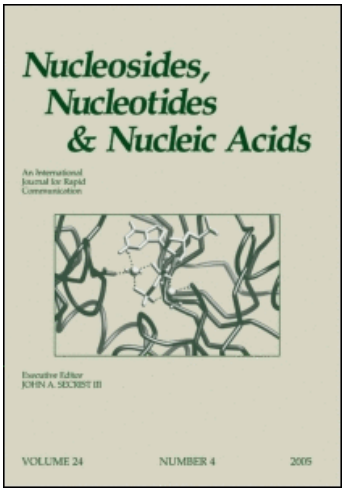

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DESIGN OF MULTIDYE SYSTEMS FOR FRET-BASED APPLICATIONS

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ABSTRACT

A new solid phase approach, based on orthogonal protective group strategy utilizing Fmoc and DMTr groups, was used to assemble linear polymeric chains with pending groups at desired locations. A compound synthesized using four different fluorophores with consequently overlapping absorption and emission spectra (pyrene, perylene, fluorescein and TAMRA) was shown to fluoresce at 570 nm when excited at 330 nm, demonstrating sequential energy transfer across four chromophores.

INTRODUCTION

Fluorescence Resonance Energy Transfer (FRET) (1) is a popular technique, which finds numerous applications in life sciences and beyond. Used to estimate distances between chromophores, FRET allows for studies of dynamic processes in nanometer-sized objects, including conformational changes and self-assembly, at low concentrations and on rapid time scales (2). The sensitivity of the technique, which works well with nanomolar concentrations, allows for both the individual components and the assemblies to be observed directly and in real time, which is better compared to NMR-based methods that require millimolar concentrations.

The currently employed version involves a donor-acceptor pair of fluorophores with overlapping excitation and emission spectra. It has previously been reported (3) that a third fluorophore incorporated between the donor and the acceptor can

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enhance the transfer efficiency over longer distances by playing a role of a 'relay station'.

We describe here a first example of a multiple FRET system in which at least four different fluorophores are involved in an energy transfer process.

RESULTS AND DISCUSSION

We designed a convenient system for solid phase synthesis of any combinations of fluorophores positioned at precise locations relative to each other. A phosphoramidite-based approach comprises step-by-step assembly of phosphotriester-linked units which are then coupled to a desired fluorophore prior to an addition of the next monomer (Fig. 1). Some of the fluorophores we planned to use are unstable to the ammonia deprotection conditions. Furthermore, for our initial experiments we wanted to have a non-charged backbone, so that the results would

