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Conference paper

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Naphthalimides for labeling and sensing applications

Abstract: Naphthalimide has now become a class of most popular fluorophores for probe design, along with coumarin, fluorescein, rhodamine, BODIPY and cyanine. This account aims at the first-year graduate students as the primary audience and showcases the versatile design principles applicable to the naphthalimide fluorophore when designing a probe or label, with focused examples from the Qian research laboratory. We also provide a general synthetic scheme to naphthalimides of various substitution patterns.

Keywords: fluorescence; ICHC-24; internal charge transfer; naphthalimides; synthesis; photoinduced electron transfer; push-pull dyes.

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Introduction

China is estimated to share 12.6 % of the world's total recoverable coal reserve. She runs the largest coal industry in the world, and consumes more coal than the rest of the world in combination [1]. As a side product of the coal industry, ca. 9 million tons of coal tar is generated yearly. Coal tar is a rich source of polyaromatic hydrocarbons. Comprehensive exploitation of coal tar is both an economically viable and environmentally benign practice. Acenaphthene is a major chemical composition present in coal tar. The most important use of acenaphthene is oxidation to produce 1,8-naphthalic anhydride, a precursor for naphthalimide based textile dyes, optical brighteners and herbicides [2]. In this tutorial, we will present our progresses towards the use of fluorescent naphthalimides in the design of fluorescent probes for biological applications.

Fluorescence imaging is a powerful technique in trafficking bio-relevant analytes with high spatiotemporal resolution and has become a routine analytical method for modern biological studies. Development of fluorescent probes for such applications has been an ongoing effort of synthetic dye chemists in the past three decades. Fluorescent probes are typically constructed by tethering a receptor to a fluorophore (Fig. 1) [3]. Upon recognition of the analyte, a fluorescence signal is produced by the fluorophore indicative of this event in way of intensity fluctuation or wavelength shift, or both. The 1,8-naphthalimide is our keen favorite fluorophore for probe design mainly owing to its convenient derivatization and versatile fluorescence modulating mechanisms.

Dyes generally embody a conjugative backbone derivatized with electron donating and electron withdrawing groups. Absorption of a photon induces the flow of electron cloud cross the conjugative backbone, a process called photoinduced internal charge transfer (ICT). Upon absorption of a photon, the dye is promoted to a high energy state, called singlet electronically excited state. Dyes have a transient lifetime in the excited states, usually in the magnitude of nanosecond, prior to its deactivation to the ground state with concomitant dissipation of the excess energy. This energy may be lost radiatively in form of fluorescence emission, or nonradiatively as heat [4].

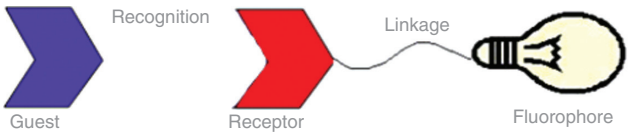


Fig. 1 A generic description of the design principle of optical probes.

Structure and fluorescence of naphthalimides

Wavelength of absorption is determined by the HOMO/LUMO band gap of a dye, which is profoundly influenced by the regiochemistry of the electron donating and withdrawing groups on its conjugative backbone. Typically, a dye will exhibit a longer wavelength absorption and higher extinction coefficient when its electron donating and withdrawing groups are in such a regiochemistry that allows efficient electron delocalization from the electron donating group to the electron withdrawing group. Such dyes are commonly referred to as push-conjugation-pull dye, or simply push-pull dye. The generic structure of 1,8-naphthalimide and related resonance structures (Fig. 2) are given below as a starting point to discuss the spectral and photophysical properties of differently substituted analogs (Table 1).

