ex} = 266 nm).

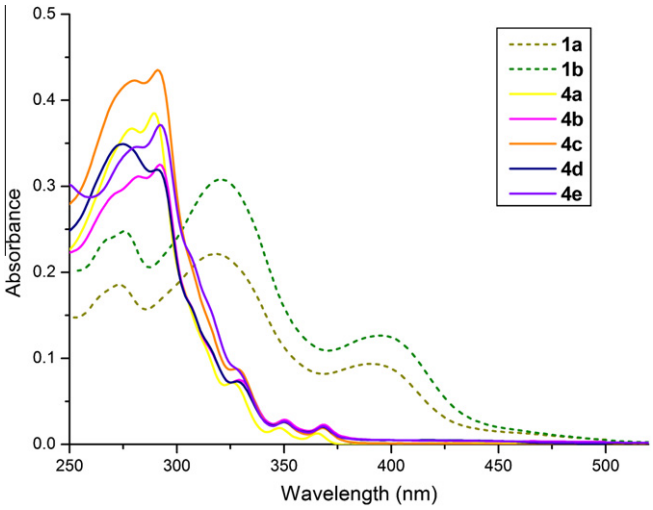


Figure 2. UV–vis spectra of **1a**,**b** and **4a–e** in dichloromethane at 1.0×10^{-5} M.

TBDMS-protected thymidine derivative by Steglich esterification in the presence of dicyclohexylcarbodiimide (DCC) and a catalytic amount of 4-dimethylaminopyridine (DMAP) to give **3c** in 20% yield. Similarly, the deoxycytidine derivative **3d** was synthesized by DCC/HOBt amide coupling in 56% yield (Scheme 3).

Regarding the magnitude of the fluorogenic effect, we measured a fluorescence quantum yield of $\Phi_f \sim 0.0011$ for the phencyclone derivative **1b**, and of $\Phi_f \sim 0.037$ for its Diels–Alder adduct **4c** (Table 1). The enhancement of fluorescence is 33-fold for this

example. The amount of fluorescence enhancements varies depending upon the substituent of maleimide derivatives (Fig. 1 and Table 1).

Moreover, the phencyclone derivatives have distinct peaks above 400 nm, which disappear after the Diels–Alder reaction. This phenomenon allows the progress of the reaction to be determined by color change. The color of the reaction turns from dark green to colorless. It is clearly seen in the UV–vis spectra that the peak in the visible range disappears (Fig. 2).

In summary, we have developed a new fluorogenic Diels–Alder reaction with maleimide systems, revealing an interesting perspective of usage of a Diels–Alder strategy in fluorogenic labeling applications. But as the adducts exhibit only relatively low quantum efficiencies and rather low excitation and emission maxima, further studies are needed to improve the optical properties of the systems. Furthermore, for biological applications the lack of their water solubility has to be overcome and these studies are currently in progress.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.01.095.

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