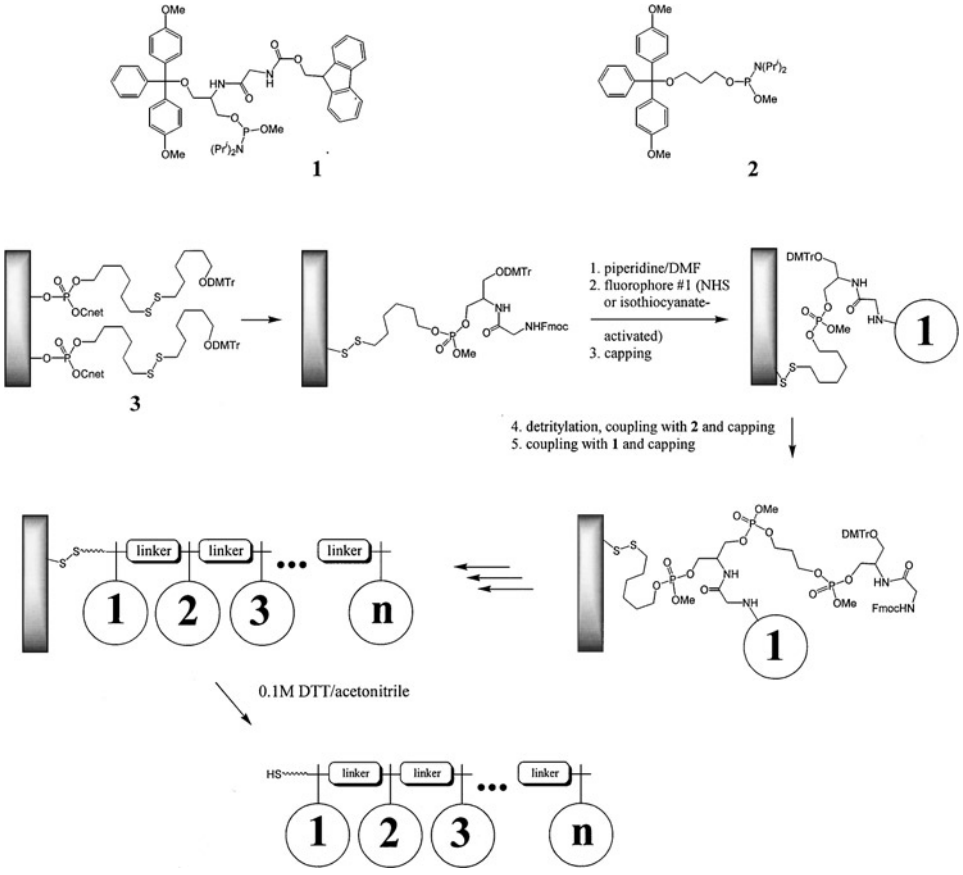

Figure 1. Synthesis of oligomer with pending reactive groups using phosphoramidites 1 and 2 and conjugation of the growing oligomer with different chromophores.

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not be complicated by unpredictable interactions between some positively charged fluorophores and negatively charged backbone. We therefore used a disulphide (**3**) rather than an ester bond as a means of anchoring the first 'monomer' to a solid support. Another undesirable side-reaction would be cleavage of oligomers from the support and removal of Cnet group when deprotecting Fmoc with 10% piperidine in DMF. Thus, in addition to a disulphide anchoring, methyl rather than cyanoethyl protective group was used to generate non-charged backbone after deprotection and cleavage. The serinol-based Fmoc-protected synthon was synthesized according to a published method (4) and phosphitylated according to a standard procedure to give **1** as a white solid, MALDI-TOF: 833.38382 Da (Calculated for: $C_{48}H_{56}N_3O_8P$: 833.38050 Da; error: 4 ppm). Rapp Polymere (*ca* 200 μm polystyrene beads grafted with polyethyleneglycol) was placed in a standard ABI oligonucleotide synthesis column and derivatised with C6 disulphide phosphoramidite (Glen Res.). Subsequent steps were according to Figure 1. After each coupling with **1**, as well as after each condensation with a fluorescent tag, we used a capping step (standard ABI capping solution) to terminate non-reacted strands. After removal of the last DMTr group, the support was briefly soaked in ammonia and the product was then cleaved by 0.1 M DTT in acetonitrile (overnight).

Our initial attempt to obtain a poly-FRET displaying compound involved consequent coupling, to a nascent oligomer chain made by repeated couplings of synthon **1**, of 7 different dyes in the following order: 1) pyrene, 2) perylene, 3) fluorescein, 4) TAMRA, 5) X-Rhodamine, 6) Naphtho-Rhodamine, and 7) Cy 5.5. The first two were used as NHS-activated trityls (5), fluorescein was in a form of FITC, all rhodamines were as NHS-esters and Cy5.5 was in a form of methyl phosphoramidite. The structure of this compound was confirmed by an absorption spectrum, were peaks corresponding to all fluorophores used were present (data not shown). The excitation wavelength (λ_{ex}) for pyrene is about 330 nm; with λ_{em} for Cy5.5 at about 694 nm, we would have a remarkable Stokes' shift of 364 nm. Nevertheless, we failed to detect any energy transfer; rather, all tags fluoresced separately when excited at their λ_{ex}. We then increased the distance between all the dyes by incorporating an additional propanediol-based linker **2** (6). The distance between each two adjacent fluorophores has thus become equal to that used in a model pyrene-perylene dimer, were 100% FRET was registered (5). Again utilizing the approach outlined on Figure 1, we synthesized a shorter compound (Fig. 2), made up of 4 different chromophores. It proved to be difficult to characterize the structures of these compounds. MALDI-TOF analyses were inconclusive, maybe partially due to earlier described effects (5) involving FRET during the MALDI-TOF process itself (the laser used in MALDI has the same wavelength (337 nm) as pyrene excitation band). The absorption spectra (Fig. 3) demonstrated the presence of all added chromophores in the same sample which, given the use of the capping step, means that all fluorophores are attached to the same molecule. We also synthesized the four fluorophores-containing conjugate in two different directions: first, by starting from the pyrene-bearing tag, followed by perylene, fluorescein and rhodamine. Another structure was synthesized in the