The imide moiety installed at the C-1 and -8 of the naphthalene core is strongly electron withdrawing and decreases the electron density at C-2, -4, -5 and -7, as exemplified by the resonance structures (Fig. 2). Therefore, attachment of an electron donating group such as NH_2 at those positions will facilitates the internal charge transfer (ICT) process and the resulting fluorophore will exhibit a longer-wavelength and often stronger absorption. Since the C-2 of the naphthalimide core could not be conveniently derivatized, the popularity of 4-aminonaphthalimide is warranted. 4-Hydroxynaphthalimide has found only occasional use in probe design, likely due to the pH sensitivity of its fluorescence at near physiological pH. An electron donating group at C-3 does not lead to as strong internal charge transfer (ICT) as at C-4. This offers qualitative explanations to the following three observations related to spectral and photophysical properties of 3- and 4-aminonaphthalimides (Table 1):

1. They display shorter absorption than 4-amino analogs;
2. Their fluorescence quantum yields are usually lower since the relatively localized electron density of 3-NH_2 quench the fluorescence of the naphthalimide core via the photoinduced electron transfer (PET) mechanism;
3. The higher electron density of 3-NH_2 results in stronger solvation cage in polar protic solvent, whose relaxation after photoexcitation is likely responsible for their redder emission.

Synthesis of naphthalimides

A focused synthetic scheme to naphthalimides of various substitution patterns, starting from acenaphthene is given below (Fig. 3). By a short cascade of electrophilic/nucleophilic aromatic substitutions, oxidation, reduction, and/or condensation, naphthalimides with monosubstitution at C-3, or C-4, or disubstitution at

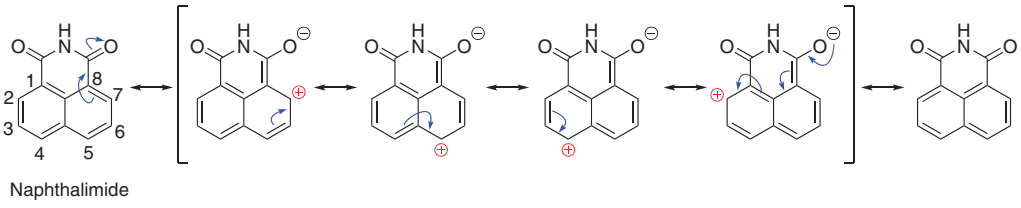
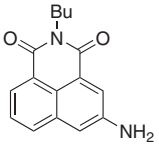
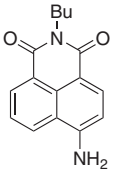


Fig. 2 Naphthalimide and its major contributing resonance structures.

Table 1 Fluorescence properties of 3-amino or 4-aminonaphthalimide [5].

Structure	λ_{abs} (nm)	λ_{abs} (nm)	ε (M^{-1} cm^{-1})	ϕ
	425	540	4.06×10^3	0.46
	434	522	6.33×10^3	0.64

Note: All data were collected in EtOH.

C-3,4, or C-4,5 could be accessed. General synthetic schemes for those compounds are described below and detailed synthetic procedures may be found in related literatures [6].

1. C-3 subsituted naphthalimides
   Industrial oxidation of acenaphthene (**1**) to naphthalic anhydride (**2**) is achieved by atmospheric oxygen with Co catalyst at elevated temperature. However, laboratory synthesis typically requires $Na_2Cr_2O_7$ or $K_2Cr_2O_7$. Further electrophilic aromatic substitution like nitration to (**3**) or halogenation occurs exclusively at C-3. Further condensation with primary amine generates various 3-nitronaphthalimides (**4**). Reduction of nitro group at C-3 by means of $SnCl_2$/HCl or Pd/C catalyzed hydrogenation gives 3-aminonaphthalimide (**5**). As indicated in Fig. 2, this $-NH_2$ is not strongly deactivated by the imido group and can be routinely acylated. Compound **5** may be exclusively halogenated or nitrated at C-4 for further derivatization.
   Compound **3** is commercially available at a reasonably affordable price from major vendors. Use of highly toxic and polluting chromate for oxidation could thus be avoided.
2. C-4 subsituted naphthalimides
   Electrophilic aromatic substitution should be done prior to oxidation of the ethylene group to modify the C-4 of acenaphthene (**1**). This is how compound **8** and **16** are synthesized. Compound **8** can condense with a primary amine to give the 4-nitronaphthalimides (**9**), which could further react with primary or second aliphalic amine to give compounds **10**. A reduction/condensation cascade yields compound **14**, the -NH_2 of which is strongly deactivated and cannot conveniently acylated or alkylated. The ortho position of the -NH_2 is activated for electrophilic aromatic substitution to compound **15**.
   Compound **8** is commercially available at a moderate price.
3. C-3,4 disubstituted naphthalimides
4. Haloacenaphthene (**11**) can be oxidized to **12**, which further condenses with amine to 4-halonaphthalimides (**17**). The halo group at the C-4 directs the nitration to occur to its ortho position to yield (**18**). The halo group in **18** is now synergetically activated by the imido and nitro group and can be easily displaced by oxygen, nitrogen or sulfur based nucleophiles. Then the nitro may be reduced to $-NH_2$ group.
5. 4-Bromo or 4-chloronaphthoic anhydride (**12**) is a very cheap reagent for this route.
6. C-4,5 disubstituted naphthalimides
   This series of compounds may be accessed from 4-haloacenaphthene (**11**) as well. Nitration of **11** furnishes **16**, which is then oxidized to 4-halo-5nitronaphthalimide (**21**). Condensation of **21** with primary usually can generate the desired imide cleanly under proper conditions to **22**. Undesired substitution of the nitro group may also occur. Substitution of **22** by amines displaces the NO_2 first to give **23**. Further displacement of the remaining halogen atom typically requires elevated temperature.
   This sequence should start from the commercial 4-haloacenaphthene (**11**).

