Recent applications of bifunctional trityl groups

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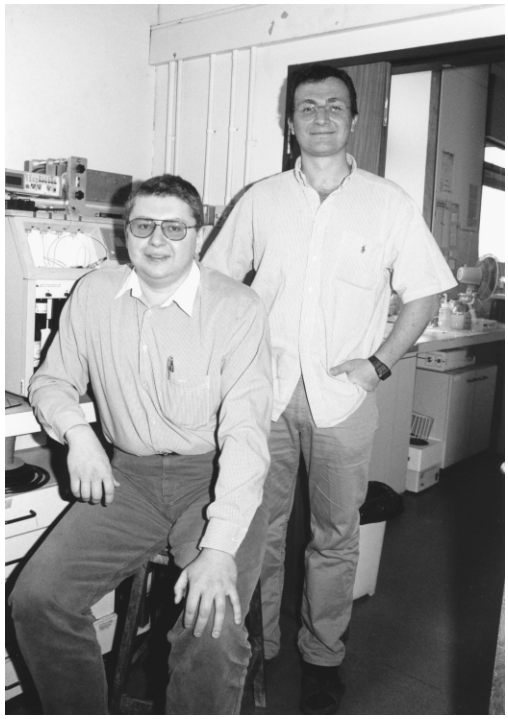
Triphenylmethyl derivatives represent an important class of dyestuffs as well as a useful family of protecting groups widely used in organic synthesis to transiently block various functional moieties. These applications are well documented and have been a subject of a number of reviews. Here we focus instead on some novel applications of a trityl which make good use of its ability to easily form a stabilised cation in combination with additional peripheral functionalities. Topics covered include applications in bioconjugation, cross-linking, mass-spectrometry, fluorescence and optics.

1 Introduction

That the triphenylmethyl (trityl) group is full of surprises was first demonstrated one hundred years ago, when it was discovered by M. Gomberg to give the first stable free radical.[1] On acidic treatment, tritanols or trityl esters easily give trityl cations. The positive charge on the α-carbon atom is stabilized by the resonance effect of three aromatic rings, and the cation stability can be further increased by introducing substituents that facilitate the charge distribution, *e.g. ortho* or *para* alkyl or alkoxy groups (Scheme 1). This particular property, which makes trityl ethers acid-labile, turned out to be useful, and the next few decades saw the trityl being developed into a major class of protective groups[2a] widely used in nucleoside, oligonucleotide, peptide and carbohydrate chemistry, and indeed in almost all other fields of organic and bioorganic chemistry. 4,4′-Dimethoxytrityl group (DMTr) has become a group of choice for 5′-O protection in solid phase DNA and RNA synthesis, while 4′-monomethoxytrityl (MMTr) is used in peptide and, more recently, peptide nucleic acid (PNA) synthesis.[2b] 4,4′,4″-Trimethoxytrityl (TMT) is used less frequently due to its low stability. For applications where an extremely low level of detection is desired, a ^{14}C-labeled DMTr group bearing 6 radioactive carbons was also employed[2c]. For similar reasons, a ^{18}F-fluorine containing trialkoxytrityl deriva-



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tive (4-(2-[^{18}F]fluoroethoxy)phenyl)bis(4-methoxyphenyl)methyl was recently used in precise neurotransmission measurements.[2d] The chemistry of trityl deprotection has not changed over the last two decades. On the instrumental side, a new method of electrochemical deprotection of DMTr groups in combinatorial synthesis utilizing arrays of microelectrodes was recently described as a new approach to manufacturing oligonucleotide arrays (DNA chips).[2e]

Triphenylmethyl-based compounds that are able to give stable quinoid forms occupy an important niche in organic dye chemistry[3] representing an old and numerous class of dyes and fluorophores. These compounds (*e.g.* xanthene fluorophores fluoresceine and rhodamines, rosolic acid, malachite green, *etc*.) are often available as functional derivatives, suitable for covalent fluorescent labeling of biomolecules.[4] These mellifluously named compounds, that remind one of the romantic early days of organic chemistry, are outside of scope of the present review.

Two basic ways of trityl synthesis predominate (Scheme 2). Grignard synthesis (**a**), starting from methylbenzoate **1** or benzophenone **4**, yields tritanol **2**. Alternatively, a twofold aromatic electrophilic substitution in acidic media (**b**) gives triarylmethane **3**, which is then oxidised into the corresponding tritanol. The tritanol can then be halogenated (for example, by refluxing in AcCl/toluene, (**c**)) or converted into alkyl ether **5**, either directly (**d**) by reacting it with alcohol in glacial acetic acid, or indirectly (**e**) by tritylating the alcohol with trityl chloride **6**. For some arylhalogenides, reaction with butyllithium (**f**) rather than the Grignard synthesis leads to the tritanol structure. Triarylmethyl halogenides can be reduced back into triarylmethanes (**g**) by dialkylamides. Another potential method of synthesis, comprising a Friedel–Crafts reaction between an aromatic component (benzene or toluene) and carbon tetrachloride (**h**), is rarely used for other aromatics because some functionalities may be lost under harsh conditions of the Friedel–Crafts reaction.

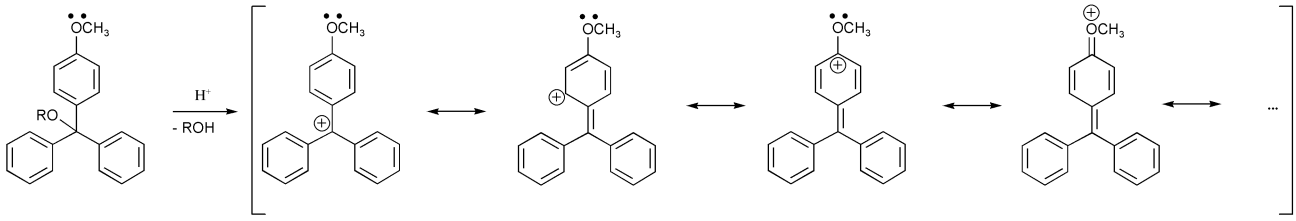
If functionalised trityls are to be obtained, functional groups are usually introduced into the intermediates, and then the trityl is assembled using pathways (**a**), (**b**) or (**f**). In some cases, functional groups may also be introduced into trityls directly.

2 Bifunctional trityl groups

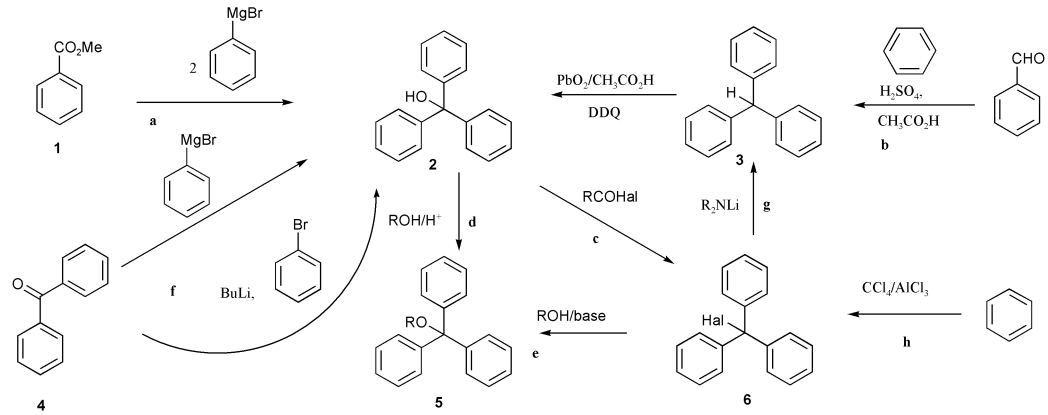
2.1 Bifunctional trityl groups as linkers for bioconjugation

The first support-bound trityl functions were prepared by Leznoff[5] and Fréchet[6] and coworkers and used for performing the multistep synthesis on solid phase. Köster was also one of the first to realise the importance of the trityl moiety equipped with a side-chain bearing another functionality, thus combining a regioselective protecting group and a linking group. Despite of the popularity of solid phase-based methods of oligonucleotide synthesis, solution phase synthesis is still sometimes employed when large quantities of the product are needed. Separating reaction products from non-reacted material by gel filtration is problematic when, especially at the earlier stages of the synthesis, the product is comparable in size to the monomers. To increase the difference between the monomers and oligomers, a 'liquid phase carrier' **8** (Scheme 3) was prepared starting from 3-hydroxyphenyl-diphenylmethanol **7**.[7] The nascent oligonucleotide chains were grown from both ends of the 'carrier' **8** after removal of the 2,2,2-trichloroethyl phosphate protecting groups, therefore dramatically increasing the difference in size of the products as compared to monomeric synthons—nucleoside 3′-phosphates. At the end of the synthesis, both strands were cleaved from the carrier by acidic treatment.

