ORGANIC LETTERS

2003
Vol. 5, No. 14
2397−2400

Syntheses of Pyridine and Bipyridine Frameworks Combining Dual Fluorescent and Magnetic Probes

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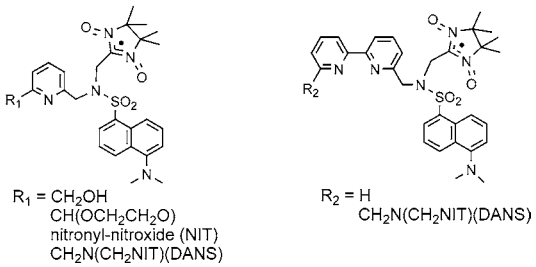
Received March 19, 2003

ABSTRACT



2,6-Disubstituted pyridines, 6-substituted 2,2′-bipyridines, and 6,6′-disubstituted 2,2′-bipyridines are readily prepared under mild conditions from 5-(dimethylamino)-1-naphthalenesulfonamide chloride (DANS-Cl) and chloromethyl-nitronyl nitroxide (CH_2Cl-NIT) starting materials and adequately functionalized building blocks. The syntheses of the pyridine molecules bearing two radicals and a DANS fragment first required the attachment of the aliphatic radical onto an aldehyde-protected dansylated compound, followed by the construction of the second aromatic radical.

The large number of biological processes for which radicals are implicated necessitates the development of improved analytical methods for tracing and quantifying these reactive species. The free radicals produced by living organisms are the result of complicated and synergetic processes, which may damage biomolecules such as proteins, lipids, and nucleic acids. These chemical reactions cause damage to mitochondria, chromosomes, and cell membranes and are believed to be responsible for a variety of human diseases.[1,2] Natural and artificial antioxidants delay or prevent such detrimental processes and, it has been demonstrated that the decomposition of lipids starts only when complete consumption of ascorbate has occurred.[3] Knowledge of the exact amount of ascorbic acid in blood plasma and erythrocytes is crucial in assessing the antioxidant capability of a human in healthy and/or pathological states. The amount of natural antioxidants and ascorbate, in particular, in food and feedstocks is a way to evaluate their nutritional benefits and efficiency. Recently, a new, reliable, sensitive, and simple method of ascorbic quantification in biological and chemical liquids was discovered, which is based on molecular probes bearing a singlet reporter.[4]

We and others have previously argued the case that modifying oligopyridines to provide various sensing properties in the molecules is a field that has great promise.[5−7] Many modifications of these platforms have been engineered, but we noticed that simultaneous implementation of dansyl

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10.1021/ol034474p CCC: $25.00
Published on Web 06/19/2003

fragments (known to be very efficient fluorophores) and nitronyl-nitroxide radicals has not been exploited to a great extent. Hideg and co-workers noticed that modulation of the fluorescence of a dansyl reporter attached to a phenyl ring is effective when a nitronyl-nitroxide (NIT) or an imino-nitroxide (IM) is present in the para position.[8] It occurred to us that new pyridine and bipyridine analogues should be used with the steady-state luminescence technique to exploit the on/off switching process to detect the presence of incoming charged species such as cations, protons, or ammonium salts. Furthermore, the chemical detection of ascorbate could be notably improved by an intramolecular cascade electron transfer within a radical/cation/ascorbate triad. We were also stimulated by our involvement in a program devoted to the development of new dyes that consists of increasing (i) the sensitivity and selectivity of the detection process and (ii) the stability and reversibility of the sensing device.

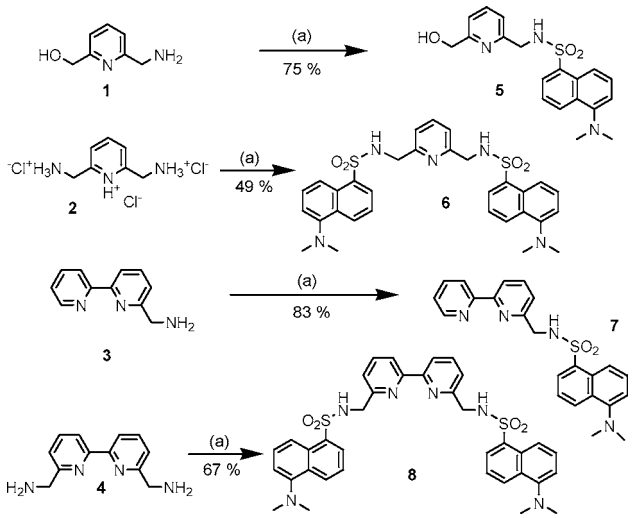
As a first step toward this goal, we have to set up synthetic protocols to prepare pyridine and bipyridine probes containing dangling radicals or more rigid aromatic NIT or IM radicals and a fluorescent dansyl fragment. The complexation center for the incoming cation or cation/ascorbate pair is provided by the pyridine or bipyridine and the N-atoms of the bridges,[9] either by the IM radical or the oxygen atom of the NIT radical.[10]