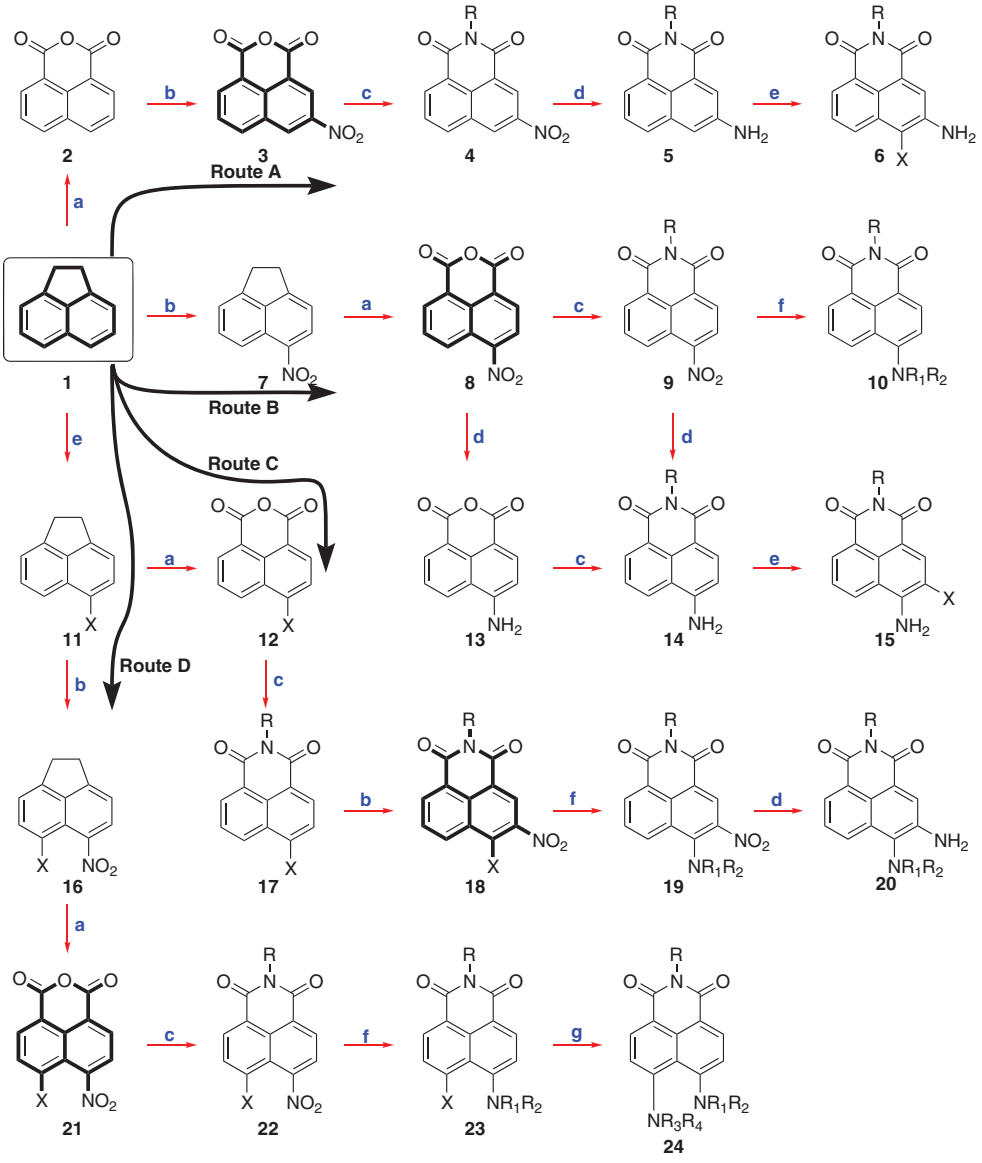


Fig. 3 (a) $Na_2Cr_2O_7$, AcOH, 75 °C; (b) H_2SO_4, HNO_3, 0 °C; (c) R-NH_2, EtOH, reflux; (d) $SnCl_2$, HCl, or Pd/C, H_2, MeOH, rt; (e) NBS, DMF, r.t. or Br_2, Et_2O; (f) HNR_1R_2, DMF or DMSO, rt; (g) HNR_3R_4, $CH_3OCH_2CH_2OH$, reflux. Note: R=alkyl; R_1, R_2, R_3 and R_4=H or alkyl; X=Cl or Br.

Probe/label design principles

Probe design via modification of –NH_2 at C-4

The fluorescence property of naphthalimide is very sensitive to the electron donating ability of the group attached to the C-4. A stronger push leads to a redder absorption/excitation maximum. Therefore, fluorescent probes may be designed by effectively modulating the electron density of the nitrogen atom at C-4 (Fig. 4). Ratiometric or dual-channel signaling may be achieved if the R group on the probe is not quenching, and the