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# Amplified Fluorescent Molecular Probes Based on 1,3,5,7-Tetrasubstituted Adamantane

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**Abstract:**

A novel series of fluorescent labels containing multiple fluorescein moieties attached to a bridgehead-substituted adamantane has been synthesized. Molecules containing two, three or four fluorescein residues were prepared and their fluorescence was measured. The rigid adamantane core prevents fluorescein moieties from intramolecular collisions, thus precluding mutual fluorescence quenching. Proposed design allows building the molecular probes with amplified fluorescence signal by combining several fluorophores in a molecule.


*Keywords:* Adamantanes; Fluorescence; Molecular design.

Enhancement of the resolution is one of the major goals in the development of molecular probe methods [1]. This can be accomplished by increasing the level of the signal obtained from the probe molecule, along with the hardware improvement and data processing sophistication. However, attempts to achieve signal amplification by accumulation of several reporter moieties in the probe molecule have not been successful for fluorescent probes. Usually, mutual quenching of the fluorescence originating from intramolecular collisions between fluorophores results in a fading, rather than in an amplification of the fluorescence for molecules with multiple fluorophores [2]. This effect is especially significant for the planar aromatic fluorophores [2,3]. We assumed that internal quenching should be reduced in those multifluorophore molecules where interactions between fluorophore moieties are sterically hindered. Molecular modeling suggests that a rigid three-dimensional

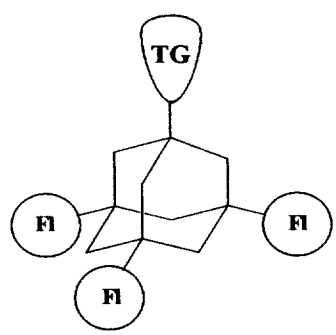


*Figure 1.* General Drawing of the Adamantane-based Molecular Probe

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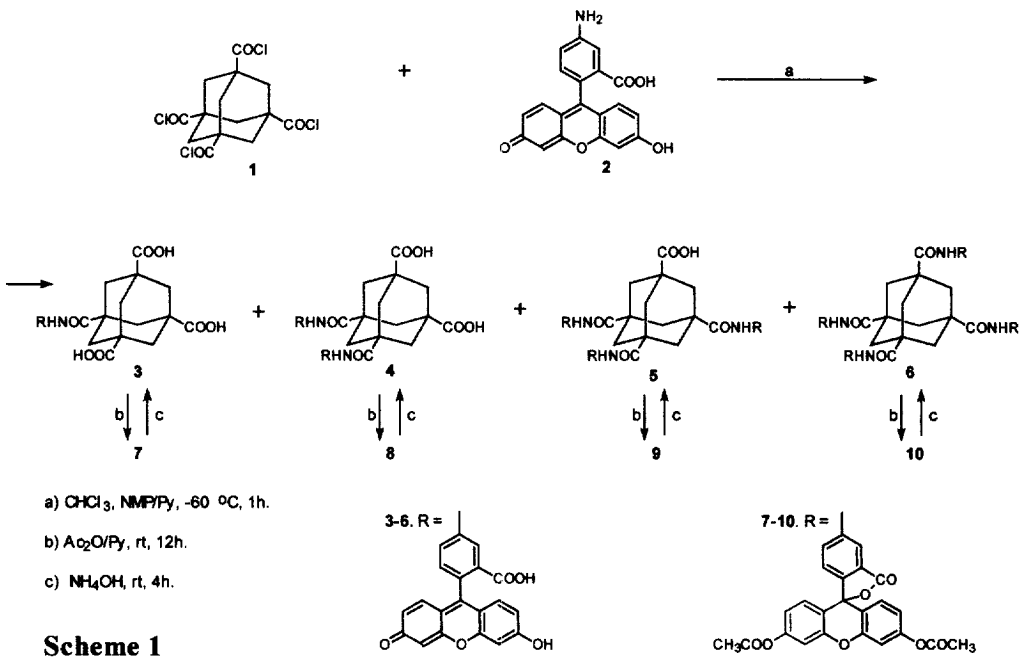
core such as adamantane, substituted in the bridgehead positions, could provide the necessary template for building probes with non-interacting fluorophores. The general drawing (Figure 1) features an adamantane-based probe having three non-interacting fluorophores (Fl) along with an extra functionality (targeting group, TG) for attaching the reporter moiety to biological molecules. Also, because of the probe's overall size, the proposed design should provide additional advantages in DNA probing, especially in fluorescence-in-situ-hybridization (FISH) diagnostics [4], by reducing the unwanted stacking of the aromatic fluorophores into the helical nucleic acid structure [5]. Herein, we report the synthesis and fluorescent properties of the multifluorophore molecules combining an adamantane core and fluorescein fluorophores.

**Synthesis.** A series of four compounds **3** – **6** having from one to four fluorescein residues connected to the adamantane core through amide linkages were prepared. All four molecules were obtained from the single reaction between adamantane-1,3,5,7-tetracarboxylic acid chloride **1** [6] and aminofluorescein **2** (Scheme 1).