Further development of the concept of functionalised trityls was achieved by the synthesis of a carboxy derivative of DMTr group (Scheme 4).[8] The starting benzophenone **9** was converted into tritanol **10**, and, after work-up and removal of oxazolyl protecting group, 4-carboxy-4′,4″-dimethoxytritanol **11** was obtained. This was transformed into carboxy-activated trityl chloride **12**, which was used for the synthesis of nucleotide monomers **13**. The monomers were used in the final step of



Scheme 1



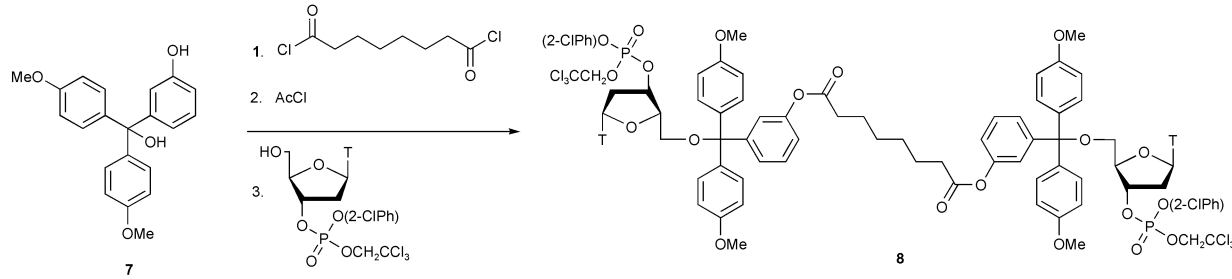
Scheme 2

oligonucleotide chain assembly. Alternatively, oligonucleotides still attached to CPG with a deprotected 5′-hydroxy group were tritylated with a DMTr chloride derivative **12**. The immobilised oligonucleotide was treated with a solution of 1,6-diaminohexane, deprotected with aqueous ammonia, and labelled with biotin. Oligonucleotide probes with acid-cleavable biotin can be used for stepwise multiplex detection of target sequences in gels.[9]

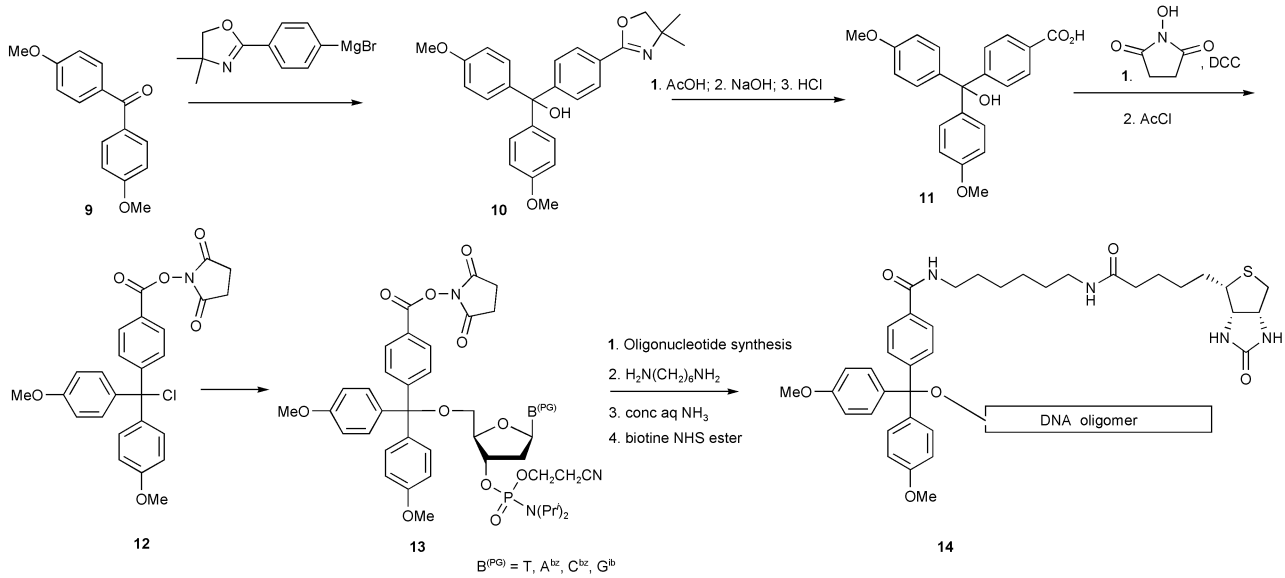
Later, an improved methodology was developed for the synthesis of modified DMTr chloride **12** and *meta* isomer **16** (Scheme 5).[10] The reaction sequence includes condensation of 3(4)-carboxybenzaldehyde with 2 mol of anisole to give triphenylmethane **15**, and subsequent carboxy activation, Pb(IV) oxidation of tertiary carbon and replacement of the hydroxyl with chloride. The nucleoside 5′ ethers from *meta* isomer **16** are more acidically labile as compared with the *para* isomer, presumably due to less pronounced trityl cation destabilisation with the *meta* carboxamide residue. A nucleotide with aliphatic amino groups prepared from DMTr chlorides **12** and **16** by a method similar to that shown in Scheme 4 was used for immobilisation on solid phase. Pure, unprotected nucleotide can be recovered on acid treatment.

The fact that the $DMTr^+$ cation has a very high molar extinction coefficient (ε_{498} = 68 700) in acidic media and can therefore be detected at low concentration was used to good effect in a composite protecting group **20**, which combines DMTr and a levulinyl part (Scheme 6).[11] Tritanol **7** was alkylated to give **17**, which was subject for further transformations to produce symmetrical anhydride **18** which was then used in the synthesis of protected nucleoside phosphoramidite **19**. The trityl group is then incorporated into synthetic oligonucleotides according to standard methods and can be selectively released upon treatment with hydrazine to yield **20**. This release can be monitored very precisely due to a remarkable molar extinction coefficient of the *meta*-substituted trityl part (ε_{513} = 78 600 for the cation) in the heterocyclic derivative **20**.

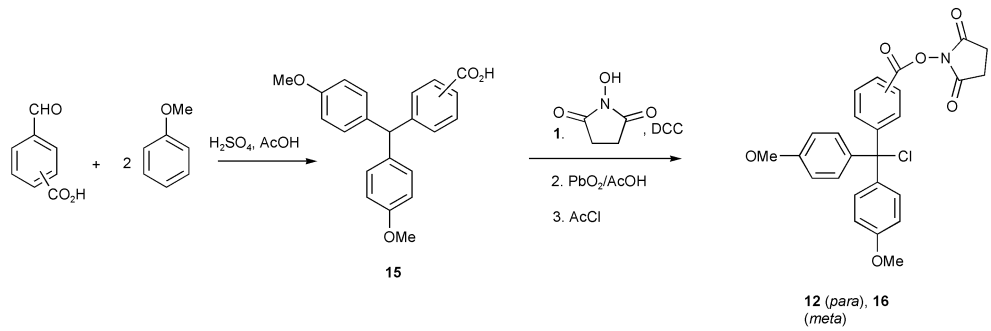
This group was further employed in combinatorial synthesis[12] of oligonucleotides in reverse (5′ to 3′) fashion using



