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Scavenging and Characterization of Short-Lived Radicals Using a Novel Stable Nitroxide Radical with a Characteristic UV−vis Absorption Spectrum

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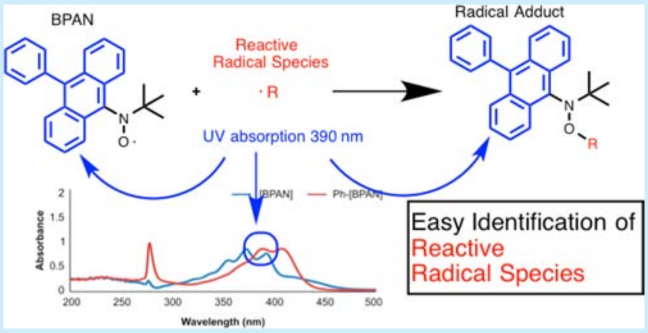
S *Supporting Information*

ABSTRACT: A stable *tert*-butyl(10-phenyl-9-anthryl)-nitroxide (BPAN) radical was newly synthesized and used for the capture/characterization of reactive radicals. Adducts obtained from the reactions of BPAN with *in situ* generated reactive radicals showed excellent stability, assuring complete isolation and purification. The structures of the adducts were established by LC−MS and NMR analyses.

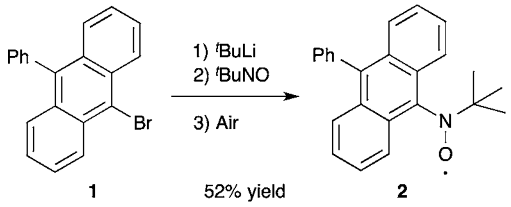


Radical intermediates play important roles in chemistry relating to the environment,[1] biological phenomena,[2] polymerization,[3] and organic syntheses.[4] However, such intermediates are short-lived and hence require sophisticated instruments for direct monitoring in an efficient manner.[5] "Radical scavenging" is a convenient alternative using stable radicals as scavenging reagents to afford stable diamagnetic adducts by reaction with short-lived radicals.[6] TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) derivatives are known to be stable nitroxide radicals and are widely used as radical scavenging reagents.[7−9] In fact, in radical scavenging chemistry, TEMPO has been used predominantly until now. TEMPO and its derivatives, 4-OH TEMPO and polymer-supported TEMPO, are commercially available. Based on the TEMPO structure, several functional radical scavenging reagents have been developed, such as profluorescent,[10] UV-detectable,[11] azaphenalene profluorescent,[12] umbelliferone,[13] and anthracene nitroxide radicals.[14] However, TEMPO adducts cannot be effectively analyzed because they do not show a specific fragmentation pattern in MS, and they are thermally unstable because of steric repulsion.[15]

We designed and synthesized a novel functional nitroxide radical with radical trapping ability, improved stability, and easy analysis. The radical adducts formed by the reaction of short-lived radicals with the developed nitroxide radical are stable enough to be characterized. It is important to balance reactivity and stability when designing nitroxide radicals as radical scavengers. To increase the stability, steric protection of the nitroxide moiety and spin delocalization are the main requisites.[16] Lesser steric hindrance to increase the reactivity of the nitroxide may decrease the stability. Therefore, an optimum balance should be achieved between the reactivity and stability. For example, *tert*-butyl phenyl nitroxide is not stable enough for isolating radical adducts[17] because (1) the steric protection by the phenyl (Ph) group is not sufficient and (2) the higher spin density at the *para*-position of the Ph group would dimerize the nitroxide itself. We replaced the Ph group with an anthracene ring containing a Ph substituent at the C10 position to prevent the dimerization. This scaffold increased the steric protection without decreasing the spin delocalization throughout the nitroxide radical. In addition, an anthracene ring is advantageous for its characteristic UV−vis absorption, facilitating UV detection by high-performance liquid chromatography (HPLC) analysis.