fluorescence brightness of both probe and product is comparable. For example, the probe (**25**) for hypoxia has an absorption/excitation maximum at 370 nm in neutral aqueous media [7]. Enzymatic and/or chemical reduction of –NO_2 to –NH_2 under hypoxic condition will trigger a cascade reaction to deprotect the –NH_2 of naphthalimide with a concomitant bathochromic shift in spectral properties (Fig. 5).

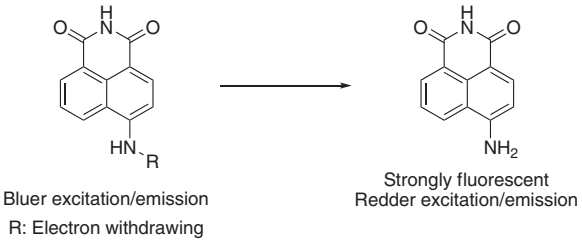


Fig. 4 General probe design by modulation of 4-NH_2 of naphthalimide.

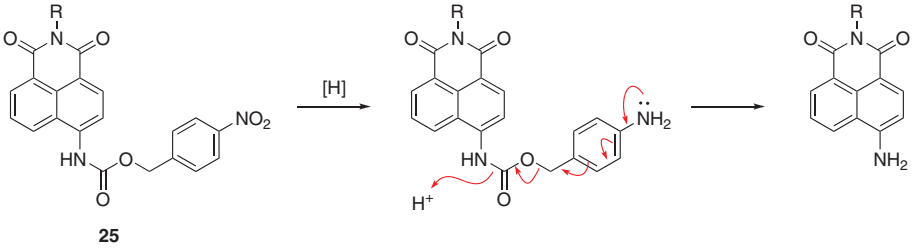


Fig. 5 Structure and detection mechanism of probe **25**.

In another example, the amino group in the probe **26** is converted into a much less electrondonating guanidinium nitrogen upon recognition of Hg^{2+} (Fig. 6) [8]. A gigantic hypsochromic shift in absorption/emission was observed.

