Synthesis of new inexpensive fluorescente water-soluble amine-reactive reagentes for biomolecules labeling: application to cultural heritage

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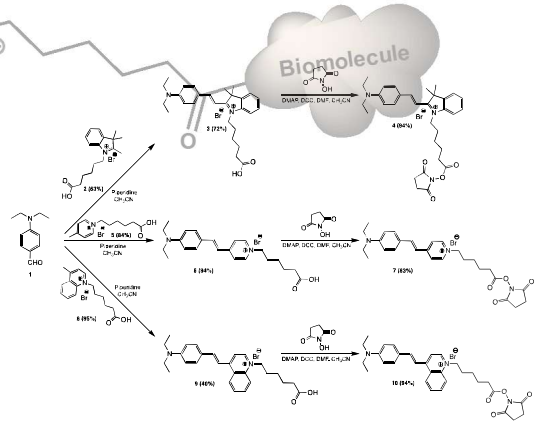
Cultural heritage assets are being continuously submitted to physical, chemical and biological degradation. Biodegradation is the major factor for their deterioration, with microorganisms being of critical importance due to their metabolism and proliferation. [1,2] Fluorescent labeling, a widely used bioanalytical technique, facilitates biomolecule detection but is often too costly for routine use. This work presents a simple and cost-effective method to synthesize inexpensive fluorescent labels from 4-diethylaminobenzaldehyde. These new labels enable improved diagnostics of biodegradation, supporting the development of preventive strategies and effective remediation in cultural heritage preservation.

DISCUSSION

In this work, we develop a simple and effective synthetic strategy to produce new inexpensive small molecules as promising fluorescent labels for biomolecules, using 4-diethylamino-benzaldehyde (**1**) as a starting material. The synthetic routes followed for the preparation of the fluorescent labels for biomolecules **4**, **7** and **10** are shown in **Scheme 1.** The aldol condensation reaction allowed the synthesis of intermediates **3**, **6** and **9,** with electron-donating groups and effective electron-accepting groups. These groups enhance π-delocalization and the push-pull character of the chromophore, crucial for effective intramolecular charge transfer (ICT). The extended π-conjugation in these molecules improves their photophysical properties, including high molar extinction coefficients and, for compounds **4** and **7**, large Stokes shifts.



Scheme 1. Structure of the synthesized fluorescent labels.

The photophysical properties of the synthesized compounds **4**, **7** and **10** are summarized in **Table 1.**

Table 1. Spectroscopic properties of 4-diethylamino-benzaldehyde derivatives.

Compounds	λ_{abs}[a] (nm)	λ_{em}[b] (nm)	Stokes shift (nm)	ε[c] ($cm^{-1}M^{-1}$)	Φ_F
1	342	385	43	19400	0.06
4	556	597	41	90000	0.57
7	490	604	114	64000	0.30
10	560	665	105	60000	0.02

[a] Absorption maxima in acetonitrile. [b] Emission maxima in acetonitrile. [c] Molar extinction coefficient at longest wavelength transition. [d] Fluorescence quantum yield in ethanol, determined using 7-diethylamino-4-methylcoumarin (Φ_F = 0,73 in ethanol) as standard.

The study utilizes 5'-AC6 amino-modified oligonucleotide sequences, EUK516-mod and EUB338-mod, which target rRNA regions of eukaryotic and prokaryotic cells, respectively, to develop fluorescent probes.

The method involves chemically labeling these oligonucleotides by forming a covalent bond between the fluorescent labels (**4**, **7**, and **10**) and the amino group at the 5' terminus of the oligonucleotides. Three of these new probes were designed to target the rRNA region of eukaryotic cells, while the remaining three were tailored for the rRNA region of prokaryotic cells (Fig. 1).

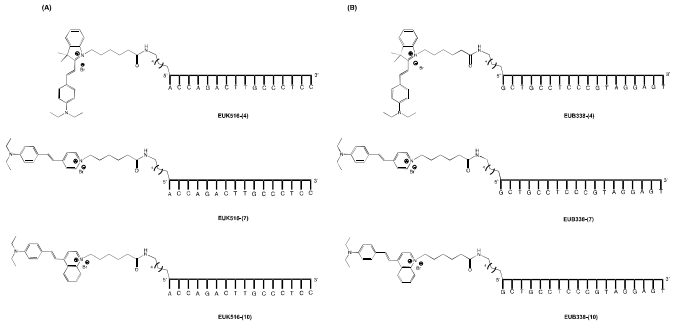


Figure 1. Fluorescent oligonucleotide probes: **(A)** Oligonucleotide probes complementary to eukaryotic cell RNA; **(B)** Oligonucleotide probes complementary to prokaryotic cell RNA.

RESULTS OF PERCENTAGE OF FLUORESCENT CELLS

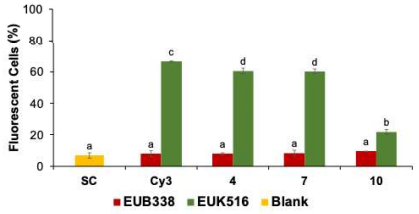


Figure 2. Percentage of fluorescent cells **(A)** from *Saccharomyces cerevisiae* cells labeled with EUK516 and EUB338 probes with different markers (**4**, **7**, **10** and **Cy3**) and their respective blank. In each assay 1000 cells were analyzed in triplicate. Values represented in A correspond to the average of flow cytometry measurements and error bars to standard deviation (± SD). In each plot, different letters located over the error bars indicate significant differences ($p<0.05$).

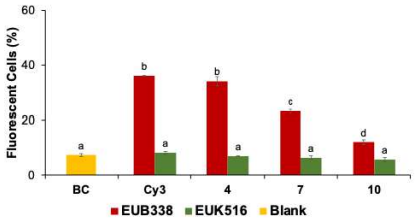


Figure 3. Percentage of fluorescent cells **(B)** from *Bacillus* cells labeled with EUK516 and EUB338 probes with different markers (**4**, **7**, **10** and **Cy3**) and their respective blank. In each assay 1000 cells were analyzed in triplicate. Values represented in A correspond to the average of flow cytometry measurements and error bars to standard deviation (± SD). In each plot, different letters located over the error bars indicate significant differences ($p<0.05$).

CONCLUSIONS

i) Fluorescent 4-diethylamino-benzaldehyde derivative labels are highly effective for tagging biomolecules with primary amine groups, achieving high yields in producing single-fluorescent-labeled oligonucleotides. ii) The labeled oligonucleotide probes are highly effective as RNA-FISH probes, enabling specific detection of microbial cells and opening opportunities for broader applications of these amino-reactive fluorescent labels. iii) The EUK516-(**4**) and EUB338-(**4**) probes demonstrated excellent specificity for labeling yeasts and bacteria, proving their exceptional utility in microbial identification through RNA-FISH.

REFERENCES

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2. Johnson, I.; Spence, M. Molecular Probes™ Handbook—A Guide to Fluorescent Probes and Labeling Technologies, 11th ed.; Life Technologies: Carlsbad, CA, USA; Thermo Fischer Scientific: Waltham, MA USA, **2010**.

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