RSC Advances

Cite this: *RSC Advances*, 2011, **1**, 573–575

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COMMUNICATION

Solid phase synthesis of ultra-photostable cyanine NIR dye library†

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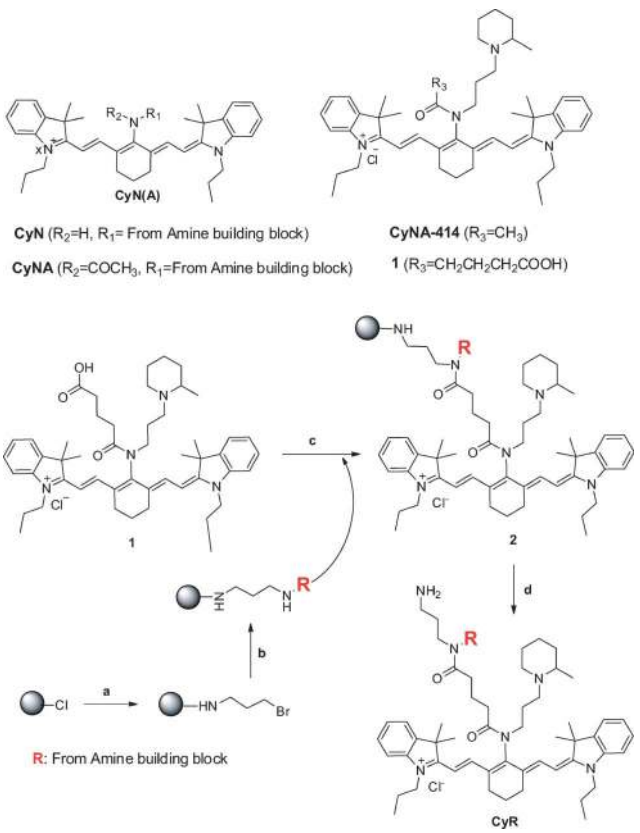
Received 21st July 2011, Accepted 21st July 2011
DOI: 10.1039/c1ra00498k

Photostability is one of the key issues in NIR dyes and we have previously reported a photostable CyNA library. Herein we report an ultra-photostable cyanine-based NIR fluorescence library, CyR, utilizing the stability component of CyNA. Efficient solid phase chemistry was also devised to provide a robust synthetic route to the new library.

Near-infrared (NIR) fluorescence (λ_{max}: 700–1000 nm) has recently received substantial attention in various chemical and biological studies.[1–2] The advantages of NIR fluorescence are the deep penetrating ability through tissue and the significant reduction of autofluorescence which often occurs in visible light emission. Therefore, NIR probes are extensively used in a broad range of biological research as *in vivo* imaging probes,[3] protein labelling agents[4] and fluorescence tags in DNA sequencing.[5] To date, a very limited number of scaffolds such as squarine,[6] quinone,[7] triphenylmethane[8] and cyanine[9] have been employed for organic NIR fluorophores. Among them, cyanine dyes[10] have attracted the most attention, due to their synthetic accessibility, broad wavelength tunability, and large molar extinction coefficient with moderate fluorescence quantum yields. However, the photostability of the dyes diminishes significantly along with the increase of the π-conjugation system. In particular, the photodegradation is a serious problem for the NIR cyanine dyes having absorbance λ_{max} longer than 700 nm.[11] To overcome this limitation, our group has recently developed a photostable NIR cyanine dye library **CyNA** in which the amine group of **CyN** was acetylated and thus the photoactivating lone pair electron was removed.[12] As a result, **CyNA** was much more stable than **CyN** in general, and **CyNA-414** was selected as the most photostable dye from the **CyNA** library (Scheme 1, upper). While a derivative of **CyNA-414** was proved to be superior to currently available commercial NIR dye in *in vivo* mouse imaging,[13] to further improve the photostability, we designed a new library utilizing the **CyNA-414** structure as the key component. Herein we report the synthesis of an ultra-photostable cyanine library (**CyR**) composed of 80 members (Scheme 1, lower) using a highly efficient and clean solid phase chemistry.