Scheme 3



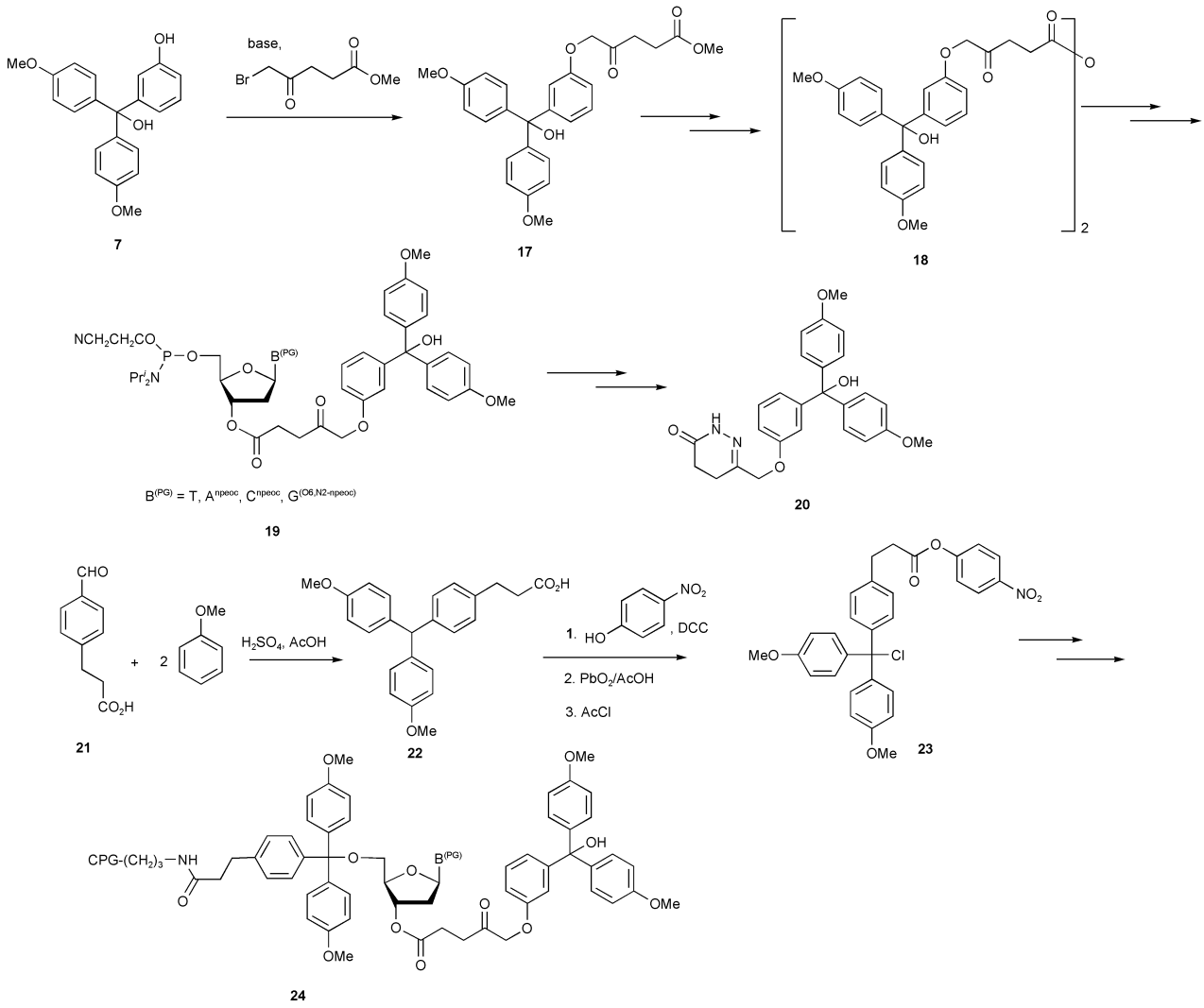
Scheme 4



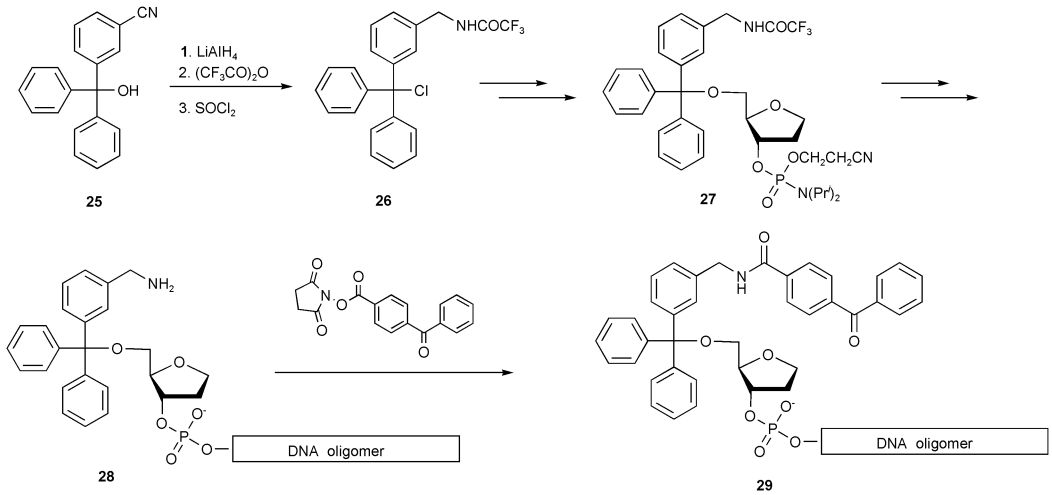
Scheme 5

synthons **19** and **24**. The anchoring to CPG support **24** was achieved utilising a *p*-nitrophenyl-activated acid cleavable cross-linker **23**, synthesised by method **b**(Scheme 2). Triarylmethane **22**, synthesised from aldehyde **21**, was activated, oxidized, and chlorinated to give **23**. A multiselective deprotection scheme allows for simultaneous combinatorial synthesis of oligonucleotides with different sequences.

Attachment of benzophenone to a trityl moiety was also reported,[13] by reduction and further transformations of *meta* cyano tritanol **25**, through the protected block **26** into a phosporamidite **27** (Scheme 7). This was incorporated into synthetic oligonucleotides according to standard protocols and the resulting amino-modified oligonucleotide **28** labelled with an activated carboxy derivative of benzophenone to give **29**. The conjugates were formed due to either hydrophobic interactions between the tritylated oligonucleotide and albumin, or photocross-linking between the protein and the *p*-benzoylbenzoic tag **29**. This benzophenone photophore can be manipu-



Scheme 6



Scheme 7

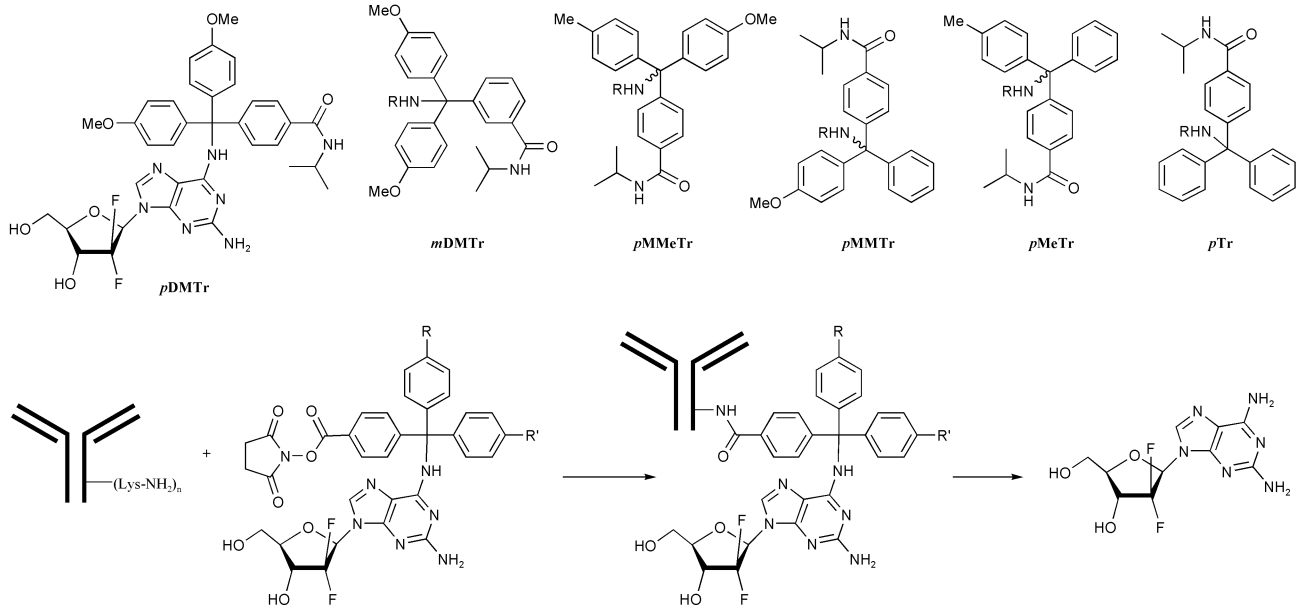
lated using ambient light and can be activated at wavelengths that cause little damage to proteins, and the efficiency of cross-linking is one of the highest known.

The concept of bifunctional trityls was employed in the synthesis of nucleoside prodrugs activatable *in vivo*. In this case, acidic lability of the trityl group was well suited for the increased acidity of most of the tumours and their environments relative to normal tissues (usually, the pH is about 0.8 units lower). Deoxydifluororibofuranosyl purines (DFP) represent a novel family of nucleoside antimetabolites with good *in vivo* antitumor activity. Unfortunately, some drug-related cardiotoxicity was also revealed. To achieve site-selective delivery, an inactive prodrug activatable in acidic media has been described.[14] Several 6-amino-tritylated 2,6-diaminopurine nucleoside prodrugs (Scheme 8) were prepared bearing trityl groups with different substituents, and the rates of acidic hydrolysis were found to be: *p*DMTr ~ *m*DMTr > *p*MMeTr = *p*MMTr >> *p*MeTr >> *p*Tr, which is consistent with the electronic arguments. These do not, however, completely correlate with the calculated charge on the presumed intermediate trityl cation, suggesting that the hydrolysis of trityl groups is also influenced by factors such as ionic strength, counterions, concentration, temperature *etc*.[14c] Interestingly, tritylation of a free nucleoside by a mixture of the corresponding trityl chloride and nBu$_4$NClO$_4$ proceeded regioselectively with no 5′- or N^2 products detected. Racemic trityls gave inseparable diastereoisomers. Some other oncolytics were also tritylated in a similar fashion.