Naphthalimides with two amino groups at C-4 and C-5 largely resembles the one chain analogs at C-4, except that their wavelengths are further red-shifted. They are particularly suitable scaffolds for probe design against various transition metal ions including Cu^{2+}, Zn^{2+}, Pd^{2+}, Cd^{2+} etc [9–12], (Fig. 7).

Probe design via modification of -NH_2 at C-3

Amino group at C-3 may also potentially be exploited for probe design in a similar fashion to the –NH_2 at C-4 (Fig. 8). However, development of ratiometric probes via this approach requires some luck because the fluorescence brightness of the probe and product are often mismatched.

For example, 3-aminonaphthalimides (**32** [13]) are moderately fluorescent at longer wavelength of 590 nm in aqueous media with a quantum yield of 0.075 (Fig. 9). Upon acylation, the fluorescence emission blue-shifted by near 70 nm and fluorescence intensity is enhanced by an impressive 16 fold. Such a high turn-on

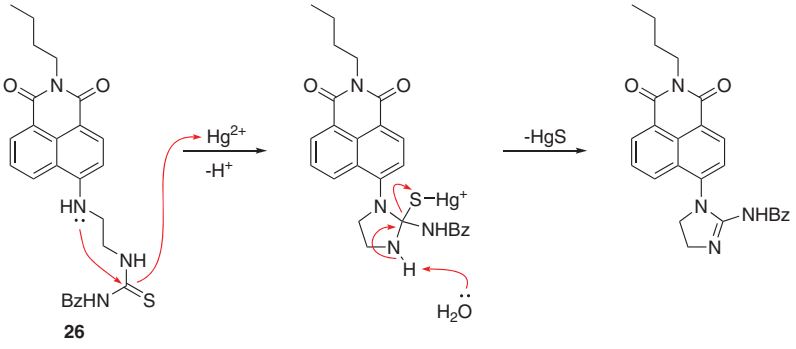


Fig. 6 Structure and detection mechanism of probe **26**.

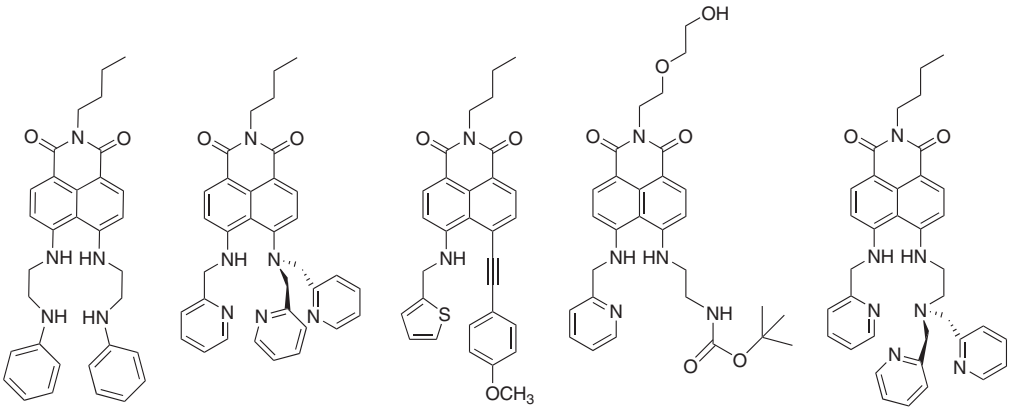


Fig. 7 Other probes of this type.

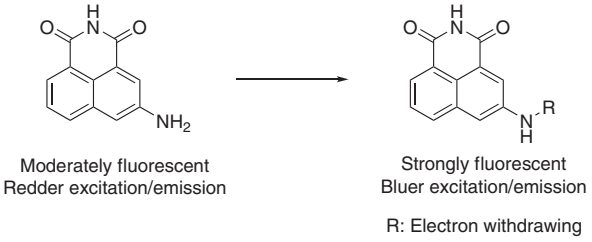


Fig. 8 General probe design by modulation of 3-NH_2 of naphthalimide.

