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Efficient Synthesis of Fluorescent Alkynyl C-Nucleosides via Sonogashira Coupling for the Preparation of DNA-based Polyfluorophores†

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Abstract

A facile and general procedure for the preparation of alkynyl C-nucleosides with varied fluorophores is presented. Sonogashira coupling was used as a key reaction to conjugate the dyes to an easily accessible ethynyl functionalized deoxyribose derivative. The new C-nucleosides were used for the preparation of DNA-based polyfluorophores.

Graphical abstract

Introduction

Fluorescently modified DNA has been extensively studied over the past years. Currently, a plethora of dye-nucleic acid conjugates is routinely used to investigate the cellular distribution, abundance, and fate of DNA and/or RNA.^{1, 2} Fluorescent labels have been proven to be versatile tools, e.g. for DNA hybridization studies,³ DNA sequencing,⁴ DNA logic gates,^{5–7} and multiplexed super-resolution microscopy.^{8, 9} One particular example involves nucleic acids that bear multiple fluorophores.¹⁰ Such polyfluorophores are currently under investigation for a broad range of applications including artificial light harvesting complexes,^{11, 12} organic photovoltaic solar cells,¹³ and biological imaging.¹⁴

†Electronic Supplementary Information (ESI) available: Experimental and spectral data for all new compounds as well as details on the synthesis and screening of the combinatorial library. See DOI: 10.1039/x0xx00000x

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Previously we have reported short single-stranded DNA-based composite fluorophores in which nucleobases are replaced by diverse organic fluorophores. These assemblies of multiple dyes on a DNA backbone, which we termed oligodeoxyfluorosides (ODFs),¹⁴ offer several advantages compared to classical fluorescent dyes. The phosphodiester backbone holds the dyes in close proximity, facilitating strong intramolecular interactions (e.g. π -stacking). Concomitantly, the photophysical properties of ODFs are frequently characterized by multiple forms of energy transfer, including Förster resonance energy transfer (FRET), or excimer, exciplex and H dimer formation.¹⁵ This results in a wide variety of emission properties. ODFs can have large apparent Stokes shifts and typically contain at least one UV-absorbing dye. As a result, multi-coloured fluorescence images can be obtained simultaneously using a single excitation and a single long-pass emission filter.¹⁴ Moreover, their oligomeric nature allows them to be assembled directly on a DNA synthesizer in thousands of combinations. Because of their polyanionic backbone, ODFs generally have high solubility in aqueous media, which is ideal for biological applications. Furthermore, they have been shown to be cell-penetrable and possess high *in vivo* stability, making ODFs desirable probes for bioimaging.^{14, 15} ODFs have also been extensively applied for sensing of small molecules and as well as metal cations and inorganic anions.^{16–20}

ODFs have proven to be versatile labels and probes for numerous applications, however an efficient and modular access to the fluorophore building blocks is still lacking, and additional orange and red emitters also are needed for better coverage of the spectrum. Moreover, fluorescent nucleosides in general are broadly useful as tools in biotechnology and biomedicine.^{21, 22} Having convenient access to such building blocks is imperative. To date, the fluorescent monomers for the synthesis of ODFs were mainly obtained by nucleophilic substitution of Hoffer's chlorosugar²³ with different metallated aromatic dyes.²⁴ In that strategy, the fluorophores are directly attached to the anomeric carbon atom of the sugar. However, major drawbacks of that approach are the relatively low coupling yields,²⁵ the limited functional group compatibility, and the generation of mixtures of α - and β -anomers, which in some cases can be difficult to separate.