Herein, we report the synthesis and application of *tert*-butyl(10-phenyl-9-anthryl)nitroxide **2** (BPAN) as a radical scavenger. BPAN was synthesized from 9-bromo-10-phenylanthracene **1**, which is commercially available, in two steps and was purified by column chromatography (Scheme 1). Lithiation of **1**, followed by reaction with ^{t}BuNO and spontaneous air oxidation, afforded nitroxide **2**, which was purified by column chromatography and recrystallization. The structure of BPAN was established by infrared (IR), electron spin resonance (ESR), and high-resolution mass spectrometry (HRMS) analyses and

Scheme 1. Synthesis of *tert*-Butyl(10-phenyl-9-anthryl)nitroxide



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melting point. In addition, the detailed structure was elucidated by ^{1}H NMR and ^{13}C NMR analyses after reduction with ascorbic acid. This synthesis is practical and scalable (see Supporting Information). The fluorescence of the anthracenyl fluorophore should be completely quenched by the adjacent nitroxide entity. However, compounds **2** and **3** exhibit small fluorescence intensities, and the quenching effect is not significant. The reason is not clear yet; therefore, further study is needed to explain this phenomenon.

We carried out a Ph radical scavenging experiment with BPAN using phenylazotriphenylmethane (PAT) as the radical precursor. Many chemical species along with the target Ph radical adduct of BPAN [**Ph-BPAN**] were present in the reaction mixture; therefore, proper monitoring of [**Ph-BPAN**] is essential during the separation and characterization by HPLC. However, the usual UV detection at 254 nm is not effective because this wavelength is not specific for the radical adduct of BPAN (Figure 1). In contrast, by taking advantage of the inherent absorption peak of the anthracene chromophore at 390 nm, BPAN and the radical adducts of BPAN could be easily observed during HPLC separation, as shown in Figure 2 (BPAN and the BPAN adduct have an absorbance at 390 nm). Further, a high-sensitivity analysis of the BPAN adduct is possible by analyzing the absorbance at 410 nm. Free rotation of the C (anthracene ring)—N bond may be suppressed by the steric hindrance in the course of the formation of the adduct; therefore, its absorption maximum is shifted to the longer wavelengths by the conjugation of anthracene (π electrons) and unshared electron pairs (N and O). Because the carbon radical adduct showed a different UV pattern, carbon radicals can be detected by *in situ* UV monitoring. We plan to study this aspect in the future.

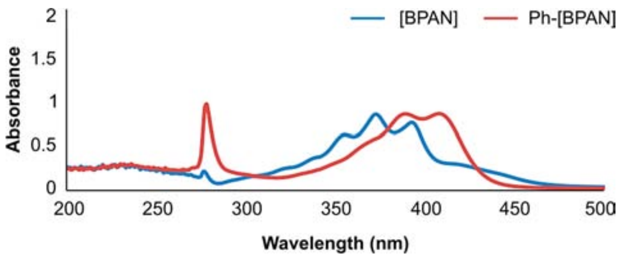


Figure 1. UV—vis spectra of [BPAN] and [Ph-BPAN] (BPAN = *tert*-butyl(10-phenyl-9-anthryl)nitroxide, Ph-BPAN = phenyl radical adduct of BPAN).

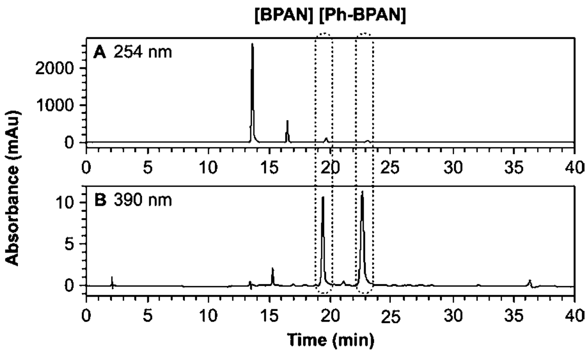
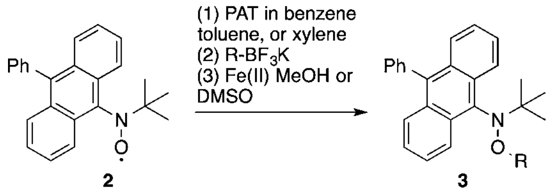


Figure 2. HPLC profile of [Ph-BPAN] monitored at (A) 254 or (B) 390 nm. HPLC = high-performance liquid chromatography, Ph-BPAN = phenyl radical adduct of BPAN.