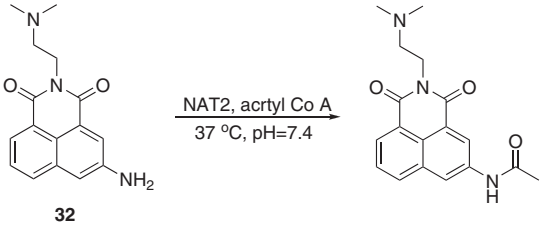


Fig. 9 Structure and detection scheme of probe **32**.

ratio is very advantageous to achieve high detection sensitivity in one hand. As exhibited by **32**, as low as 13 nM of NAT1 could be unambiguously detected.

In another probe (**33**) for hypochlorite [14], a deacylation is trigger by the recognition event (Fig. 10). It was expected to exemplify an emission increase at 570 nm and decrease at 460 nm. Intriguingly, emissions at both wavelengths were enhanced. This is because the probe emission was quenched by PET mechanism from the electron rich aniline moiety. Upon oxidation, both blue emitting **34** (or **35**) and long wavelength emitting **36** were generated, leading to the emission enhancement at both channels.

Probe design via fluorophore-linker-receptor principle

The photoinduced electron transfer (PET) mechanism is probably by far the most popular for designing a probe. It has a wide scope for both analyte and fluorophore. The general principle is that a fluorophore is

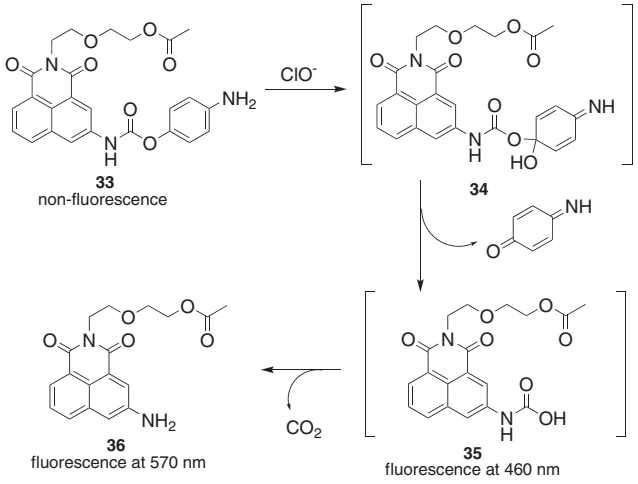


Fig. 10 Detection mechanism of probe 33.

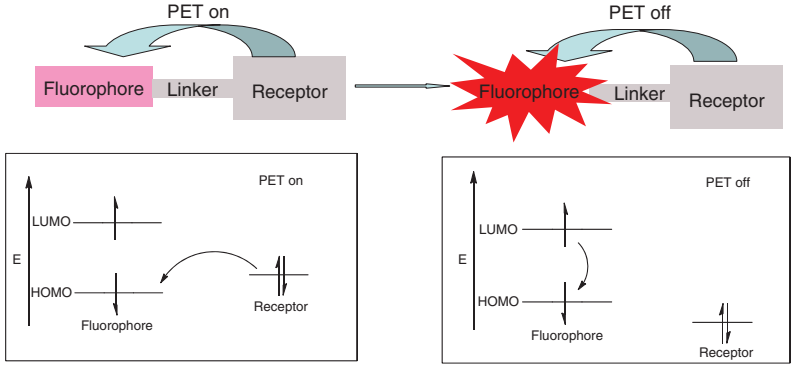


Fig. 11 General probe design by modulation of naphthalimide via PET mechanism.

attached to an electron rich receptor (Fig. 11). Upon photoexcitation of the fluorophore, an electron is promoted from the HOMO to the LUMO, yielding an oxidative "hole". If a pair of electron of higher energetic level is in proximity, an electron transfer from the lone pair to the "hole", a formal oxidation, will occur. This eliminates the radiative deactivation pathway of the excited state and hence the fluorescence emission. Upon recognition of the analyte, the high energetic level of the lone pair is lowered to an extent that the electron transfer can no longer occur to restore the fluorescence emission of the fluorophore.

