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Synthesis and Photochromic Properties of Azido Analogues of Spiropyran and Spirooxazine as Nucleic Acid Labeling Reagent

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Abstract: A series of photo active azido analogues have been synthesized and their photochromic properties have also been investigated by UV-Vis spectrum. It will be used for the rapid and reliable preparation of large amounts of stable, non-radioactive labeled DNA and RNA hybridization probes. And it is supposed to be easily detected for its photochromic properties.

Keywords: Nucleic acid, photochromic, label, azido, spiro.

Nucleic acid hybridization probes have become indispensable to molecular biology for the detection of specific, complementary nucleic acid sequence. Probes are commonly labeled with the radioisotopes ^{32}P, ^{125}I or ^{3}H, but stability, safety and detection problems have stimulated interest in the development of non-radioactive probes[1-3].

Forster[4] *et al*. firstly synthesized a photo active analogue of biotin, which was called photobiotin. Upon brief irradiation with visible light, photobiotin forms stable linkage with nucleic acid for its very active azido group. And the labeled probes can be recognized by colorimetric detection procedure[5]. This versatile, chemical labeling procedure can be used for the rapid, small or large-scale preparation of stable probe. Moreover macromolecules exhibiting photoswitchable physical or chemical properties are extensively as information storage and signal amplification materials. Various means have been used to photoregulate biotransformations by light-switchable enzyme active site[6,7] and protein backone[8] by photochromic components and immobilization of enzyme in photochromic copolymers[9]. In this report the similar light labeling method was introduced. Instead of biotin, spiropyran and spirooxazine were used as light labeling reagents for their good photochromic and anti-fatigue properties[10, 11]. This method not only facilitated the detection of nucleic acid probes, but also made it possible to adjust the structural and functional characteristic of the labeled nucleic acid by ultraviolet light for its photochromic properties.

Results and Discussion

Firstly 4-chloro-1-nitrobenzene was selected as the starting compound. After a series of

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reactions 4-fluoro-3-nitrophenyl azido was synthesized by the method of Fleet[12] *et al.* And 4-fluoro-3-nitrophenyl azido was easily reacted with diamine in dry ethyl ether at room temperature. After 24 hours, the TLC on silica gel showed the reaction to be completed. The solvent was removed and the red oil was dissolved in 0.5 mol/L NaOH solution. The product was extracted with ethyl acetate and washed with water. After being dried over Na_2SO_4 overnight and the solvent was removed to give the crude product, which was used without further purification.

So the product N-(4-azido-2-nitrophenyl)-N'-diamine was obtained and it could be coupled with equal molar photochromic compounds spiropyran or spirooxazine with DCC (N, N'-dicyclohexylcarbodiimide)/DMAP (4-dimethylaminopyrimidine)[13] as the catalysts in dichloromethane to form corresponding amides **1-4**[14]. These compounds were purified with TLC(dichloromethane: methanol=50:1 as eluent). The compounds **1-4** could form stable linkage with bases of nucleic acid for its azido group upon irradiation with visible light and the labeled nucleic acid could be detected. Further more the structural and functional characteristic of nucleic acid could be adjusted by ultraviolet light irradiation for that the azido derivatives possess photochromic properties. All the reactions described above were carried out in the dark.

The photochromic properties of the products obtained were investigated simply by their UV-Vis spectra in selected solvent and in solid state before and after the irradiation of ultraviolet light. The results showed that compounds **1-4** had good photochromic properties in solution and solid state, which were similar with the spiro photochromic compounds themselves. For example, the absorption of compound **1** in methanol in the visible region rose greatly after ultraviolet irradiation.