We also examined the radical-trapping ability of BPAN using a previously reported method (Table 1).[18] The reaction of the ^{t}Bu radical generated from ^{t}BuBF$_3$K and CuCl$_2$ with BPAN afforded the corresponding adduct **3** in 98% yield (Table 1, entry 4). A secondary alkyl radical, isopropyl radical, was also successfully trapped by BPAN (Table 1, entry 5). Although the hexyl radical was generated slowly, BPAN reacted with it to afford the corresponding product in moderate yield (Table 1, entry 6). The reaction proceeded efficiently in the case of allyl and pinene radicals as well (Table 1, entries 7 and 8).

Table 1. Summary of Radical Trapping Experiments Using BPAN with Various Reactive Radicals and the Characteristic Mass Spectral Fragmentation Patterns of BPAN Adducts[a]



entry	method	R	yield (%)	MS fragments (ESI)
1	PAT	Ph	78	326
2	PAT	Bn	59	325, 284 + R
3	PAT	*O*-xylene	81	325, 284 + R
4	R-Bf$_3$K	^{t}Bu	98	268, 284, 325, 341
5	R-Bf$_3$K	isopropyl	85	268, 325, 284 + R
6	R-Bf$_3$K	hexyl	81	268, 284 + R
7	R-Bf$_3$K	allyl	94	268, 325, 284 + R
8	R-Bf$_3$K	pinene	94	325, 284 + R
9	Fenton	Me	98	268, 325
10	Fenton	Ch$_2$Oh	37	268, 284, 325, 341

[a] PAT = phenylazotriphenylmethane, BPAN = *tert*-butyl(10-phenyl-9-anthryl)nitroxide, DMSO = dimethyl sulfoxide, ESI = electrospray ionization.

Moreover, BPAN could capture the radicals formed in the Fenton reaction, in which hydroxyl radicals are generated from aqueous hydrogen peroxide and Fe(II).[19] When the Fenton reaction was carried out in the presence of dimethyl sulfoxide (DMSO), methyl (Me) radicals were generated quantitatively,[20] and BPAN captured them efficiently (Table 1, entry 9). In the presence of methanol, the Fenton reaction afforded CH$_2$OH radicals, and BPAN reacted with them to afford the corresponding adduct in a moderate yield (Table 1, entry 10). This moderate yield is probably because of the side reactions of BPAN with hydroxyl radicals.[21]

In addition to the HPLC analyses, the BPAN adducts were characterized by liquid chromatography—mass spectrometry (LC—MS, electrospray ionization (ESI)). In addition to observing the parent ion clearly in each case, characteristic mass spectral fragmentation pathways could be observed for the BPAN adducts (Table 1). Five representative fragment ions were observed in each case: (1) 268, (2) 284, (3) 325, (4) 341, and (5) 284+R (Figure 3); these fragment ions were obtained by the bond scission at the (1) N—O and N—^{t}Bu bonds, (2) N—^{t}Bu and O—R bonds, (3) N—O bond, (4) O—R bond, and (5) N—^{t}Bu bond, respectively. These characteristic mass spectral fragmentation pathways could not be observed in radical trapping experiments using cyclic nitroxides such as TEMPO. Therefore, BPAN is advantageous for facilitating characterization using both UV and LC—MS. Moreover, it is possible to determine the type

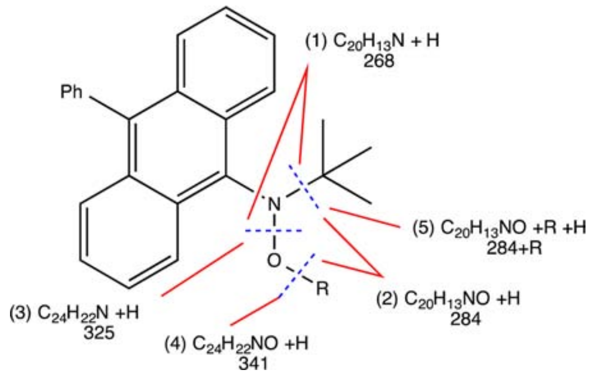


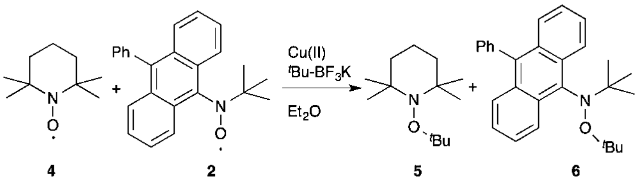
Figure 3. Mass spectral fragmentation patterns of BPAN adducts (BPAN = *tert*-butyl(10-phenyl-9-anthryl)nitroxide).

