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Tritylisation of pyrene, perylene and coronene: a new family of switchable fluorescent labels

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Abstract

The synthesis of novel fluorescent labels based on pyrene, perylene and coronene is described. Due to the trityl-type structure, their fluorescence may be reversibly switched on and off by changing the pH. This property can be used to expand the palette of fluorophores available for multicolour DNA detection on DNA chips. Some FRET and surface chemistry applications are also demonstrated. © 2000 Elsevier Science Ltd. All rights reserved.

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As part of a programme to optimise the manufacture and use of DNA chips we developed a new family of fluorescent tags derived from polycyclic aromatic hydrocarbons (PAHs). Both single fluorophore[1] and energy transfer[2]-based fluorescence detection methods find wide applications in the analysis of nucleic acids. Some PAHs have certain advantages over the fluorophores currently used to label DNA, such as fluorescein: they are less prone to photobleaching and have high molar absorbance and high quantum yields. Furthermore, molecules are available with a range of excitation and emission maxima and large Stokes shifts. We chose pyrene,[3] perylene[4] and coronene[5] as PAHs with useful fluorescent properties. The introduction of a carbinol–carbocation switchable element would allow one to controllably turn the fluorescence on and off by changing the pH. The conversion of these PAHs into trityl-type structures also seems advantageous due to the non-planar conformation of triarylmethanols, which would prevent the π–π stacking interactions with a resulting increase in the solubility.

Friedel–Crafts acylation of PAHs with 2,4-dimethoxybenzoyl chloride in presence of $AlCl_3$ in DCM for pyrene or chlorobenzene[6] for perylene and coronene gave 1-acylpyrene **2a**, 3-acylperylene **2b** and acylcoronene **2c** (Scheme 1). These ketones were converted into the corresponding

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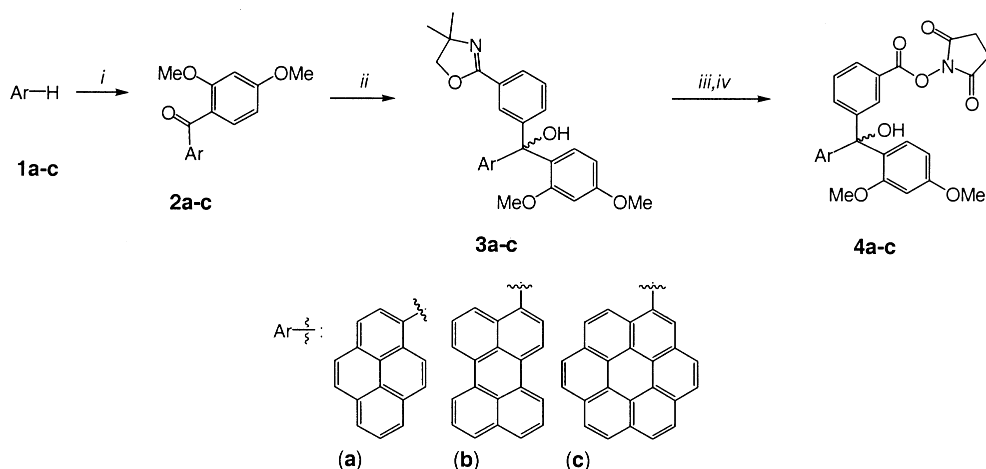
R,*S*-oxazolyltritanols **3a–c** using appropriate phenylmagnesium bromide as a nucleophile in Grignard synthesis. Further conversion[7] yielded *N*-hydroxysuccinimide (NHS)-derivatives **4a** (as pale yellow), **4b** (as dark red) and **4c** as yellow solids.[8] NHS-esters **4a–c** can be used for labelling of biomolecules or for derivatisation of surfaces. The labelling procedure would involve reacting **4a–c** with an amino group(s)-containing analyte; we therefore synthesised[7b,c] the corresponding model butylamides **7–9** (Fig. 1) for fluorescence studies.