To circumvent these obstacles, we have now investigated whether ODFs that feature alkynyl-tethered fluorophores could be used instead. The Sonogashira cross-coupling has previously been shown to be a versatile reaction to link (hetero)aromatic compounds and dyes to an ethynylated sugar.^{26–28} To test the scope of the Sonogashira approach, several organic fluorophores with diverse absorption/emission spectra were chosen as coupling partners: 1-bromopyrene,^{28, 29} 3-bromoperylene,^{28, 29} a styryl-pyran dye,^{14, 30} a Nile red derivative,^{31–33} and two distyryl-BODIPY dyes.^{34, 35}

Results and discussion

Synthesis of fluorophore-labelled C-nucleosides

The ethynyl functionalized deoxyribose derivative **8**, which served as a central building block for the synthesis of all fluorophore-*C*-nucleosides,^{36, 37} was prepared by following previously reported procedures (Scheme 1).^{38–41} The α -anomer **8** is readily available on multi-gram scale as the major isomer from inexpensive 2-deoxy-D-ribose (**1**) through a 7 step procedure. In essence, the deoxyfuranose **1** was converted into the dibenzylated

derivative **4**. The alkynylated sugar **6** was obtained according to Adamo *et al.* by addition of ethynylmagnesium bromide to sugar **4** and subsequent ring closure with tosyl chloride.³⁹ Upon debenzylation and tritylation of the primary alcohol, alkyne **8** was isolated in high yield. This alkyne was successfully used for Sonogashira coupling with aromatic bromides, iodides, or triflates of various fluorophores (Scheme 2). Alkyne **8** was used in slight excess (1.5 equiv.) to compensate for oxidative Glaser homocoupling,³⁹ which could diminish the yield of the desired coupling product in the presence of traces of oxygen. The conjugates **9a–f** were obtained in good yields (50–86%). Subsequently, the phosphoramidites **10a–f** were isolated in excellent yields (83–98%) by following a standard phosphitylation procedure.⁴² The presented strategy allows for late-stage incorporation of the respective dyes minimizing losses that typically accumulate over the course of multi-step syntheses. This is particularly crucial if the fluorophores are not readily available. After the cross-coupling, the phosphoramidite derivatives can be accessed in one more step without additional protection group manipulation.

Synthesis of oligodeoxyfluorosides (ODFs)

The phosphoramidites **10a–f** were used for the synthesis of a one-bead-one-compound combinatorial library of tetrameric ODFs via standard split-and-pool procedure.²⁹ In addition to the fluorescently labelled building blocks, the abasic spacer monomer S was incorporated to allow for different intramolecular distances between the fluorophores. In total, the seven monomers gave rise to a library that theoretically contains 2401 different sequences. The library was synthesized on PEG-grafted polystyrene beads to enable screening under aqueous conditions. The screening was done with an epifluorescence microscope equipped with a single long-pass filter (cut-on wavelength 420 nm). Fluorescence images were taken with an excitation wavelength of 340–380 nm. The brightness of the beads varied significantly and a considerable part of the combinatorial library was completely nonfluorescent. Bright beads with diverse emission colours were selected and the respective ODF sequences were obtained by using previously reported electrophoretic tags,⁴³ which were installed during the library synthesis (see Supplementary Information).

Examination of the brightest bead sequences revealed some general trends. Most of the sequenced beads contained at least one pyrene monomer Y. As for previous combinatorial ODF libraries, pyrene serves as an antenna harvesting light in the UV region.⁴⁴ Additionally, pyrene is well known to form excimers/exciplexes with other fluorophores (e.g. another Y or E)^{45, 46} and it can also potentially function as a FRET donor for through-space energy transfer to an appropriate acceptor dye.^{47, 48} If the styryl-pyran dye K was incorporated, the respective ODFs showed yellow to orange emission. Beads with red emission were found rather frequently, but the brightness of those beads was typically limited. Those beads that displayed a more intense red colour were composed of ODFs sequences which included the Nile red monomer N as a key feature. However, ODF sequences with notable fluorescence that contained either the BODIPY monomer C or D could not be identified.