of radicals captured by analyzing the mass spectral fragmentation pattern. For example, although a peak was observed at 325 in each case, a signal was observed at 268 only when an alkyl substituent was present.

Another advantage of BPAN as a scavenger for short-lived radicals is the ease of characterization of the resulting adducts by soft ionization mass spectrometry. Because the anthracene moiety of BPAN can be used as a matrix in matrix-assisted laser desorption/ionization (MALDI),[22] the BPAN adducts could be analyzed without any matrix (see Supporting Information).

The radical trapping abilities of TEMPO and BPAN were compared by carrying out a competition experiment where the ^{t}Bu radical was scavenged (Scheme 2). A mixture of 1 equiv of TEMPO and 1 equiv of BPAN was treated with 2 equiv of ^{t}Bu-BF_3K. The ^{t}Bu radical trapping ratios were determined from the ^1H NMR integration values of each radical adduct (see Supporting Information). When 62% of BPAN was consumed, the BPAN-to-TEMPO adduct ratio was 52:48. Although the reaction was allowed to progress until no starting material remained, the adduct ratio did not change, implying that the radical trapping abilities of BPAN and TEMPO were nearly identical.

Scheme 2. Comparison of ^{t}Bu Trapping Abilities between TEMPO and BPAN



To analyze the trapping of reactive radicals comprehensively, it is necessary to evaluate both the reactivity of the radicals and the stability of the adducts generated. Consequently, the stabilities of the BPAN and TEMPO adducts obtained by the reaction with ^{t}Bu radical were examined. When the ^{t}Bu-BPAN adduct (compound **6**) was heated at 150 °C for 1 h, no decomposition was observed. In contrast, the ^{t}Bu-TEMPO adduct (compound **5**) decomposed upon heating at 150 °C for 1 min. Therefore, the BPAN adduct had greater stability than the TEMPO adduct. This is because of less steric hindrance around the reaction center of the nitroxide radical in BPAN. Although BPAN is less sterically hindered than TEMPO, the reactivity of BPAN was the same as that of TEMPO, because BPAN is more electronically delocalized than TEMPO.

Notably, radical scavenging experiments by BPAN can be applicable and very useful when multiple radical species are generated. Benzene, toluene, and xylene (BTX) are hazardous materials. Moreover, BTX gases have also been reported to form secondary pollutants in the troposphere, including compounds leading to photochemical smog and tropospheric ozone formation.[23] It has been suggested that radical species may be involved. Radical scavenging experiments using BPAN, where the Ph radical was generated by the thermal decomposition of PAT in a benzene, toluene, and *o*-xylene mixture, were carried out, and the adducts obtained were analyzed and characterized by LC−MS. Both the Ph radical adduct and adducts of radicals generated by the hydrogen abstraction from toluene and *o*-xylene were obtained. The ratio of the adducts (Ph/benzyl/*o*-methylbenzyl = 11:32:57) was determined by LC−MS. Because of high radical capture capability and characteristic UV absorption at 390 nm, the adducts of radical trapping reactions could be easily characterized even when complex mixtures were formed.

In summary, we developed a novel stable radical scavenging reagent, BPAN, containing an anthracene moiety. BPAN could be easily prepared from commercially available reagents in two steps. It could be used to capture diverse radical species; further, the adducts obtained with BPAN were stable and could be easily analyzed by HPLC and MS analyses. Hence, the proposed reagent has great potential for use in radical trapping and characterization. One limitation of BPAN is that it is insoluble in water; therefore, radicals generated in aqueous solutions cannot be trapped by BPAN. Water-soluble radical scavengers based on BPAN will be developed in the future.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures and characterization data for new compounds described. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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