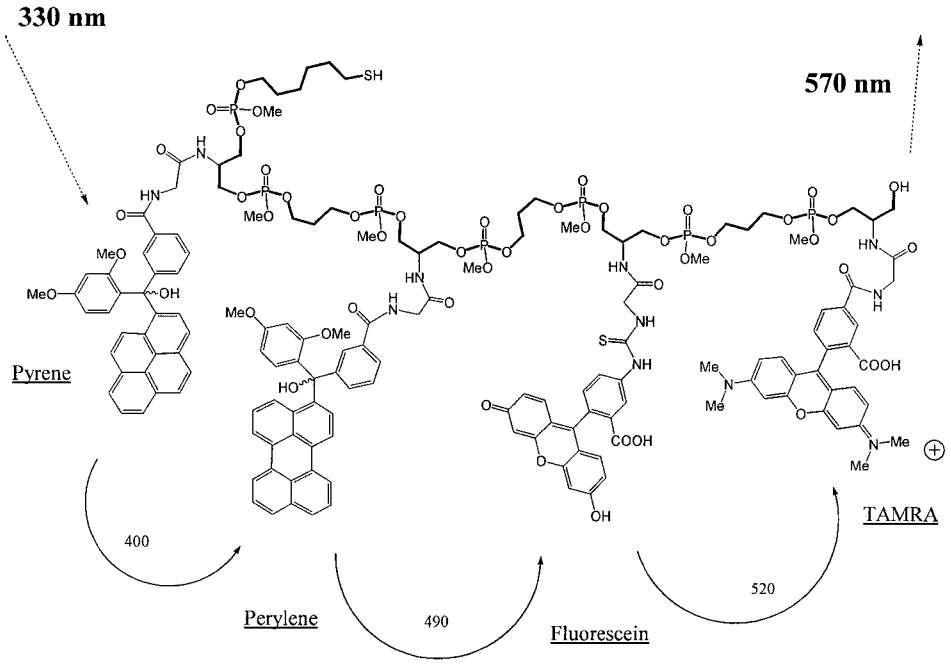

Figure 2. Structure of oligomer bearing four different chromophores with overlapping absorption and emission spectra.

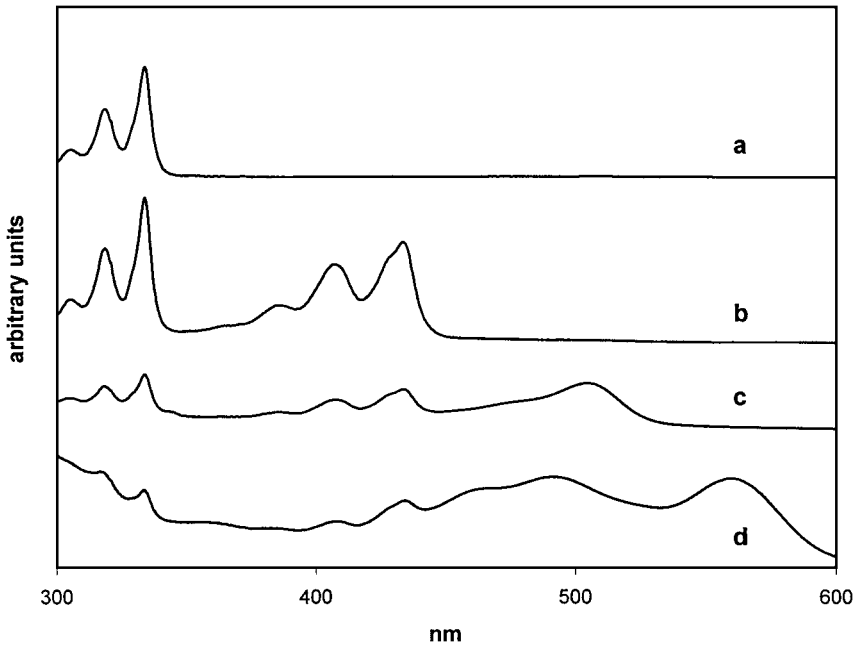

Figure 3. Absorption spectra of the 1- (**a**), 2- (**b**), 3- (**c**) and 4- (**d**) mer compounds synthesized according to Figure 1. The samples were taken from the pool of Rapp beads after each coupling with a fluorophore, cleaved from the support by DTT and analysed by UV spectroscopy. As expected, attachment of each chromophore adds a characteristic peak to the spectrum: **a**, pyrene; **b**, pyrene + perylene; **c**, pyrene + perylene + fluorescein; **d**, the final structure with all four dyes, see Figure 2.

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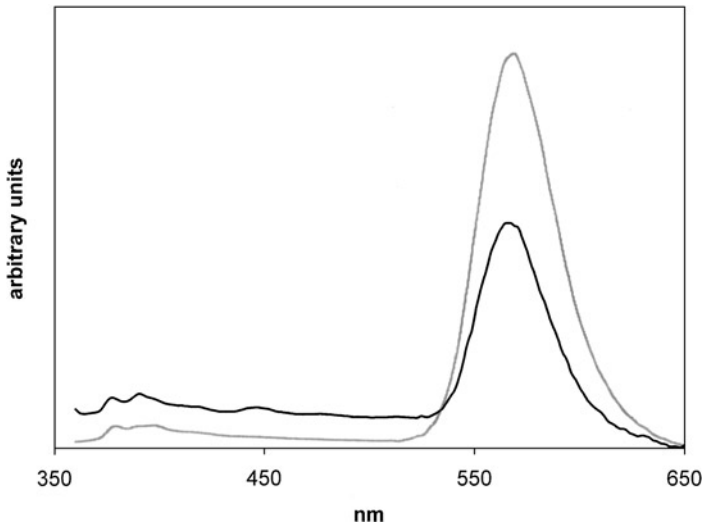