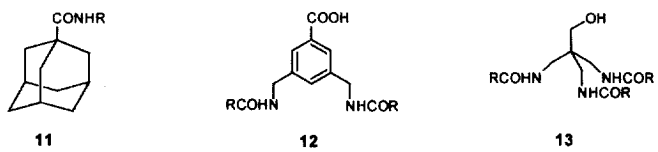



**Scheme 1**

Due to a lack of discrimination between the carbamoyl chloride groups, the reaction with amine **2** results in a mixture of amides **3** – **6**. The ratio of the products **3** - **6** depends upon stoichiometry and reaction conditions. Oligomeric by-products from side-acylation of the fluorescein phenolic hydroxyls were cleaved by mild basic hydrolysis of the reaction mixture. In order to separate the products by conventional chromatography they were acylated *in situ* to

give a mixture of less polar and more soluble diacetyl fluorescein derivatives **7 – 10**. Chromatographic separation of the acetyl derivatives **7 - 10** followed by ammonolysis yielded individual compounds **3 – 6**. When five-fold excess of starting amine **2** was employed, the yields of the products were as follows: monoamide **3**, 6%; diamide **4**, 20%; triamide **5**[1], 26%; tetraamide **6**, 18%.

**Fluorescence study**. Fluorescent spectra of compounds **3 – 6** were recorded for $10^{-6}$ M solutions in 50 mM phosphate buffer (pH 8). 1-Adamantanecarboxylic acid fluoresceinamide **11** as well as compounds **12** and **13** (Figure 2; R = fluorescein) having two and three fluorescein residues separated by a flexible spacers were prepared for comparison. They were synthesized by acylation of corresponding amines [7] with fluorescein-5-carboxylic acid NHS ester [8].




**11** **12** **13**

*Figure 2.* Model compounds used in fluorescence study

The fluorescence intensities of adamantane derivatives **3 - 5** (emission maxima at 516 nm; excitation at 490 nm) were almost linear to the number of fluorescein residues (Figure 3, value for fluorescein (**fl**) is listed for comparison). The deviation by tetraamide **6** is probably caused by low solubility.

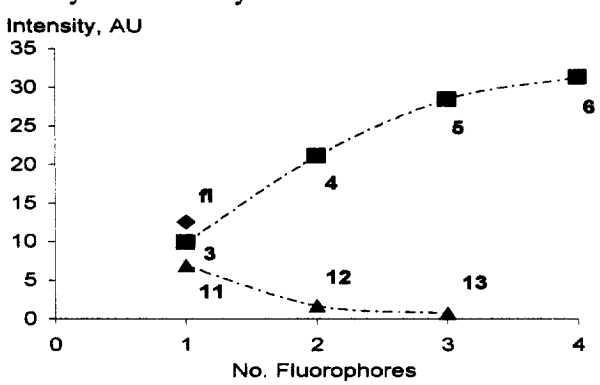



*Figure 3.* Fluorescence intensities (arbitrary units) of the studied compounds at 516 nm (excitation at 495 nm)

In contrast to adamantane derivatives, fluorescence intensity of the compound **12** with two fluorophores was only 20% of that for monoamide **11**. The intensity dropped another 2.5 times

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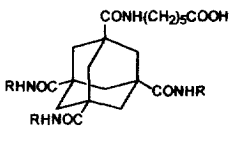
[1]Selected data: mp. dec. above 300 °C; IR (KBr): 1113, 1179, 1208, 1462, 1606, and 1639s $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$) δ 2.21 (br.s., 6H), 2.50 (br.s, 6H), 6.59 (m., 12H), 6.62 (br., 6H), 7.24 (d, 3H), 8.03 (d., 3H), 8.41 (s, 3H), 9.93 (s., 3H, N-H)10.30 (br.s., 3H, O-H), 12.80 (br.s., 1H, OH). HPLC (det. @ 254 nm): 99.9% pure. ES MS, m/e: Calcd $(M+1)^+$ for $C_{74}H_{49}N_3O_{20}$ 1300.3. Found: 1300.5.

in a compound **13** with three fluorescein residues. Because the UV absorbance values at 495 nm for these compounds were linear to the number of fluorescein residues, the decrease in fluorescence intensities was obviously caused by intramolecular quenching.

**Triamide 5 in synthesis of molecular probes.** The remaining adamantane carboxylic group in the triamide **5** is accessible and reacts with aliphatic amines under standard activation protocols (NHS/DCC, HOBt/EIA, *etc*). The resulting products, such as the acid **14** with $C_5$ spacer or labeled nucleoside **15** (Figure 4, R = fluorescein) display fluorescent intensities about three times that of fluorescein. These derivatives are currently under study as precursors for probes for biological research and FISH diagnostics.

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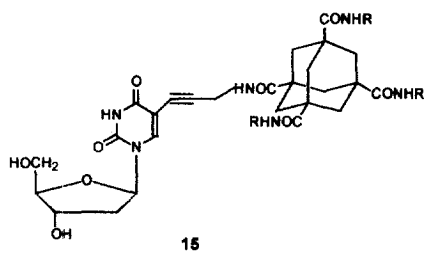


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*Figure 4.*

In summary, we found that a rigid adamantane core eliminates intramolecular interactions between fluorophores and, as a consequence, mutual fluorescence quenching. This effect enables the construction of molecular probes with amplified fluorescence signal.

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