Scheme 1

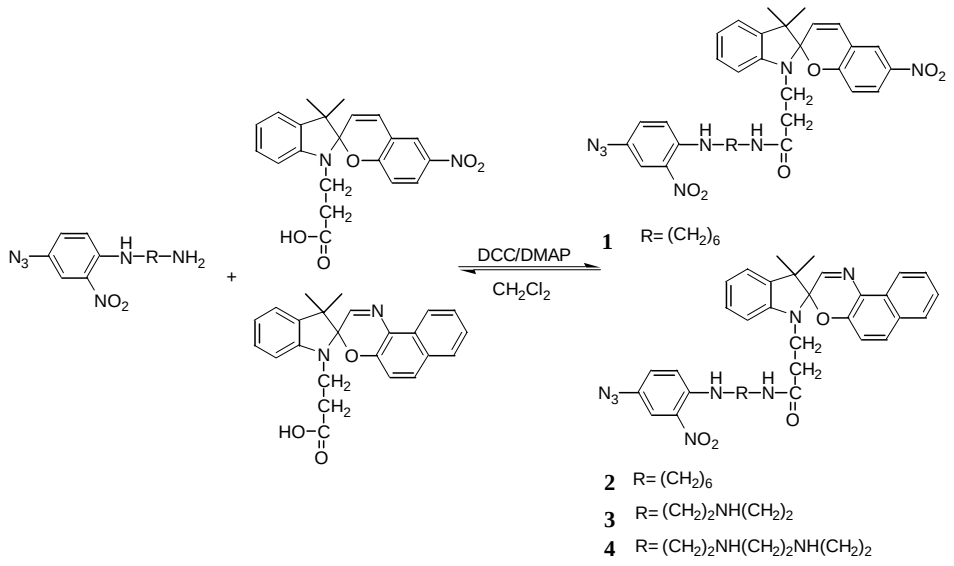

Figure 1 The UV-Vis absorption of compound **1** in methanol

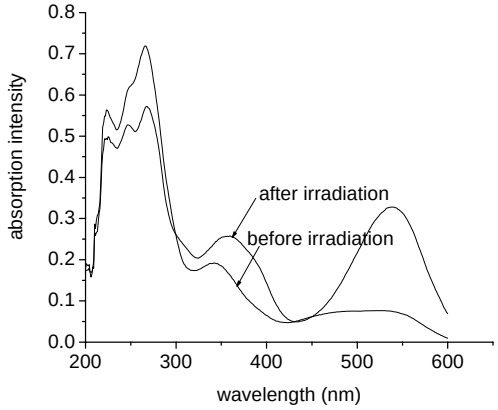

Table 1 The wavelength of maximum absorption of compound **1-4** in the visible region in the different solvents before and after irradiation

Compound	λ max (nm)					
	Dichloromethane		Acetone		Methanol	
	before	after	before	after	before	after
1	469	None	464	464	None	538
2	467	None	464	464	462	None
3	467	449.5	461.5	456	459.5	469.5
4	459.5	431.5	458.5	453.5	458.5	465.5

The great color difference was also observed before and after irradiation in the solid state. This result facilitated the detection of labeled nucleic acid. The UV-Vis spectra in selected solvent revealed that absorption varied greatly in the region 400-600 nm before and after irradiation as listed in **Table 1**, which made it possible to adjust the different functional and structural characteristic of labeled nucleic acid. The further research is being carried out in our lab to label nucleic acid with the above nucleic acid labeling reagent. It would be helpful to the preparation of nucleic acid probes and the investigation of biology, especially to the exploration of biological intelligent materials.

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    Data of **1**: ^{1}H NMR ($CDCl_3$, 200 MHz, δ ppm) 1.11 (s, 3H, CH_3), 1.23-1.68(m, 11H, CH_3, $NHCH_2$*(*CH_2*)*$_4$NH), 2.36-2.47(m, 2H, CH_2CO), 3.11-3.29(m, 4H, $NHCH_2(CH_2)_4CH_2NH$), 3.48-3.70(m, 2H, CH_2CH_2CO), 5.50(br, 1H, NHCO), 5.79-5.84(d, 1H, *J*=10.6Hz, Vinyl-H), 6.59-6.80(m, 2H, ArH), 6.82-6.92(m, 3H, 2*ArH*, Vinyl-H), 7.04-7.16(m, 3H, ArH), 7.83-7.84(d, 1H, *J*=2.4Hz, ArH), 7.96-8.01(m, 3H, 2*ArH*, ArNH). IR(KBr, ν cm^{-1}): 2932, 2862, 2118, 1647, 1569, 1519, 1481, 1336, 1272, 1157, 1090, 1021, 951, 808, 747. MS: *m/z* 641.1 (M+1).

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