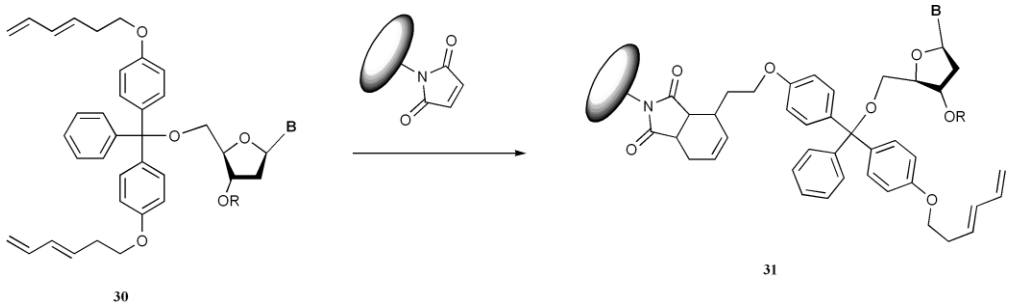
Rather than temporarily blocking the activity (and toxicity) of a compound until it gets to its destination point, the trityl can serve as a linker between the drug and a moiety that directs it to the target, such as a monoclonal antibody that recognises tumour-associated antigens. As was the case in a previous example, a strong correlation was found between the cytotoxicity of the drug and the lability of the trityl group.

A trityl group which can covalently react with a derivatized resin, membrane or soluble polymer support can be useful for anchoring the intermediates or full length products during oligonucleotide synthesis in solution, thus allowing for a larger scale synthesis. The 3′- or 5′-derivatives of di-(3,5-hexadienoxy)trityl nucleoside phosphoramidites or H-phosphonates (**30**;Scheme 9) as well as 2,4-hexadienoxytrityl derivatives (not shown) can be efficiently trapped onto solid supports bearing activated dienophiles utilising Diels–Alder cycloaddition reaction, which goes to completion in 3 h for **31** and is somewhat slower for the 2,4-derivative (24 h). The diene modified trityl group is assembled from the alkylation of dihydroxybenzophenone with 3,5-hexadienol, followed by a Grignard reaction.[15]

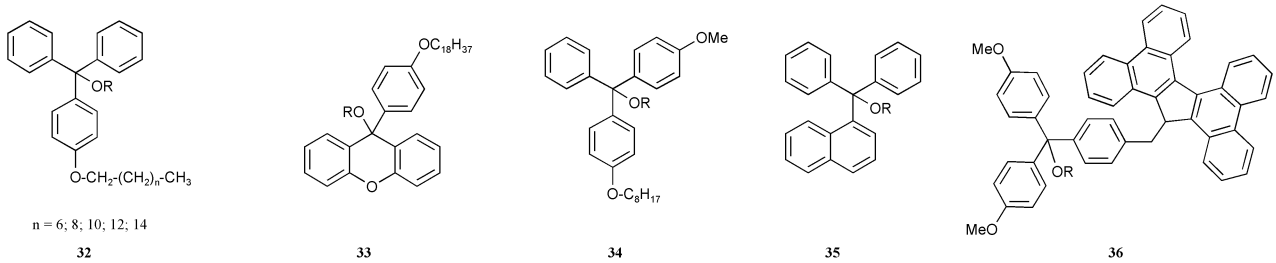
In modern oligonucleotide synthesis, hydrophobicity is routinely harnessed to assist in HPLC separation of the final length oligonucleotides from truncated failure sequences which are capped and therefore lack the terminal DMTr group, which decreases their retention time. To increase the lipophilicity, and hence the retention time for the full length sequences, thus improving their separation, a series of more hydrophobic MMTr-derivatives **32**[16] and a hydrophobic Px derivative **33**[17] were prepared and used for 5′-protection. To reduce the degree of depurination while removing **32** from purified oligos, a more labile analogue **34** was also prepared.[18] A different aryl group rather than an aliphatic carbon chain was used to produce a more hydrophobic analogue **35**,[19] followed by a 4-(17-tetrabenzo[a,c,g,i]fluorenylmethyl)-4′,4″-DMTr group **36**,[20] which, according to the authors, is twice as hydrophobic as DMTr in terms of retention time values. For some applications, a simple increase in the amount of DMTr groups per oligonucleotide leads to the same increase in the retention time.[21]



Scheme 8



Scheme 9



2.2 Applications of trityls in mass-spectrometry

The importance of mass-spectrometry has risen substantially in recent years, mainly due to the increasing demand from newly-born research fields such as combinatorial chemistry, genomics and proteomics. The main requirement of mass-spectrometric analysis, that the analyte be easily ionisable, makes highly stabilized (and therefore easy to form) trityl cations a valuable tool in mass-spectrometry. High desorption properties of trityl-based compounds can be harnessed to good effect to give rise to several new types of useful mass-spectrometry-related products.

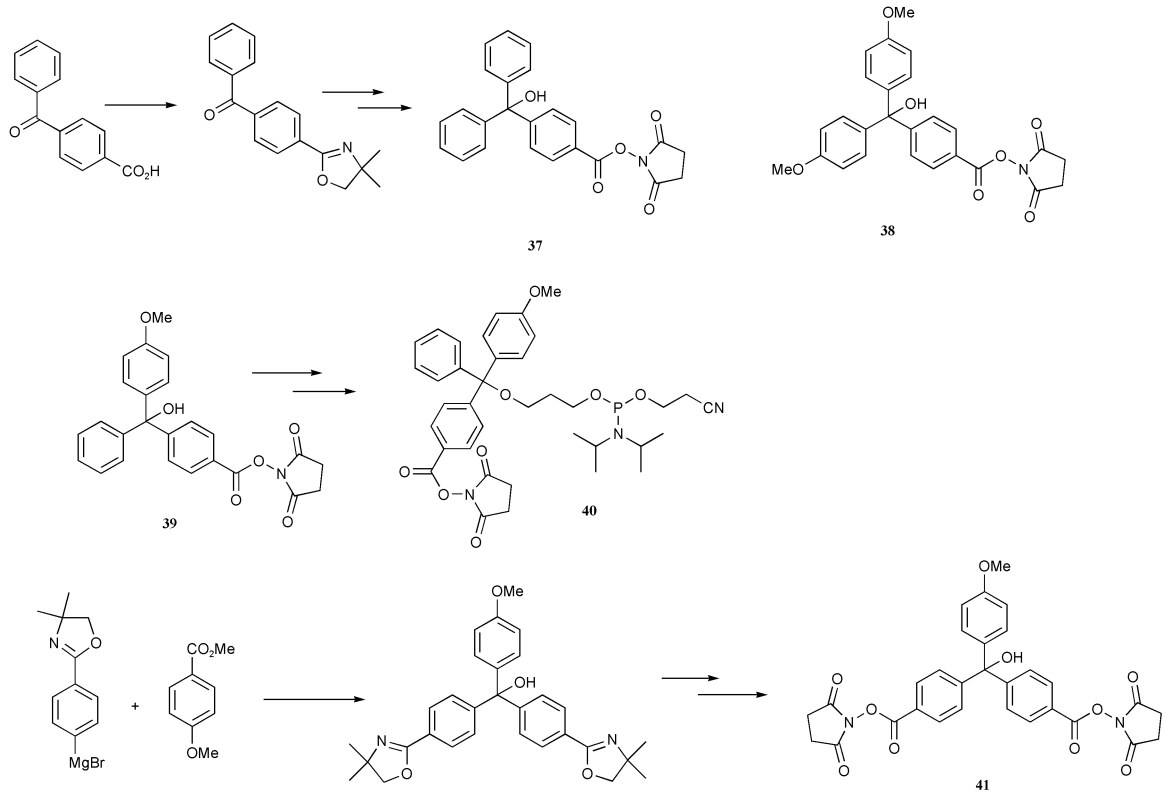
2.2.1 Trityl mass-tags: encoding of combinatorial libraries. The characteristic signal of the $DMTr^+$ cation (monoisotopic peak, exact mass 303.139 Da) is frequently present in mass-spectra of DMTr-containing compounds, suggesting that derivatives of trityl groups with different masses could serve as unique markers in combinatorial chemistry.

Two methods of combinatorial synthesis of large numbers of compounds on solid supports currently predominate: spatially addressable arrays (such as DNA chips), in which synthesis steps are performed simultaneously on sets of physically separated starting materials or areas; and bead libraries, consisting of mixtures of insoluble beads each of which carries a single compound, usually synthesized using the 'split-and-mix' method. Although bead libraries are quickly screened, their application is limited if the compound on a selected 'hit' bead cannot be readily identified.

Encoding the beads was suggested as a way around this, with the tags employed being identifiable by HPLC, GC, MS, IR, NMR. However, none of these can match mass-spectrometry in terms of the detection range and the speed of detection. With satisfactory resolution achievable for tags 4–5 Da apart, the easily available mass-range of 500–2500 Da would allow for a simultaneous analysis of 300–400 mass-tags.