We developed two Hg^{2+} sensors [15] (**37** and **38**, Fig. 12) by assembly of three fragments, 4-aminonaphthalimide fluorophore, an ethylene glycol chain and an ortho-diaminophenyl based receptor. Notably, the probe **38** exhibits a much lower basal fluorescence quantum yield (φ = 0.007) compared to that of 37 (φ = 0.032). This may be accounted for by considering the dipole moment of the 4-aminonaphthalide scaffold. When the direction of this dipole moment is aligned with the electron transfer process as in the probe **38**, the PET is enhanced and leads to better fluorescence quenching. Otherwise, they cancel out each other and lead to a compromised PET as in the probe **37**. We have also developed many other such probes [16], which will not be detailed here (Fig. 13).

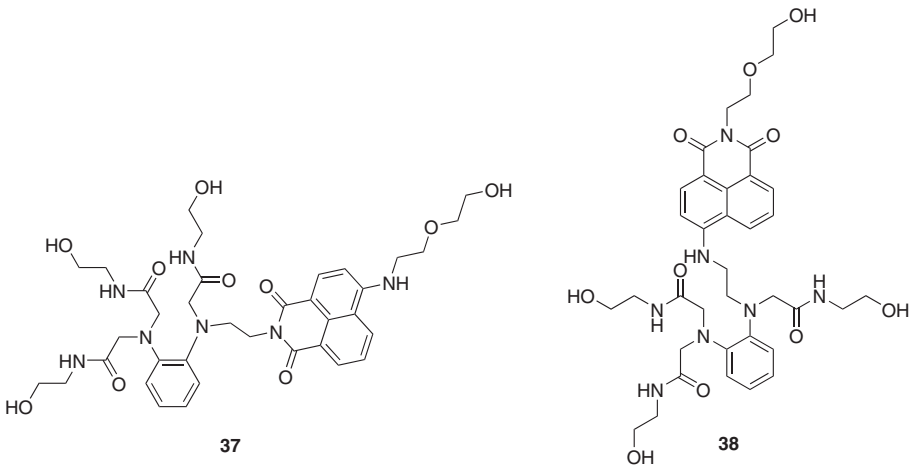


Fig. 12 Structure of probes **37** and **38**.