In the present Letter, we outline a synthetic strategy for the grafting of one or two nitroxide radicals onto dansylated pyridines and bipyridines. As a matter of fact, dansyl moieties have been covalently grafted onto N-protected α-amino acids,[11] nitroxides,[12] aminotroponimiate rings,[13] tripodal complexants,[14] a variety of macrocyclic platforms such as calix[4]arenes,[15] α-cyclodextrin,[16] and 1,4,7,10-tetraaza-cyclododecane,[17] and poly(propyleneamine) dendrimers,[18] and these scaffoldings are very popular as fluorogenic reagents for fluorescence switching in optical sensors.[19]

In our case, the choice of a dansyl dye reflects the thinking that it would act as a very efficient label[20] and the radical is an effective electron acceptor in artificial sensing devices. The synthetic routes have been adapted to ensure that the DANS fragment is attached first to the target in order to easily trace the compounds and to provide the opportunity with which to attach a methylene-bridged NIT radical at the end of the reaction sequence.

Access to this family of ligands requires the synthesis of key building blocks **1**−**4**, which have been prepared according to literature procedures,[21] from the corresponding bromo derivatives using a Delepine reaction, consisting of a nucleophilic attack of hexamethylenetetramine followed by an acidic hydrolysis. Quite selective monosulfonation of the methylamino fragment could be achieved readily in the presence of stoichiometric amounts of DANS-Cl under anhydrous and ambient conditions. Triethylamine is used to quench the nascent acid (Scheme 1).

Scheme 1[a]



[a] Reaction conditions: (a) DANS-Cl (1 equiv) with compounds **1** and **3** or DANS-Cl (2 equiv) with compounds **2** and **4**, CH_3CN, TEA, rt.

All these dansylated molecules appeared to be highly luminescent and perfectly stable in solution and in the solid state during prolonged periods at ambient conditions.

After some experimentation, we were very pleased to find that dansylated molecules **5**−**8** could be directly employed in the preparation of N-substituted methylene-bridged nitroxide radicals (Scheme 2). Compounds **10**−**13** bearing electron-donating and moderately electron-withdrawing substituents in the same sidearm were produced by using a procedure previously tested in the O-alkylation of calix[4]-arene[22] and/or the N-alkylation of Kryptospin macrocycles.[23]

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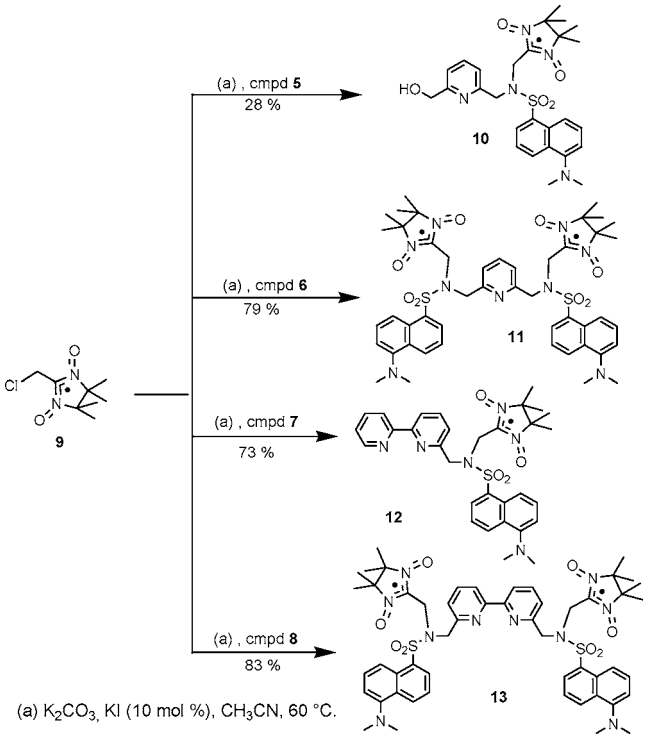
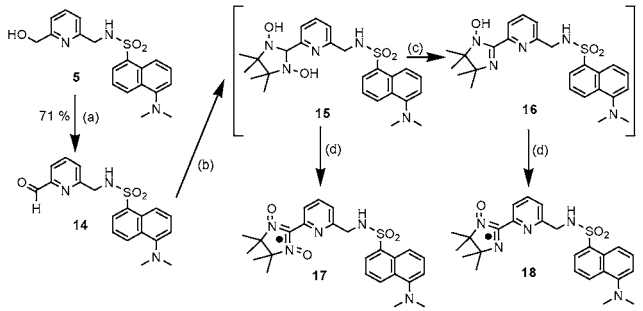
Scheme 2[a]