Appropriately derivatised trityls, with the detection limits in lower femto- to upper atto-moles (determined by mass-spectrometric analysis of DMTr monolayers on glass: M. Shchepinov *et al*., unpublished) were prepared (Scheme 10) and used to encode for combinatorial oligonucleotide libraries.[22,23]

A series of pro-tags **37**, **38**, **39**, **41** (Scheme 10) all bearing an activated carboxy group were prepared according to methods ***a*** and ***f*** (from Scheme 2). Upon treatment with amines, they yield tags of different masses, which fly as cations.[22] No difference was detected for the signal intensity for tritanols as compared to the corresponding trityl ethers (or trityl chlorides) when using laser ionisation instead of acidic treatment, suggesting photocleavage by the laser irradiation as a good alternative to acidic cleavage. The bis-activated tritanol derivative **41** reacts with two amines, thus extending the mass-range covered. To incorporate the tags during the combinatorial oligonucleotide synthesis (for a detailed scheme, see refs. 22,24), a special phosphoramidite **40** was developed, which provides similar



Scheme 10

reactivity to the standard phosphoramidites. The molar ratio between **40** and a standard amidite it is premixed with in solution will therefore be retained on the solid support after condensation. Upon coupling, each batch of beads was subjected to treatment with a different amine. The mass-protag MMTr(NHS) (**39**) was employed, which is compatible with DMTr-based oligonucleotide chemistry because it is more than 50 times more stable to acid than the DMTr group. A combinatorial library of short oligonucleotides was manufactured on a four-column DNA synthesizer using split-and-mix strategy. The total number of tags required to encode all possible oligonucleotide 8-mers is 4 (four bases) × 8 = 32. A typical reading from a single bead (selected by hybridization with a labeled target), which can be translated into an oligonucleotide sequence, is shown on Fig. 1.

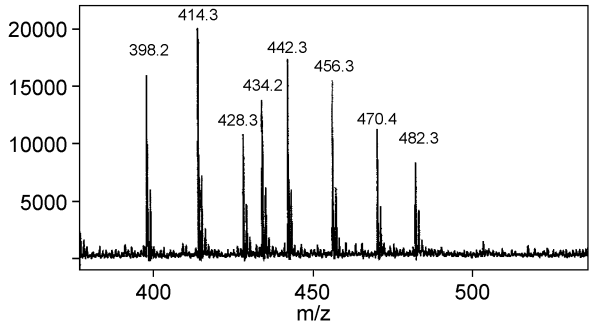


Fig. 1 LDI-TOF of a set of MMTr(amide)-based tags encoding for an 8-mer oligonucleotide on a single Rapp bead. (Reprinted from ref. 22 with permission from Elsevier Science.)

Trityl cations can be released by acidic treatment and detected by mass spectral analysis with or without matrix. Alternatively, the cations can be generated directly by laser irradiation,[22] which permits direct detection of tagged DNA on surfaces, for example, when hybridised at different positions of a DNA chip.

Rather than being introduced through a combinatorial process, the trityl tags may also be used for post-synthetic (non-combinatorial) labeling of oligonucleotides or indeed any other (bio)molecules for their subsequent highly sensitive detection by mass-spectrometry. This way, the tags may be conjugated with compounds whose synthesis is not compatible with trityl group (*i.e.* involves strong acids).

Similar trityl pro-tag-based reagents can also be employed in combinatorial peptide synthesis.[24]

2.2.2 Trityl mass-tags as 'trityl ladders': calibration of mass-spectrometers and high precision mass measurements. It has become a standard practice in modern organic chemistry to mass-spectrometrically characterise new compounds with the precision of at least 5 parts per million, which for a compound with the mass of a few hundred Da would mean a complete match between theoretically calculated and observed masses for at least two digits after the decimal point. To achieve that degree of precision, one needs mass-markers which possess a very high desorption rate (in other words, fly well), and can potentially cover a long range of masses. The peptide-based mass-markers, oligonucleotides or dextrane derivatives presently used possess neither of these two properties to the extent desired.

Trityl mass-markers with unique masses are easy to make, just by treating activated trityl blocks like Tr(NHS) (**37**), MMTr(NHS) (**39**), DMTr(NHS) (**38**) and MMTr(2NHS) (**41**) with appropriate amines. The exact masses for compounds with MW of 350–800 Da can be routinely measured with a precision of 0.5–1 ppm using trityl mass-tags as markers, whereas with standard peptides the precision is usually 5–7 ppm.[24] Thus, trityl-based mass-tags are a mass-spec equivalent of DNA/RNA ladders, routinely used in gel electrophoresis. The calibration curve for the majority of modern mass-spectrometers is not linear. It is therefore highly desirable to have more than two mass-markers in the same experiment.

At the same time, one wouldn't want the markers to interfere with the analyte, decreasing its desorption rate. The mixtures of trityl-based tags ('trityl ladders'), which possess an almost uniform desorption rate regardless of the amine used and can be detected at a very low concentration, seem to be the ideal choice (Fig. 2).

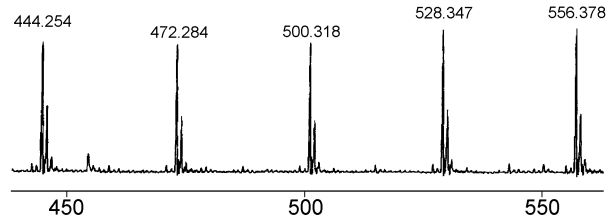


Fig. 2 An example of a 'Trityl Ladder' produced by reacting DMTr(NHS) pro-tag **38** with amines containing odd numbers of carbons (heptylamine (444), nonylamine (472), undecylamine (500), tridecylamine (528) and pentadecylamine (556)). (From ref. 24.)

2.2.3 Other mass-spec applications. Another potential application of activated trityl blocks can be to activate compounds which are usually difficult to analyse, like carbohydrates, which will increase their desorption rate and will make it easier to identify them mass-spectrometrically. Since they are superior to tertiary ammonium salts, trityl groups bearing a reactive function on a side-chain would be ideal for 'uniforming' signal intensities of complex mixtures with applications in such fields as genomics and proteomics.

It was found that tritanols do not require matrix for matrix assisted laser desorption ionisation mass-spectrometrical (MALDI MS) detection,[22] and therefore the abbreviation for the technique can be reduced to LDI MS. Direct ionisation of a trityl by a laser shot also does not require acidic treatment and is therefore technologically beneficial. The major signals present in the mass spectra originate from the corresponding trityl cations. This demonstrates that laser light used in modern MALDI-TOF mass-spectrometers (340 nm) causes heterolytic scission of the tertiary carbon–oxygen (or carbon–nitrogen) bond, liberating trityl cations that fly in the electromagnetic field. The photochemical ionisation has been studied for some triarylmethyl cyanides,[3,25] but in this case cations were stabilised with two *p*-dimethylamino groups. Apparently, the increase in absorbance of aromatics makes trityl compounds more susceptible to photoionisation. For example, for 9-phenylxanthen-9-yl (pixyl, Px)[26] derivatives, both alcohol[27] and ethers[28] are photoreactive (very recently, substituted thioanalogues, 9-phenyl-2,7-dibromo- and 9-phenyl-3-methoxythioxanthe ethers were suggested as superior photocleavable 5′-O-protecting groups for nucleosides in oligonucleotide synthesis[28*b*]). Indeed, compounds **42**–**45** containing highly absorptive residues of polycyclic aromatic hydrocarbons gave distinct $[M - OH]^+$ peaks in LDI mass spectra on ionisation with 340 nm laser.[29] Interestingly, the $[M - 2OH]^+$ (and not the expected $[M - 2OH]^{2+}$) peak was detected for compound **45**, thus suggesting possible formation of a monocation-monoradical from **45**.

2.3 Modified trityls: colour and fluorescence

Trityl cations (triarylmethinium ions) have strong absorbance in the visible wavelength range and are thus coloured. Anything

from ceric sulfate spray with subsequent heating, for more stable tritanols and trityl ethers, to simple exposure to volatile acid vapours, for less stable ones, produces bright coloured spots on the TLC plate, simplifying detection.

The DMTr cation absorbance ($\varepsilon_{498\ nm}$ = 68 000 to 76 000) is routinely used for loading measurement of solid support for oligonucleotide synthesis with the first attached nucleoside as well as for stepwise yield monitoring in the course of nucleotide chain assembly.[30] Interestingly, a 'sulfuric analogue' of a $DMTr^+$ cation, where both oxygens are replaced by sulfur, is somewhat less stabilized, but thioethers exert a much stronger bathochromic effect on the UV/Vis spectrum.[31] Coloured cations from various trityls were used for encoding in oligonucleotide synthesis.[32] Each triarylmethyl group was used for 5′-*O* protection of a particular nucleoside in solid phase procedure. The colours of cations in nitromethane solution are given below.