Scheme 1. Reagents and conditions: (i) 2,4-dimethoxybenzoylchloride/$AlCl_3$ in DCM (a, 86%) or PhCl (b, 53%; c, 48%); (ii) 3-(4,4-dimethyl-1,3-oxazoline-2-yl)phenylmagnesium bromide/THF, reflux; (a, 73%; b, 64%; c, 49%); (iii) 80% AcOH, (or TFA, 72 h, for c), at 70°C, 48 h, then reflux in 20% NaOH in EtOH/H_2O, 3 h; a, 96%; b, 93%, c, 85%; (iv) DCC, NHS, dioxane/THF (a, 91%; b, 87%; c, 79%)

A modified trityl group bearing a pyrenyl residue in place of one of the phenyls has fluorescent properties similar to non-modified pyrene.[9] Triarylmethyl cation derived from tritanol by an acidic treatment (Scheme 2) would have completely different fluorescence properties, while remaining covalently linked to a probe molecule if attached to it through a side-chain.[7] These features are combined in compounds **4a–c**. The pH-threshold for the formation of trityl carbocations (like **6**) from corresponding tritanols at low pH can be controlled by electron withdrawing or donating groups on the aromatic rings.[10] Two methoxy groups and one carboxyl group give **7–9** an acidic stability similar to that of a standard DMTr and MMTr groups.[11] The UV spectra of **7–9** were of the same shape but slightly red-shifted (3–10 nm) as compared to the starting PAHs.[2,3]

Multicolour detection (use of more than one fluorophore in one reaction) is a useful feature of fluorescent dyes; it enables different sequences to be detected simultaneously and has been used to good effect in DNA sequencing,[1] fluorescence in situ hybridisation (FISH)[12] and differential gene expression analysis on DNA chips.[13] The size of the palette is limited by the overlap of the excitation and emission spectra; it has proved difficult to use more than four colours in FISH and two colours are normal in expression analysis. An advantage of trityl-based fluorescent tags is the potential to ‘switch’ the spectra on and off by simply changing the pH. The magnitude of the shifts is very large. For example, moving from neutral or alkaline to acidic pH shifts the excitation maximum of the pyrene-based compound **7** from 346 to 711 nm (Fig. 1), the property earlier used to generate triarylmethyl carbocations with a variety of different colours.[14]