Figure 4. An energy transfer obtained for a tetramer (Fig. 2), λ_{ex} = 330 nm, 10^{-6} M in DCM. (black line). Some residual fluorescence of pyrene (400 nm) and perylene (450 nm) is also visible, due to the presence of truncated oligomers. Grey line, a fluorescence spectrum of the tetramer synthesized starting from TAMRA, λ_{ex} = 330 nm, 10^{-6} M in DCM. Only the full-length product would contain the pyrene moiety, and the energy transfer is therefore more efficient. The concentrations used were determined based on the trityl yield measurements of the couplings.

same way, but starting from rhodamine, and ending with pyrene. These two different structures had almost identical absorption spectra. This time, we were able to observe an energy transfer across all four chromophores (Fig. 4) for both structures. For an excitation at 330 nm (pyrene), a fluorescence at 570 nm (TAMRA) was detected, which equals to a Stokes' shift of 240 nm. A difference in the fluorescence spectra for the two compounds can be explained by decreasing yields of attachment of consecutive fluorophores, which depends on phosphoramidite coupling, Fmoc removal and fluorophore conjugation yields. If the first fluorophore to be attached will play a role of a donor (the 'blue' one), then the decreased yields of attachment of the following tags would lead to an excessive fluorescence of the truncated species, decreasing the yield at the longest wavelength. If on the contrary the longest wave-length emitting chromophore (the 'red' one) is attached first, then the only chains to contain a starting, shortest wave-length fluorophore will be the full-length chains and therefore the efficiency of the transfer would be higher.

CONCLUSIONS

We have developed a new solid phase-based method of assembly of the oligomer structures bearing pending chromophore groups. The groups can be positioned at defined distances away from each other, by controlling the lengths of spacers (like **2**) incorporated between the pending group-containing monomer units of **1**. These compounds were developed to test a possibility of sequential energy

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transfer through more than two dyes routinely used as donor and acceptor in FRET techniques. In the previously reported example (3), three fluorophores were used. Non-charged oligomers, synthesized using methyl-protected phosphoramidites in combination with disulphide anchoring, were cleaved from the support (Rapp polymere) by DTT and the composition was confirmed by UV spectrometry. The distance separating adjacent chromophores was found to be important. No energy transfer was detected when the distance was less than 8 Å. We found that the distance between the attachment sites of two adjacent fluorophores should be at least 14 Å for an energy transfer to take place. Presumably, when the fluorophores are too densely packed, or/and arranged in a slightly distorted way, as in the case of tritylised pyrene and perylene (5), too close a proximity may prevent their dipoles from being positioned at the optimal distance/orientation for a FRET to occur. A good example is cyclophane structures, which lack resonance energy transfer properties even when two appropriate chromophores are rigidly fixed facing each other at a close distance.

The ability to carry out the FRET process in this cascade way can be advantageous for many applications, such as allowing one to monitor the energy transfer process over much greater distances or in light harvesting (7). Alternatively, the process may be used to monitor the assembly of several units (more than two). Employing 'switchable' fluorophores like 'tritylized' pyrene or perylene can exert an additional degree of control over this multiple FRET: turning their fluorescence on and off by a gentle pH change (5) may reversibly interrupt the energy transfer process. It is likely that each pair of chromophores should be separated by a defined distance different from that for other pairs, to achieve the optimal yields. Quantitative studies of the efficiency of the sequential energy transfer process, such as quantum yield and environment sensitivity, and its limits (in terms of the maximum number of the units in the chain) are currently under investigation.

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REFERENCES

1. Förster, T. *Ann. Phys.*, **1948**, *2*, 55–62.
2. De Silva, A. P., et. al. *Chem. Rev.*, **1997**, *97*, 1515–1555.
3. Kawahara, S.-I.; Uchimaru, T.; Murata, S. *Chem. Commun.*, **1999**, 563–564.
4. Shchepinov, M. S.; Case-Green, S.C.; Southern, E.M. *Nucl. Acids Res.*, **1997**, *25*, 1155–1161.
5. Shchepinov, M. S., et. al. *Tetrahedron Lett.*, **2000**, *41*, 4943–4948.
6. Seela, F.; Kaiser, K. *Nucl. Acids Res.*, **1987**, *15*, 3113–3129.
7. Officer, D. L.; Burrell, A. K.; Reid, D. C. *Chem. Commun.*, **1996**, 1657–1658.

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