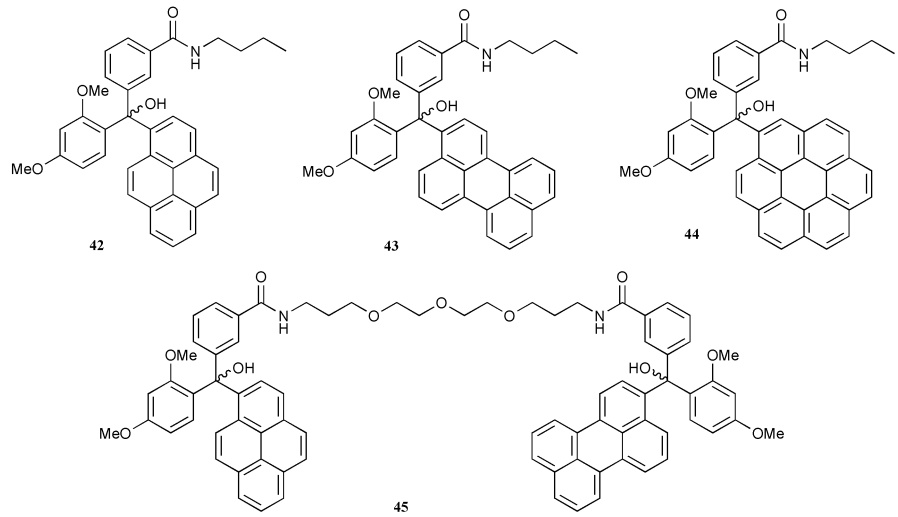
The latter work was ahead of its time because there turned out to be no need to separately monitor the couplings of four different bases during routine oligonucleotide synthesis, since all four would give nearly quantitative yields. The situation changed recently, however, when combinatorial synthesis required the development of new encoding techniques.

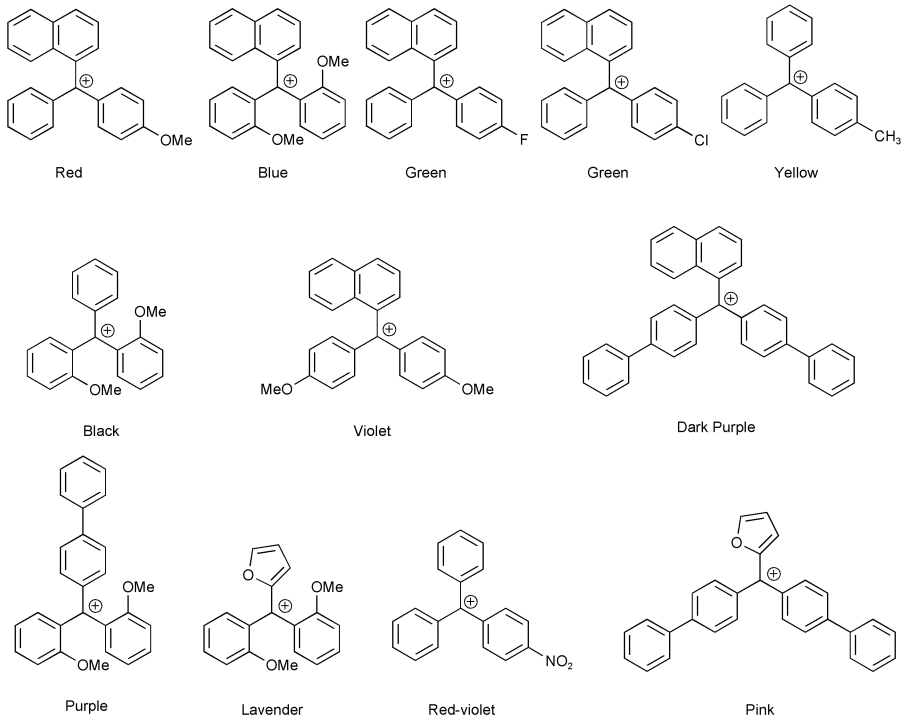
The large value of the extinction coefficient of the DMTr cation is utilised for quantification of amino groups on solid supports. The commercially available (Pierce) water-soluble sulfo form of NHS-activated butyric acid bearing the DMTr moiety **46** reacts with amino groups on the surface, with subsequent spectrophotometric detection of $DMTr^+$. Isothiocyanate **47** is also used as an amine-reactive reagent.[33]

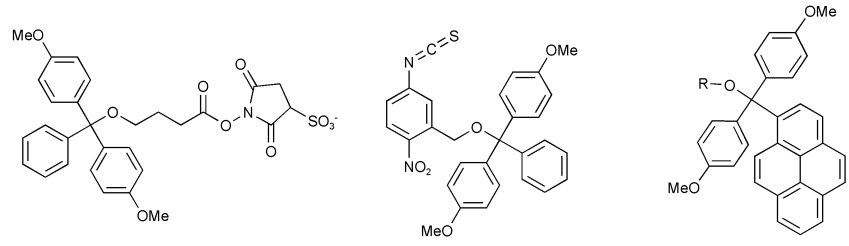
The use of a DMT derivative of levulinyl protecting group[9] for colorimetric detection has already been mentioned (Scheme 6).

The first use of fluorescent molecules as a part of a triarylmethyl protecting group was the synthesis of a modified DMTr group bearing a pyrenyl residue in place of phenyl. Ethers **48** can be detected by fluorescence (down to 10^{-10} M) and their stability is similar to that of DMT derivatives.[34]

Fluorescent trityl derivatives **49**–**51** were prepared[29] from corresponding aryl-2,4-dimethoxyphenyl ketones by a reaction sequence similar to that shown in Scheme 4. These reagents, containing the fluorescent polycyclic aromatic hydrocarbons pyrene, perylene, and coronene, are suitable for derivatisation of aliphatic amino groups, *e.g.* in biomolecules and on solid surfaces. The compounds in the form of triarylmethanols are highly fluorescent, but in the form of triaryl cations are not. The







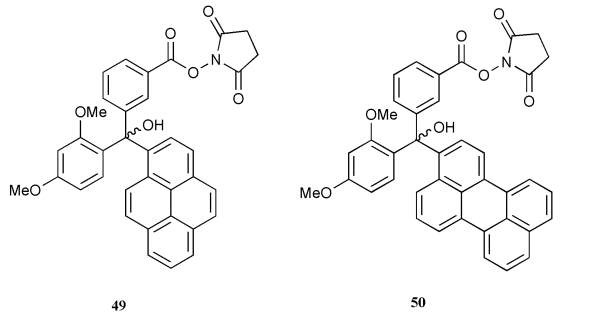
46 47 48

fluorescence can be switched off by acid treatment, and restored with alkali. The pH-threshold for the ionisation depends on the nature of the hydrocarbons and differs slightly between these compounds. A triarylmethyl cation derived from tritanol by an acidic treatment still remains covalently linked to a probe molecule if attached to it through a side-chain, thus representing a new family of switchable fluorescent labels for amino group(s)-containing analyte.

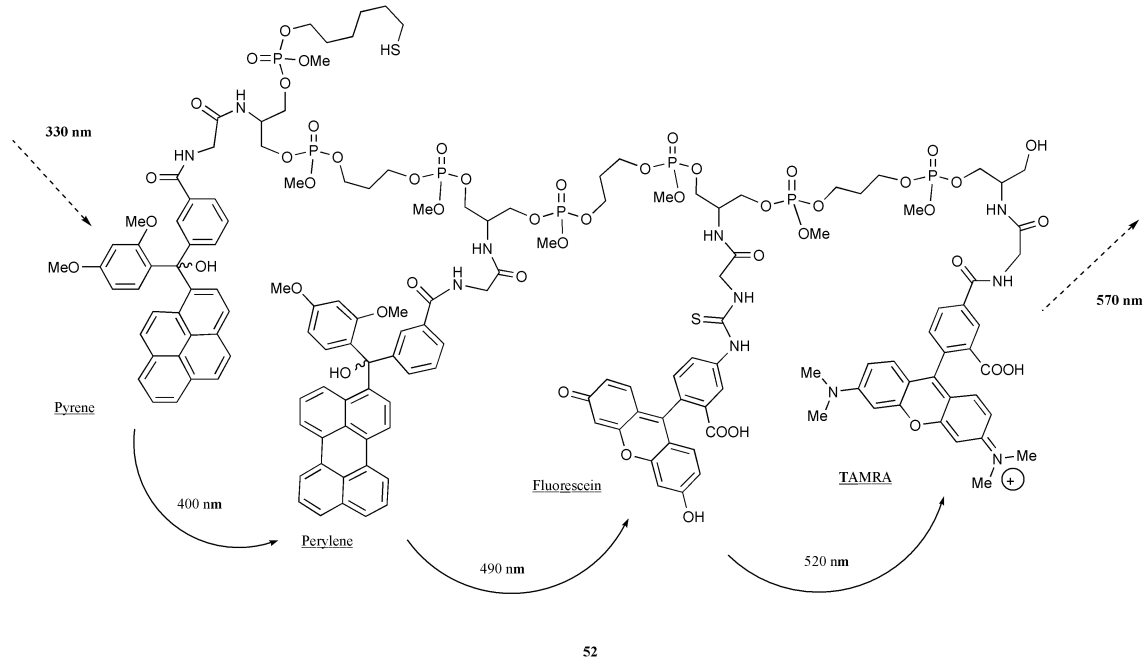
This switcheable fluorescence can be used to improve the discrimination of labels: first, by increasing the accuracy of the intensity measurements, and second, by increasing the potential number of colours in the palette. For example, targets can be labelled with two fluorophores having similar excitation 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 the 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.