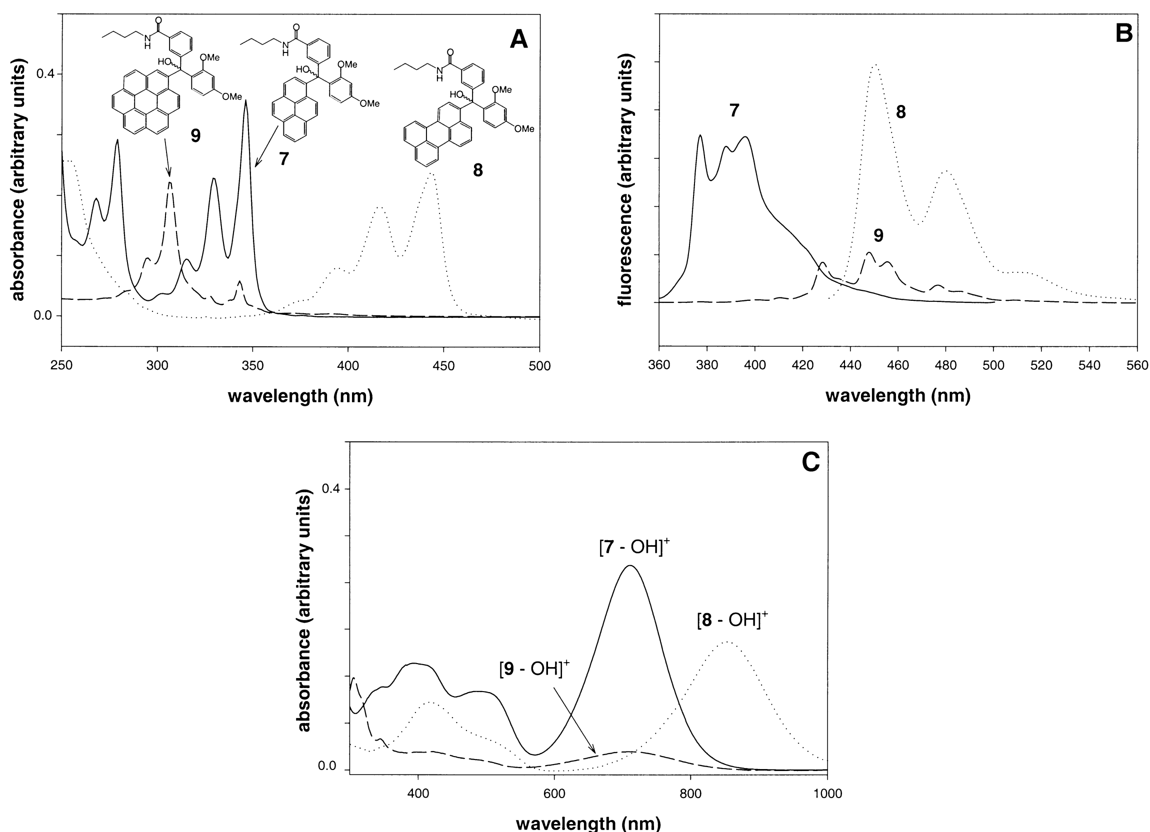
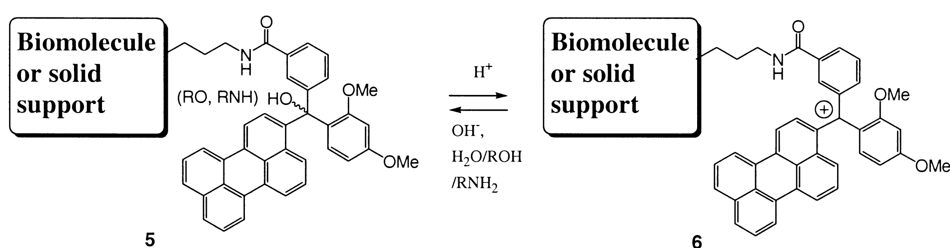


Figure 1. **A**. Absorption spectra of butylamides **7** (solid line), **8** (dotted line) and **9** (dashed line) (10^{-5} M in DCM). **B**. Fluorescence spectra of butylamides **7**–**9** (10^{-6} M in DCM); excitation wavelengths: 330 nm (**7**), 420 nm (**8**), 343 nm (**9**). **C**. Absorption spectra of cations of butylamides **7**–**9** (10^{-5} M in 1% TFA/DCM)



Scheme 2.

Trityl carbocations do not fluoresce in the range detected for the corresponding tritanols. This property can be used to improve the discrimination of labels: first by increasing the accuracy of intensity measurements; and, second, increasing the potential number of colours in the palette. For example, targets can be labelled with two fluorophores having similar excitation and emission spectra, but only one of which is switchable by pH change. After hybridisation, measurements are taken at two pH values: under ambient conditions and after exposing the array to acidic vapour, which is enough to switch the emission of fluorescent trityls off immediately, but reversibly. Using

a single excitation source, both fluorophores emit at neutral pH but only one will emit in acid. These two measurements alone would be enough to distinguish the two patterns of hybridisation. But a third measurement, using a source which excites the second fluorophore in acid, can give more analysis. In this way it may be possible to double the number of labels that can be used together.