Scheme 3[a]



[a] Reaction conditions: (a) MnO_2, CH_2Cl_2, rt; (b) *N*,*N*′-dihydroxy-2,3-diamino-2,3-dimethylbutane, MeOH, rt; (c) SeO_2, rt , MeOH; (d) $NaIO_4$, CH_2Cl_2, H_2O, rt.

The procedure requires the presence of KI in catalytic quantities, which allows an in situ exchange of the chloro substituent to the iodo one, ensuring an efficient N-alkylation of the sulfonamide linker (Scheme 2). The absence of this coreagent dramatically decreases the rate of the reaction, and many intractable side-products were obtained due to longer reaction times and the required higher temperature. It was soon established that with KI, the reaction is completely regiospecific and no pyridine or NMe_2 alkylation was observed. This is probably due to the relatively low p*K* value of the sulfonamide and the mild experimental conditions. It is worth pointing out that for derivative **5** bearing a benzylic alcohol function, the yield is significantly lower compared to other compounds. In this case, the O-alkylation could not be excluded, but the compound could not be properly isolated possibly due its inherent instability.

This is a convenient and versatile protocol because of the mild conditions and the tolerance of various functions such as pyridine, tertiary amine, and sulfonamide groups. The success achieved in these N-alkylations prompted us to evaluate the potential of derivatives **5** and **10** to act as precursors for increasing the molecular complexity via construction of a second radical on the pyridine side. The synthesis of **17** and **18** may proceed through (i) the oxidation of the primary alcohol to the carbaldehyde, (ii) a condensation step with *N*,*N*′-dihydroxy-2,3-diamino-2,3-dimethylbutane,[24] (iii) dehydration of the *N*,*N*′-dihydroxyimidazolidine to the *N*-hydroxyimidazolidine, and (vi) phase transfer oxidation to the corresponding radicals.[25] Oxidation of compound **5** to the formyl derivative **14** is straightforward and proceeds with an excess of MnO_2 under aerobic conditions. Condensation with the dihydroxylamine provides the expected imidazolidine ring **15**, which could be dehydrated in situ or after isolation to the unsaturated derivative **16**. Both derivatives were readily oxidized in biphasic media using aqueous $NaIO_4$ to afford the blue-violet radical[26] **17** and the orange-red imino radical **18** (Scheme 3).[27]

The next interesting target was the alkylation of the remaining sulfonamide function in **17** and **18** by an additional radical as previously engineered in Scheme 2. However, the preparation according to this procedure failed probably due to bimolecular redox reactions between the electron-demanding aromatic nitroxides and the more electron-rich radical **9**. A nice solution to this was found when we discovered that the protection of the aldehyde function in **14** via a cyclic ketal (compound **19**) allowed the alkylation reaction to occur smoothly providing the monoradical derivative **20** in 78% isolated yield (Scheme 4).

Interestingly, the one-pot deprotection and condensation of compound **20** in the presence of the protonated form of *N*,*N*′-dihydroxy-2,3-diamino-2,3-dimethylbutane afforded, after phase transfer oxidation, the target bis-radical **21** bearing a dansyl, an aliphatic radical, and an aromatic radical. It is worth pointing out that the preparation of compound **21** could not be properly achieved from derivative **10** by a sequence of reactions involving (i) oxidation, (ii) condensation with *N*,*N*′-dihydroxy-2,3-diamino-2,3-dimethylbutane, and (iii) phase transfer oxidation using $NaIO_4$ or heterogeneous oxidation with PbO_2. Furthermore, we were unable to prepare the imino radical analogue of compound **21** using a similar route. It is surmised that in this case, the high reactivity of the synthetic intermediates toward redox reactions is the limiting step.