Fluorescent residues in modified trityls are suitable for many common applications, *e.g.* fluorescence resonance energy transfer (FRET). The fluorescent spectrum of the model compound **45**[29] in dichloromethane shows only perylene fluorescence upon pyrene excitation, confirming an efficient energy transfer from pyrene to perylene (whereas 1 : 1 mixture of model butylamides **42** and **43** shows superposition of the pyrene and perylene fluorescence).

Reagents **49** and **50** were used in the assembly of system **52** for the demonstration of a multiple FRET process.[35] Sequential energy transfer across all four chromophores was observed. This structure, which is an example of the stepwise energy transfer, taking place between more than two participating chromophores, also allows for an additional degree of control over the process. Indeed, if one of the FRET-participating residues is based on a trityl structure, then there is a possibility of reversibly switching it off by temporarily cationizing that



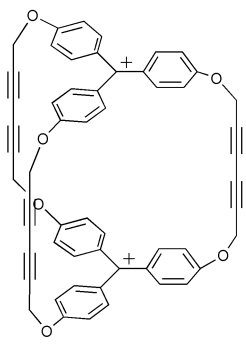
49 50 51



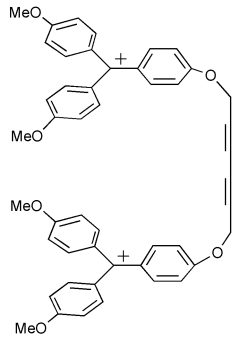
52

residue (such as pyrene or perylene in **52**). This will stop (or start) the FRET at certain point. The process may find applications in nanotechnology and the light-harvesting systems based on dendrimers, allowing one to switch between different incoming or outcoming 'power lines' while focusing energy on a particular residue such as azobenzene.[36]

Interestingly, there was no energy transfer detected when no propanediol linkers were placed between each pair of fluorophores, suggesting a need for a certain degree of freedom of the fluorophores for the energy transfer to occur. These steric considerations are essential since trityls are relatively bulky structures. It is particularly important for cations, where all three rings should be in the same plane for a maximum resonance stabilization of the positively charged α-atom to occur. An increased stiffness of the cage may be responsible for the fact that 'triply bridged' cryptand-like triphenylmethyl dication **53** (prepared from Rosolic acid) differs in colour from its linear model compound **54** in $SbCl_5/CCl_4$,[37a] although a difference in alkoxy substituents as a cause for that colour change cannot be ruled out.

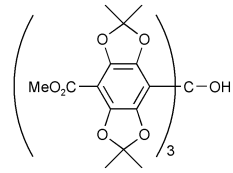


53

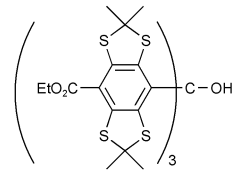


54

Steric hindrance is particularly pronounced in trityls having *ortho*- and/or *meta*- substituents in one or more rings. Recent 'extreme' examples include a hexa-isopropilidenyl derivative **55** and its thioanalog **56**, which both become water-soluble upon saponification of the ester groups,[37b] as well as tris(2,4,6-trichlorophenyl)methyl and tris(2,4,6-trichloro-3,5-dinitrophenyl)methyl compounds.[37c]



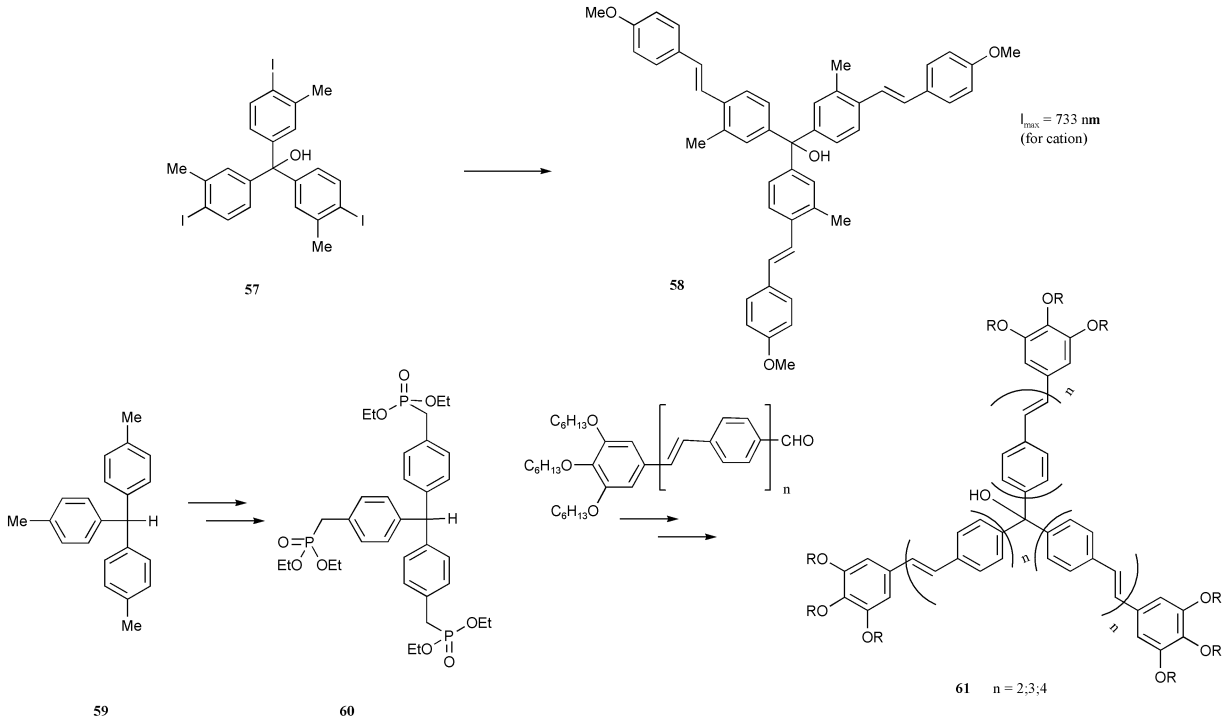
55



56

There is a need for materials possessing novel combinations of properties. For instance, near-infrared (NIR) absorbing dyes (λ_{max} > 700 nm) can potentially be used in optical imaging systems, thermal writing displays, infrared photography, as filters for NIR lasers, *etc*. Presently used compounds consist of strong donor and acceptor parts connected through conjugated chains, such as phthalocyanines or polymethines, which are rather unstable and expensive. Since carbocations are the strongest known acceptor groups, by extending their π-conjugation some existing triphenylmethane dyes can be converted into suitable candidates in a cheap and efficient way, for example,[38a] starting from Crystal Violet-derived **57** (Scheme 11). Such a substantial bathochromic shift (for Crystal Violet, λ_{max} = 588 nm) is caused by the extended π-conjugation. The principle of raising the bathochromic shift by increasing the level of conjugation was extended in **61**,[39] where up to 3 phenylenevinylene units were attached to each arm of a trityl core. Interestingly, the product of the Wittig–Horner reaction between *tris*-diethoxyphosphorylmethyl block **60**, which was obtained from **59**, and an aldehyde was readily oxidized into corresponding carbinol **61** in the presence of air. The tetrafluoroborate salts of newly synthesised trityls were obtained by treating the tritanols with HBF_4 with subsequent precipitation from acetic anhydride. The hexyloxy chains were used to enhance the solubility.

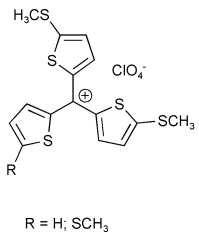
Other donors were also employed as terminal groups in trityl-based systems. Replacement of anisyls in **58** with 4-(ferrocenyl) residues gives a metallocene dye with λ_{max} = 1068 nm,[38b] whereas 4-(triphenylamine) groups[38c] produce slightly blue shifted NIR dyes (λ_{max} = 954 nm).



57 **58** **59** **60** **61**

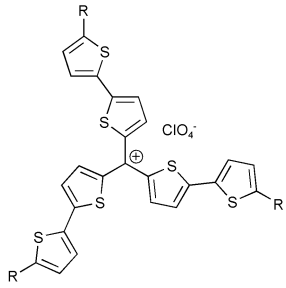
Scheme 11

For applications in non-linear optics and conducting polymers, series of thienylic and oligothienylic trityl analogues were prepared. Interestingly, there was almost no difference in absorbance for **62** (λ_{max} ~ 600 nm) regardless of the number of SMe (or even NMe_2) groups at 5-positions of thienyls,[40a] as one would expect from the substantial differences of their Hammett σ_x-values. However this correlates well with the earlier data on triphenylmethinium ions.[31]



R = H; SCH_3

62



R = H; SCH_3; SEt; SPr; SBu; 2-Thienyl

63

The attractive combination of second-order optical non-linearities (of octupolar origin) in combination with almost complete transparency in the useful (visible) frequency range for triarylmethyl cations, and interesting electrochemical properties of star-shaped conducting polymers (based on oligothienyls) which could be useful for a wide range of potential applications led to the development of oligothienylic trityls **63**.[40b,c] High off-resonant second-order non-linear coefficients have been reported, although further optimization of the donor and acceptor groups is needed. For the reasons currently being investigated,[40a] the reported absorbance data (λ_{max} ~ 350–370 nm for all substituents) differ substantially from the data obtained for **62**.