To evaluate the suitability of our fluorescent labels as components for fluorescence resonance energy transfer (FRET), a model compound **10** was synthesized[8] by the stepwise acylation of 4,7,10-trioxa-1,13-tridecanediamine with **4b** followed by **4a** in DCM. While the absorption spectra for both non-ionised and bis-cationic forms essentially represent a superposition of **7** and **8** (Fig. 2A), **10** fluoresces only at 450, 480 and 515 nm when excited at the pyrene absorption maximum of 330 nm (Fig. 2B), with no detectable fluorescence of pyrene (at 377, 388 or 396 nm). When mixed in equimolar amounts, **7** and **8** retain their own fluorescence properties (data not shown). This suggests a possibility of designing fluorescent labels having increased Stokes' shifts by arranging the necessary fluorophores (perhaps even more than two) in the vicinity of each other.[15] Furthermore, additional control can be achieved by making some of these parts more acid-labile than the others, so that some selected components of the chain may be reversibly switched off by decreasing the pH. Mass-spectrum (LDI-TOF) of **10** (calculated exact mass for $C_{78}H_{70}N_2O_{11}$: 1210.50) showed, apart from the molecular ion, two fragments, lacking one (1193.46) and two (1176.47) hydroxyl groups. The fact that the latter flies in mono-charged (mono-cationic) and not in bis-cationic form[7b,c] (the signal for 1176.49/2 = 588.25 was not detected) suggests some unusual interactions, perhaps FRET during the LDI-TOF process, initiated by laser irradiation at 340 nm, which is almost a perfect match with the absorption maximum for **10**.

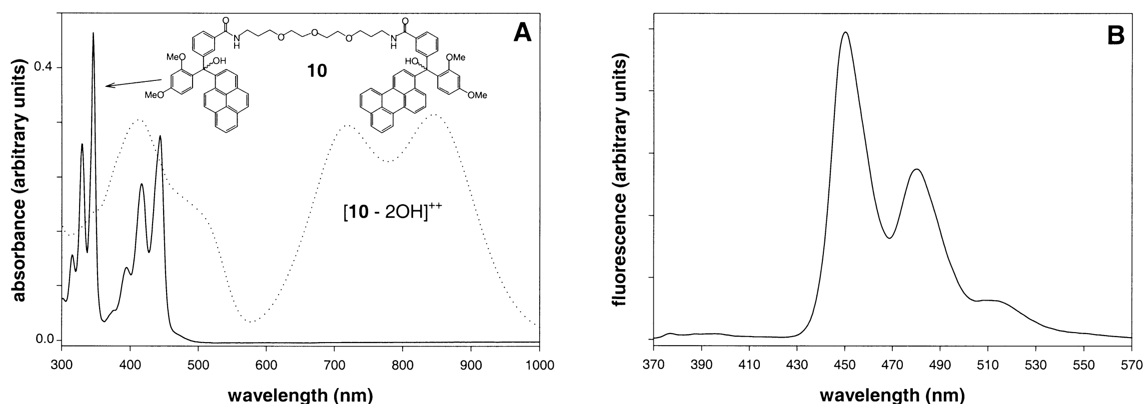


Figure 2. **A**. Absorption spectra of **10** (10^{-5} M in DCM) in non-ionised (solid line) and bis-cationic (dotted line) forms (in 1% TFA/ DCM). **B**. Fluorescence spectrum of bis-chromophore **10** (10^{-6} M in DCM); excitation wavelength: 330 nm

To demonstrate the reversibility of the fluorescence in real time, acid was generated using a microelectrode technique. A series of 20 linear iridium microelectrodes 40 μm wide (80 μm between centres) on a silicon dioxide wafer were manufactured using photolithography methods. A glass microscope slide was silanized with 3-aminopropyltrimethoxysilane and treated overnight with a 0.1 M solution of **4b** in THF. This slide was then washed and placed atop of the micro-electrode array, separated by ca. 3 μm layer of an electrolyte (hydroquinone in CH_3CN). Confocal

microscopy was used to examine the fluorescence emission at 520 nm with Ar/Kr laser excitation. Application of voltage across adjacent electrodes generated protons electrochemically at the anodes, resulting in a lower pH and a corresponding decrease in fluorescent emission at 520 nm (Fig. 3). Upon switching off the microelectrodes, the fluorescent emission reverts to the starting, voltage-off condition, demonstrating the reversibility of the acid-catalysed carbocation conformation. Aminated polypropylene can also be used instead of glass (data not shown).