The previous **CyNA** library was synthesized by solution phase chemistry and the laborious purification step was unavoidable. Inspired by an efficient solid phase chemistry reported for cyanine dyes,[14] we designed a new solid phase route to secure a pure NIR cyanine dye library (**CyR**) in high speed manner by minimizing a crucial purification step. In order to synthesize the **CyR** library, firstly, 3-bromopropylamine was loaded to the 2-chlorotrityl resin quite efficiently, and then, a broad range of structural diversity was introduced through a series of primary amines (Chart 1). Here, the nucleophilic displacement of bromide occurred in *N*-methyl-2-pyrrolidone (NMP) solvent under moderate heating conditions, with



Scheme 1 Synthesis of **CyR** library. *Reagents and conditions*: (a) DIEA, THF, 3-bromopropylamine, r.t., 12 h; (b) DIEA, RNH_2, NMP, 70 °C, 12 h; (c) HATU, DIEA, r.t., 24 h; (d) 2% TFA in DCM , r.t., 10 min.

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† Electronic Supplementary Information (ESI) available: synthetic procedures and characterization data (NMR, HR-MS) for **CyR** derivatives; additional photostability data for the **CyN, CyNA** and **CyR** compounds and spectral data for **CyR** library. See DOI: 10.1039/c1ra00498k

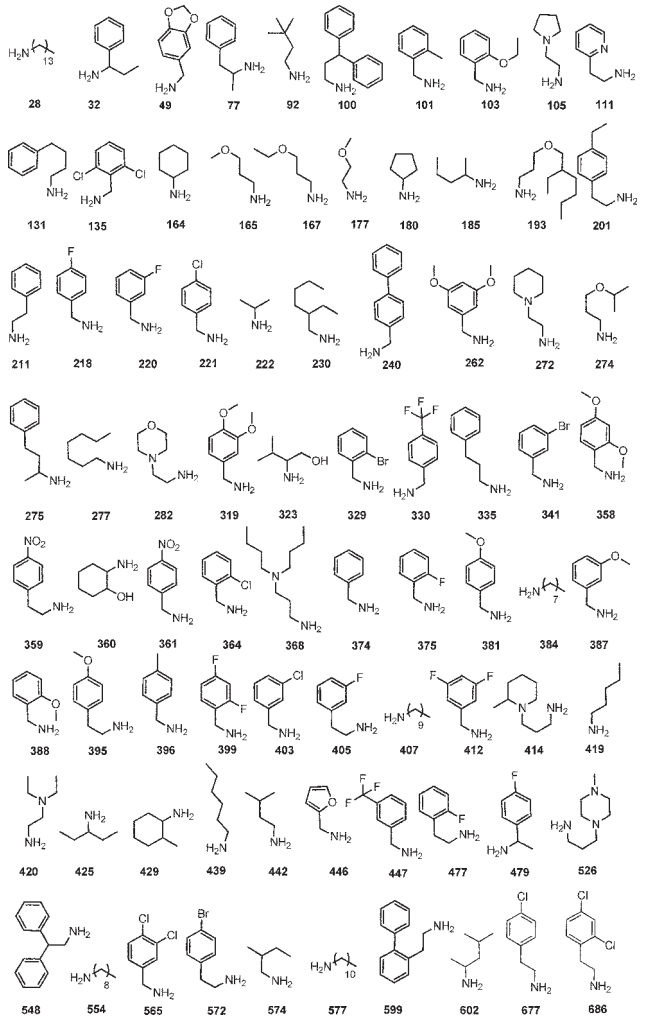

Chart 1 Amine building blocks of the **CyR** library.

primary amines to lead structurally diversified solid supported secondary amines. The key intermediate **1** (**CyNA-414** derivative)[13] was coupled to this solid phase amine building block by using a standard acid–amine coupling protocol.[15] The final step of acidic cleavage from the solid supported compounds **2** yielded 80 very pure members of the **CyR** library (average purity is 94% without further purification at 365 nm, Table S1†). It is noteworthy that the current synthetic protocol provides a linker motif with the amino group incorporated in the cyanine scaffold in addition to the structural diversity. The amino linker will be useful for further derivatization of the library compounds with various reporter or affinity tags for bio-conjugation depending on the biological study requirements. The photophysical properties of the 80 members of the **CyR** library were measured with the maximum absorption wavelengths from 806–807 nm and maximum emission wavelengths varying from 821 nm to 825 nm. The average quantum yield for the **CyR** compounds is around 10% in DMSO solution (Table S1†).