3 Conclusions

Novel technologies are being developed at the interface of traditional disciplines of science and engineering. The trityl group seems to possess several useful properties, which are all based on the fact that its cation is very efficiently stabilized. Its applications in the emerging fields may rely on the stability of the cationic form (useful in mass-spectrometry) and the very mild conditions of its formation (switchable fluorophores, cleavable conjugation reagents).

4 Acknowledgement

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5 References

1 M. Gomberg, *Ber.*, 1900, **33**, 3150.
2 (*a*) T. W. Greene and P. G. M. Wuts, *Protective Groups in Organic Synthesis*, 3rd Edition, Wiley, New York, 1999; (*b*) D. A. Stetsenko, E. N. Lubyako, V. K. Potapov, T. L. Azhikina and E. D. Sverdlov, *Tetrahedron Lett.*, 1996, **37**, 3571; (*c*) M. S. Shchepinov, S. C. Case-Green and E. M. Southern, *Nucl. Acids Res.*, 1997, **25**, 1155; (*d*) R. Schirrmacher, W. Hamkens, M. Piel, U. Schmitt, H. Lüddens, C. Hiemke and F. Rösch, *J. Labelled Cpd Radiopharm*, 2001, **44**, 627; (*e*) R. D. Egeland, F. Marken and E. M. Southern, *Analyt. Chem.*, 2002, **74**, 1590.
3 D. F. Duxbury, *Chem. Rev.*, 1993, **93**, 381.
4 Molecular Probes Catalog & Handbook, 9th Edn., 2002 (www.probes.com/handbook).
5 (*a*) J. Y. Wong, C. Manning and C. C. Leznoff, *Angew. Chem., Int. Ed. Engl.*, 1974, **13**, 666; (*b*) T. M. Fyles and C. C. Leznoff, *Can. J. Chem.*, 1976, **54**, 935; (*c*) C. C. Leznoff, *Acc. Chem. Res.*, 1978, **11**, 327.
6 J. M. J. Fréchet and L. J. Nuyens, *Can. J. Chem.*, 1976, **54**, 926.
7 J. Biernat, A. Wolter and H. Köster, *Tetrahedron Lett.*, 1983, **24**, 751.
8 B. D. Gildea, J. M. Coull and H. Köster, *Tetrahedron Lett.*, 1990, **31**, 7095.
9 H. Köster, S. Beck, J. M. Coull, T. Dunne, B. D. Gildea, C. Kissinger and Th. O'Keeffe, *Nucleic Acids Symp. Ser.*, 1991, **24**, 318.
10 E. Leikauf, F. Barnekow and H. Köster, *Tetrahedron*, 1995, **51**, 3793.
11 E. Leikauf and H. Köster, *Tetrahedron*, 1995, **51**, 5557.
12 E. Leikauf, F. Barnekow and H. Köster, *Tetrahedron*, 1996, **52**, 6913.
13 A. Bidaine, C. Berens and E. Sonveaux, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 1167.
14 (*a*) V. F. Patel, J. N. Hardin, J. J. Starling and J. M. Mastro, *Bioorg. Med. Chem. Lett.*, 1995, **5**, 507; (*b*) V. F. Patel, J. N. Hardin, G. B. Grindey and R. M. Schultz, *Bioorg. Med. Chem. Lett.*, 1995, **5**, 513; (*c*) V. F. Patel, J. N. Hardin, J. M. Mastro, K. L. Law, J. L. Zimmermann, W. J. Ehlhardt, J. M. Woodland and J. J. Starling, *Bioconjugate Chem.*, 1996, **7**, 497.
15 W. Pieken and L. Gold, 1999, *US Pat*. 6,001,966.
16 H.-H. Görtz and H. Seliger, *Angew. Chem., Int. Ed. Engl.*, 1981, **20**, 681; H. Seliger and H.-H. Görtz, *Angew. Chem., Int. Ed. Engl.*, 1981, **20**, 683; G. Schmidt, R. Schlenk and H. Seliger, *Nucleosides Nucleotides*, 1988, **7**, 795.
17 M. Kwiatkowski and J. B. Chattopadhyaya, *Nucl. Acids Symp. Ser.*, 1984, **14**, 299.
18 K. C. Gupta, R. K. Gaur and P. Sharma, *J. Chromatogr.*, 1991, **541**, 341; R. K. Gaur, *J. Chromatogr.*, 1991, **549**, 207.
19 R. L. Letsinger and J. L. Finnan, *J. Am. Chem. Soc.*, 1975, **97**, 7197.
20 R. Ramage and F. O. Wahl, *Tetrahedron Lett.*, 1993, **34,**, 7133; R. Ramage, A. R. Brown, C. McInnes, S. L. Irving, S. G. Love, T. D. Pallin, T. W. Muir, G. Raphy, K. Shaw, F. O. Wahl and J. Wilken, in: *Innovations and Perspectives in Solid Phase Synthesis, Peptides, Proteins and Nucleic Acids Biological and Biomedical Applications*, ed. R. Epton, Mayflower, Birmingham, 1994.
21 M. S. Shchepinov, I. A. Udalova, A. J. Bridgman and E. M. Southern, *Nucl. Acids Res.*, 1997, **25**, 4447.
22 M. S. Shchepinov, R. Chalk and E. M. Southern, *Tetrahedron*, 2000, **56**, 2713.
23 M. S. Shchepinov, R. Chalk and E. M. Southern, *Nucleic Acids Symp. Ser.*, 1999, **42**, 107; M. S. Shchepinov, R. Chalk and E. M. Southern, in: *Innovations and Perspectives in Solid Phase Synthesis, Peptides, Proteins and Nucleic Acids Biological and Biomedical Applications*, ed. R. Epton, Mayflower, Birmingham, 2001, 207.
24 M. S. Shchepinov, *Glen Report*, 2000, **13**, 4.
25 M. L. Herz, *J. Am. Chem. Soc.*, 1975, **97**, 7133.
26 J. B. Chattopadhyaya and C. B. Reese, *J. Chem. Soc., Chem. Commun.*, 1978, 639.
27 P. Wan, K. Yates and M. K. Boyd, *J. Org. Chem.*, 1985, **50**, 2881.
28 (*a*) A. Misetic and M. K. Boyd, *Tetrahedron Lett.*, 1998, **39**, 1653; (*b*) M. P. Coleman and M. K. Boyd, *J. Org. Chem.*, 2002, **67**, 7641.
29 M. S. Shchepinov, V. A. Korshun, R. D. Egeland and E. M. Southern, *Tetrahedron Lett.*, 2000, **41**, 4948.
30 M. J. Gait, *Oligonucleotide Synthesis: a Practical Approach*, IRL, Oxford, 1984.
31 R. Breslow, L. Kaplan and D. LaFollette, *J. Am. Chem. Soc.*, 1968, **90**, 4056.
32 E. F. Fisher and M. H. Caruthers, *Nucleic Acids Res.*, 1983, **11**, 1589.
33 S. S. Chu and S. H. Reich, *Bioorg. Med. Chem. Lett.*, 1995, **5**, 1053.
34 J. L. Fourrey, J. Varenne, C. Blonski, P. Dousset and D. Shire, *Tetrahedron Lett.*, 1987, **28**, 5157.
35 M. S. Shchepinov and V. A. Korshun, *Nucleosides Nucleotides Nucleic Acids*, 2001, **20**, 369.
36 D. M. Junge and D. V. McGrath, *Chem. Commun.*, 1997, 857.
37 (*a*) F. Vögtle, I. Michel, R. Berscheid, M. Nieger, K. Rissanen, S. Kotila, K. Airola, N. Armaroli, M. Maestri and V. Balzani, *Liebigs Ann.*, 1996, 1697; (*b*) T. J. Reddy, T. Iwama, H. J. Halpern and V. H. Rawal, *J. Org. Chem.*, 2001, **67**, 4635; (*c*) J. L. Torres, B. Varela, E. Brillas and L. Julia, *Chem. Commun.*, 2003, 74.
38 (*a*) S. Sengupta and S. K. Sadhukhan, *J. Chem. Soc., Perkin Trans. 1*, 2000, 4332; (*b*) S. Sengupta and S. K. Sadhukhan, *J. Mater. Chem.*, 2000, **10**, 1997; (*c*) S. Sengupta, *Tetrahedron Lett*, 2003, **44**, 307.
39 H. Meier and S. Kim, *Eur. J. Org. Chem.*, 2001, 1163.
40 (*a*) A. Noack and H. Hartmann, *Chem. Lett.*, 2002, 644; (*b*) F. Cherioux, L. Guyard and P. Audebert, *Adv. Mater.*, 1998, **10**, 1013; (*c*) S. Brasselet, F. Cherioux, P. Audebert and J. Zyss, *Chem. Mater.*, 1999, **11**, 1915.