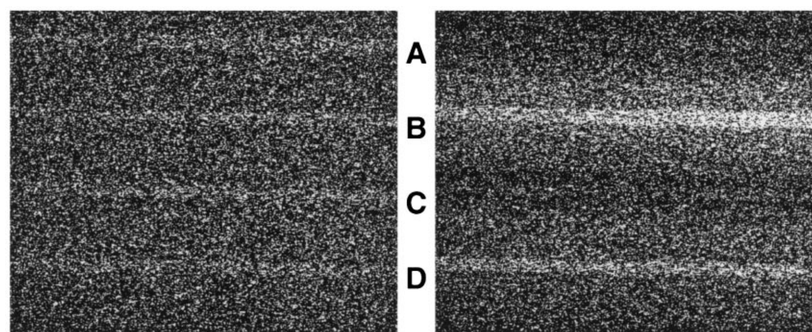


Figure 3. Fluorescent response of an aminated glass surface coated with **4b** (Scheme 2) to acid generated electrochemically above horizontal microelectrodes, observed using confocal microscopy. **Left**, no voltage applied. The signal consists of fluorescence from **4b** on the surface and reflection from iridium-covered electrodes A–D. **Right**, the voltage (1.2 V between cathode B and anode C and 0.8 V between cathode D and anode C) is applied. The acid generated on the anode C leads to conversion of **4b** into cationic form with consequent disappearance of the signal

At present, we are preparing the corresponding phosphoramidite derivatives of **4a–c** to facilitate their incorporation into synthetic oligonucleotides.

Acknowledgements

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References

1. Smith, L. M.; Sanders, J. Z.; Kaiser, R. J.; Hughes, P.; Dodd, C.; Connell, C. R.; Heiner, C.; Kent, S. B. H.; Hood, L. E. *Nature* **1986**, *321*, 674–679.
2. (a) Cardullo, R. A.; Agrawal, S.; Flores, C.; Zamecnic, P. C.; Wolf, D. E., et. al. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 8790–8794. (b) Tyagi, S.; Kramer, F. R. *Nat. Biotechnol.* **1996**, *14*, 303–308. (c) Lewis, F. D.; Zhang, Y.; Letsinger, R. L. *J. Am. Chem. Soc.* **1997**, *119*, 5451–5452. (d) Masuko, M.; Ohuchi, S.; Sode, K.; Ohtani, H.; Shimadzu, A. *Nucleic Acids Res.* **2000**, *28*, e35. (e) Mao, C. D.; Sun, W. Q.; Shen, Z. Y.; Seeman, N. C. *Nature* **1999**, *397*, 144–146. (f) Dobrikov, M. I.; Gainutdinov, T. I.; Vlassov, V. V. *Nucleosides Nucleotides* **1999**, *18*, 1517–1518.
3. Reines, S. A.; Cantor, C. R. *Nucleic Acids Res.* **1974**, *1*, 767–786.
4. Johansson, L. B.-A.; Molotkovsky, J. G.; Bergelson, L. D. *J. Am. Chem. Soc.* **1987**, *109*, 7374–7381.
5. Rohr, U.; Schlichting, P.; Bohm, A.; Gross, M.; Meerholz, K.; Brauchle, C.; Mullen, K. *Angew. Chem., Int. Ed.* **1998**, *37*, 1434–1437.