The electronic absorption spectra in dichloromethane of two sets of ligands exhibit an intense maximum in the range

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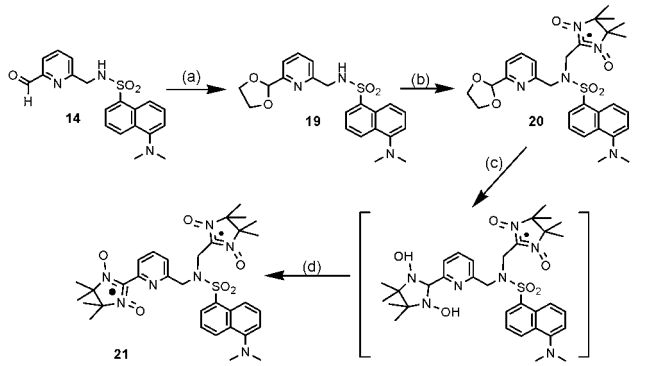
Scheme 4[a]



[a] Reaction conditions: (a) $HOCH_2CH_2Oh$, *p*-TsOH, benzene; (b) compound **9**, K_2CO_3, KI (10 mol %), CH_3CN, 60 °C; (c) *N*,*N*′-dihydroxy-2,3-diamino-2,3-dimethylbutane sulfate salt, MeOH, rt; (d) $NaIO_4$, CH_2Cl_2, H_2O, rt.

220−300 nm with a molecular absorption coefficient lying between 10 000 and 25 000 M^{-1} cm^{-1}, followed by a less intense maximum at about 344 nm (Figure 1). The intensity of this latter band is proportional to the number of dansyl (ϵ = 3400 M^{-1} cm^{-1} per unit). The absorption spectrum of the molecules carrying a nitronyl nitroide radical shows a weak, broad, and structureless band in the range 540−580 nm with ϵ = 300−800 M^{-1} cm^{-1} (Figure 1b). On the basis of literature data, the peaks at the highest energy, which dominate the absorption spectra of all these compounds, may be assigned to spin-allowed $\pi-\pi^*$ transitions centered in the bipyridine and pyridine parts of the molecules with some contribution from $n\rightarrow\pi^*$ transitions involving the polypyridine sites.[28,29]

Furthermore, the band at lower energy (around 344 nm) is typical of the absorption of the dansyle fragment,[20] while the lowest band responsible for the blue color of the nitroxide radicals is characteristic of $n\rightarrow\pi^*$ transitions as previously speculated by Ullman and co-workers.[26,27] The emission properties and the comparison of the quantum yields for emission of the nitroxide species with their precursors is currently in progress, and sensing results will be reported in the due course.

In summary, we have shown that employment of dansylated pyridine and bipyridine skeletons can provide an easy entry into N-substituted molecules with a radical adduct. In the case of 2,6-disubstituted pyridines, protection of the aldehyde function allows the application of the same protocol for the radical grafting, while further condensation with the diprotonated form of the dihydroxylamine allows the preparation of a hybrid biradical with a dangling dansyl fragment. Further work is in progress to extend the synthetic ease of the present methodology to the preparation of more complex molecular scaffoldings and to probe these dual fluorophore/radicals in the sensing of various cations as well as ascorbate/cation pairs.

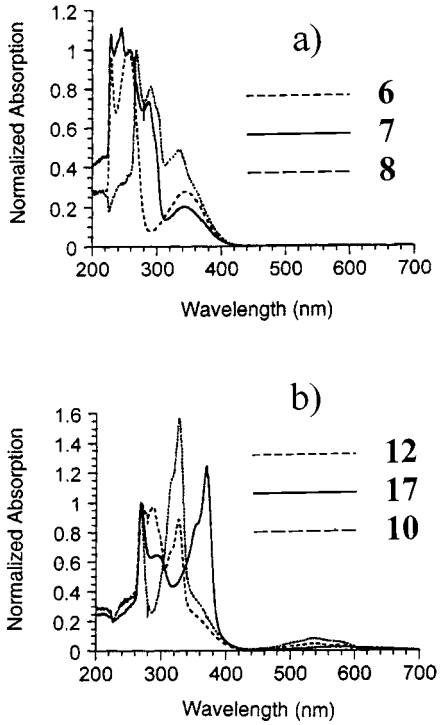


Figure 1. (a) Normalized absorption spectrum in dichloromethane solution of various mono- and bis-dansylated molecules. (b) Normalized absorption spectrum in dichloromethane solution of various molecules carrying both dansyl and nitroxide fragments.

Acknowledgment. This work was supported by the CNRS and by ULP. We thank Dr. Loïc Charbonnière and Professor Michael Chetcuti for critical readings of this manuscript.

Supporting Information Available: Experimental procedure and characterization data for the key derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.

OL034474P

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