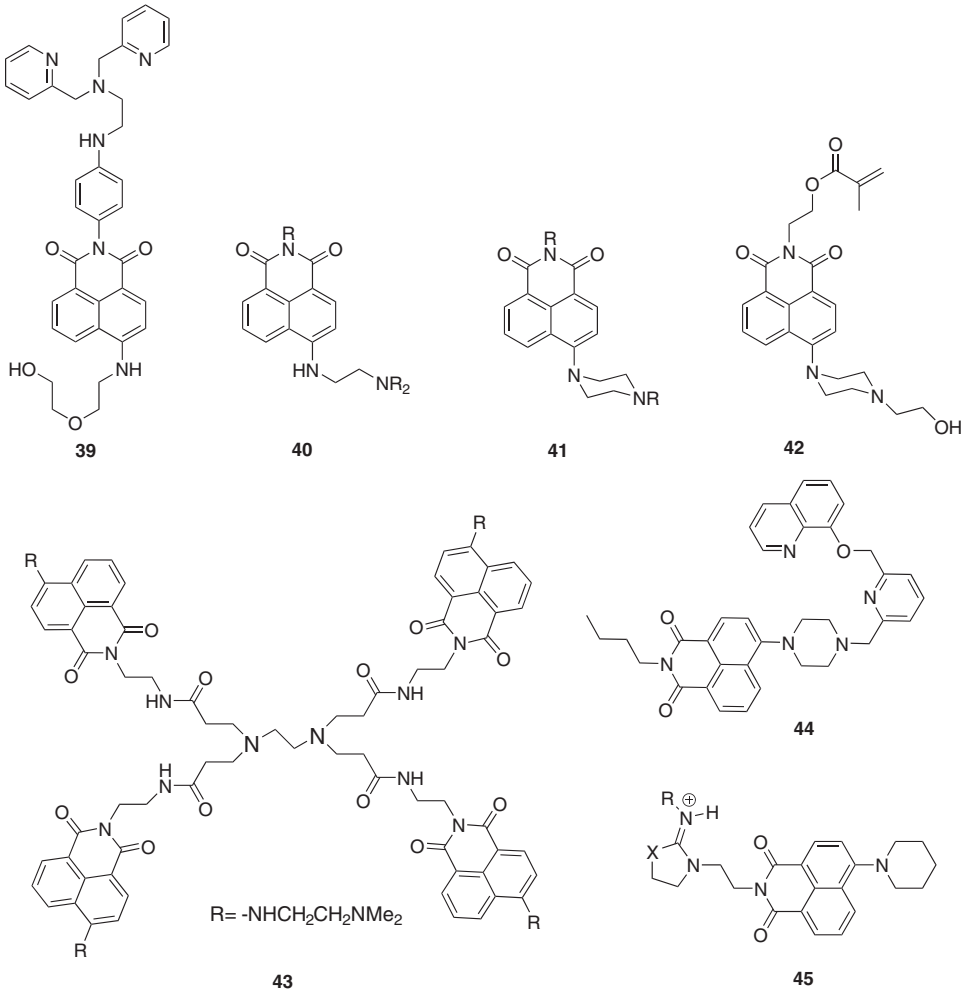


Fig. 13 Probes **39** [16a], **40** [16b], **41** [16b], **42** [16c], **43** [16d], **44** [16e] and **45** [16f].

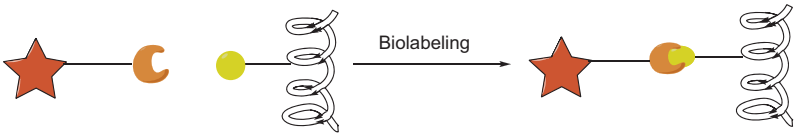


Fig. 14 Biolabeling scheme.

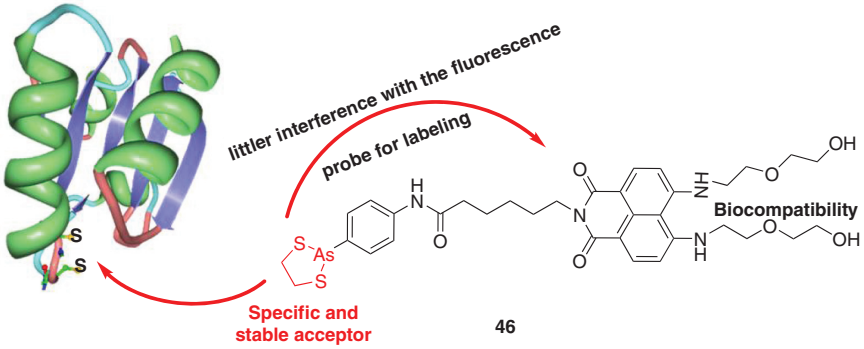


Fig. 15 Structure of scheme of labeling with probe **46**.

Naphthalimide as a fluorescent label

Labeling of a biomolecule necessitates the covalent attachment of the bimolecule of interest by a fluorophore, usually in the biological medium. Available chemistry include Huisgen cycloaddition [17], strain promoted cycloaddition [18], staudinger ligation [19], thiol-ene ligation [20], and more [21]. For such applications, change in fluorescence is not mandatory. Since the free probes could be washed out. However, if the labeling of the target molecule is accompanied by a fluorescence turn-on, efforts could be minimized since a stringent wash to eliminate the free probe is no longer necessary [22].

We developed a cyclic dithiaarsanes based vicinal dithiol containing proteins (Fig. 14) [23]. To modulate the lipo/hydrophilicity, two ethylene glycol chains were integrated. Since attachment of amino group at carbon 3 will quench the naphthalimide fluorescence via PET mechanism, two chains are installed at C-4 and -5, respectively. A relatively flexible and long alkyl chain was inserted between the ligation moiety and the fluorophore because the dithiaarsane is known to quench the fluorescence. The feasibility of this label for vicinal dithiol was demonstrated on a model thioredoxin protein (Fig. 15).

Summary

It is our hope that this account could provide a very necessary starting point for young graduate students who wish to build probes with naphthalimide, with focused examples from our own research group. And we sincerely apologize for not being able to cover all the other beautiful probes in the literatures.

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