6. Zieger, H. E. *J. Org. Chem.* **1966**, *31*, 2977–2981.
7. (a) Gildea, B. D.; Coull, J. M.; Koster, H. *Tetrahedron Lett.* **1990**, *31*, 7095–7098. (b) Shchepinov, M. S.; Chalk, R.; Southern, E. M. *Nucleic Acids Symp. Ser.* **1999**, *42*, 107–108. (c) Shchepinov, M. S.; Chalk, R.; Southern, E. M. *Tetrahedron* **2000**, *56*, 2713–2724.
8. Compound **4a**: ^{1}H NMR (200 MHz, $CDCl_3$, δ): 8.66 (d, 1H, $J=9.4$ Hz, arom.); 8.25–7.85 (m, 8H, arom.); 7.4–7.15 (m, 4H, arom.); 6.65 (s, 1H, arom.); 6.32 (m, 2H, arom.); 6.12 (s, 1H, O*H*); 3.77 (s, 3H, OCH_3); 3.74 (s, 3H, OCH_3); 2.85 (br.s, 4H, CH_2). MS (LDI-TOF): 585.3 (MI), 568.3 (MI-OH). R_f (2% MeOH in $CDCl_3$): 0.38. Compound **4b**: ^{1}H NMR (200 MHz, $CDCl_3$, δ): 8.27 (d, 1H, $J=9.6$ Hz, arom.); 8.15 (m, 6H, arom.); 7.95 (d, 1H, $J=9.4$ Hz, arom.); 7.72–7.4 (m, 7H, arom.); 6.6 (s, 1H, arom.); 6.35 (m, 2H, arom.); 6.22 (br.s, 1H, O*H*); 3.8 (s, 3H, OCH_3); 3.77 (s, 3H, OCH_3); 2.88 (br.s., 4H, CH_2). MS (LDI-TOF): 635.2 (MI), 618.2 (MI-OH). R_f (2% MeOH in $CDCl_3$): 0.55. Compound **4c**: ^{1}H NMR (200 MHz, $CDCl_3$, δ): 8.98–6.55 (m, 18H, arom.); 6.15 (br., 1H, O*H*); 3.86 (s, 3H, OCH_3); 3.84 (s, 3H, OCH_3); 2.94 (s, 4H, CH_2). MS (LDI-TOF): 683.29 (MI), 666.45 (MI-OH). R_f (2% MeOH in $CDCl_3$): 0.26. Compound **10**: ^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.58 (d, 1H, $J=9.6$ Hz, arom.); 8.41 (br. s, 1H, N*H*); 8.33–7.96 (m, 12H, arom.); 7.9–7.7 (m, 7H, arom.); 7.5 (m, 2H, arom.); 7.4–7.2 (m, 6H, arom.); 6.78 (d, 2H, $J=7.9$ Hz, arom.); 6.72–6.63 (m, 3H, 2 arom., 1N*H*); 6.44 (dd, 2H, $J_1=2.3$ Hz, $J_2=8.7$ Hz, arom.); 6.26 (s, 1H, O*H*); 6.10 (s, 1H, O*H*); 3.74 (s, 6H, OMe); 3.52 (s, 3H, OMe); 3.45 (s, 3H, OMe); 3.45–3.35 (m, 12H, OCH_2); 3.23 (m, 4H, NCH_2); 1.69 (m, 4H, $CH_2CH_2CH_2$).
9. Fourrey, J. L.; Varenne, J.; Blonski, C.; Dousset, P.; Shire, D. *Tetrahedron Lett.* **1987**, *28*, 5157–5160.
10. Smith, M.; Rammler, D. H.; Goldberg, I. H.; Khorana, H. G. *J. Am. Chem. Soc.* **1961**, *84*, 430–440.
11. The acidic stability of **7**–**9** (2% TsOH/CH_2Cl_2) was as follows: DMTrOH $<$ **7** $<$ **9** $\sim$ MMTrOH $<$ **8** $<$ TrOH.
12. Klinger, K. W.; Landes, G.; Dackowski, W.; Leverone, B.; Lopez, L.; Locke, P.; Ward, D.; Ried, T. *Cytogenet. Cell Genet.* **1991**, *58*, 2149.
13. Schena, M.; Shalon, D.; Davis, R. W.; Brown, P. O. *Science* **1995**, *270*, 467–470.
14. Fisher, E. F.; Caruthers, M. H. *Nucleic Acids Res.* **1983**, *11*, 1589–1599.
15. Lee, L. G.; Spurgeon, S. L.; Heiner, C. R.; Benson, S. C.; Rosenblum, B. B.; Menchen, S. M.; Graham, R. J.; Constantinescu, A.; Upadhya, K. G.; Cassel, J. M. *Nucleic Acids Res.* **1997**, *25*, 2816–2822.