To evaluate the photostability of **CyR** in comparison with **CyNA** and **CyN**, we examined the decrease of fluorescence intensity of the compounds under a xenon lamp (6 W cm^{-2}) for 8 h. The average fluorescence intensity decreases of **CyNA** and **CyN** compounds[12] were around 17% and 70% respectively, whereas the newly

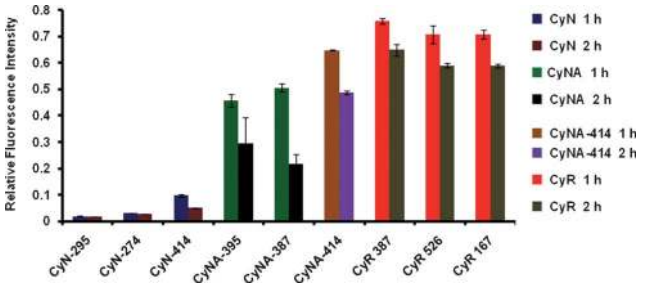

Fig. 1 Photostability assessment under strong UV irradiation in each 10 min interval for 2 h of **CyN, CyNA** (including **CyNA-414**) and 3 selected **CyR** compounds. 1% DMSO in 10 mM HEPES buffer (pH 7.4) was used to dissolve the compounds at a final concentration of 10 μM. The bar graphs exhibit mean fluorescence intensity after 1 h and 2 h. Values are means ± s.d. acquired from three experiments.

Table 1 Photodecomposition rate constant of three selected **CyR** compounds under the strong UV light

Compound	k (s^{-1})
CyNA-414	11.16×10^{-5}
CyR 167	8.16×10^{-5}
CyR 387	7.00×10^{-5}
CyR 526	8.33×10^{-5}

synthesized **CyR** library shows only 5% decrease on average (Table S1) reflecting the superior photostability in general. To differentiate the relative photostability among **CyR** compounds, we introduced a stronger light condition (UVP Blak-Ray® B-100AP high intensity mercury lamp, 100 W, 365 nm) to all the **CyR** compounds and monitored the relative fluorescence intensity (F/F_0) at 1 h and 2 h (Table S2†). Three most stable **CyR** compounds (**CyR 167**, **CyR 387**, **CyR 526**) were selected and further compared with representative **CyN** and **CyNA** compounds including **CyNA-414** (structures available in Tables S3, S4, S5†) and the results are summarized in Fig. 1. It clearly shows that the three selected **CyR** compounds exhibit much better photostability compared to **CyN** and **CyNA**, even the previous best compound **CyNA-414**. A further detailed kinetic analysis was performed for the three **CyR** dyes in comparison with **CyNA-414** and the kinetic constants were measured (Table 1, Fig. S1, S2, S3, S4†) showing the superior photostability of new **CyR** compounds to **CyNA-414**.

In summary, we designed and demonstrated an efficient solid phase synthesis of an ultra-photostable NIR fluorescence library, **CyR**. The reported synthetic procedure is quite straightforward and practical to be applied to much bigger library construction with almost no purification step required. Therefore, the **CyR** library and derivatives might be an extremely useful toolbox to develop novel *in vivo* bioimaging probes.

Acknowledgements

The authors are grateful to the intramural funding from A*STAR (Agency for Science, Technology and Research, Singapore) Biomedical Research Council and the National University of Singapore (NUS) for the financial support (Young Investigator Award: R-143-000-353-123).

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