During the library screening, beads with interesting emission properties were selected, decoded and subsequently resynthesized to study their photophysical properties in more

detail. The synthesis was done on 3'-phosphate CPG to yield ODFs with an additional phosphate group at the 3' end to enhance water solubility. A polar propanediol appendix (monomer P, Fig. 1) was added to the 3' and 5' ends of the tetrameric ODFs to add charge and discourage hydrophobic self-association. The resynthesized ODF sequences were: PYYSSP, PSSYEP, PKYYYYP, PSKKKP, and PYYKNP. As with DNA, ODF sequences are named by using one-letter abbreviations (5' to 3' convention, Fig. 1).

Spectral properties

The spectroscopic properties of the resynthesized ODFs were compared with those of the *C*-nucleosides **9a–f** (Table 1 and 2, Fig. 2 and 3). The spectra of the *C*-nucleosides were recorded in organic solvents, and phosphate-buffered saline (PBS) was used for the ODFs. The absorption spectra of the *C*-nucleosides covered most of the visible electromagnetic spectrum and displayed medium to high quantum yields. Due to the presence of multiple chromophores, the absorption spectra of the ODFs were more complex as expected. However, the fluorescence spectra were typically dominated by a single emission band. Notably, the ODFs PYYSSP and PSSYEP showed very intense excimer and exciplex emission, with broad and unstructured emission bands around 500 nm. This is in contrast to the fluorescence spectra of the pyrenyl nucleoside **9a** and the perylenyl nucleoside **9b**, which display sharp emission lines around 380 and 460 nm. Similar findings were reported for the analogous β -anomers of the dimers YY and YE by Inouye *et al.*²⁸ The styryl-pyran-containing sequences PKYYYYP and PSKKKP had a broad emission band with a maximum around 600 nm. The fluorescence spectrum of the styryl-pyran triad PSKKKP was slightly bathochromically shifted as compared to PKYYYYP. The latter showed a similar styryl-pyran fluorescence and weak, narrow emission lines which are characteristic for monomeric pyrene. But interestingly, pyrene excimer emission could not be detected, which implies that its excitation energy is efficiently transferred to the styryl-pyran dye. Overall, the K-containing ODF in combination with Y monomers showed a large Stokes shift of 210 nm from the ~350 nm pyrene absorption band.

Surprisingly, the Nile red-containing ODF PYYKNP was barely fluorescent in PBS. Long-wavelength emission could only be detected when the ODF was directly excited at the Nile red absorption band (ca. 600 nm). Since Nile red is known to have strong solvatochromic behaviour,^{32, 49, 50} i.e. red-shifted absorption and emission and decreased quantum yield in polar solvents, the photophysical properties of this ODF were also investigated in methanol. The less polar organic solvent led to an increased brightness of the Nile red emission and also enabled energy transfer upon excitation around 325 nm. Yet the quantum yield did not exceed 1%. For comparison, the tetrameric ODF NSSS, bearing just one Nile Red monomer, was synthesized to examine how this fluorophore behaves in aqueous media, if it is not interacting with other dyes. Interestingly, the quantum yield reached almost 5% in PBS (see Supplementary Information, Table S1), which is noticeably higher than the quantum yield of PYYKNP.

Hence, the presence of the other dyes has a detrimental impact on the quantum yield of Nile red. We further hypothesize that the hydrophobic core of the polystyrene-based beads might have interacted with the Nile red moiety and thereby mitigated the polarity of the ODF

environment during the aqueous library screening conditions. Consequently, Nile red-containing sequences like PYYKNP could be brighter in their solid-supported form, leading to false-positive hits during the library screening.

The tetramers CSSS and DSSS were synthesized to study the photophysical behaviour of the BODIPY-bearing monomers in aqueous solution (see Supplementary Information, Table S1 and Fig. S38). Surprisingly, no fluorescence could be detected for either ODF sequence, although the BODIPY-containing nucleosides **9e** and **9f** had intense emission in organic solvents (see Table 1). Water-induced fluorescence quenching appears to be a likely explanation, which has also been previously described for similar BODIPY dyes.⁵¹ We thus conclude that the BODIPY monomers C and D account for the significant amount of nonfluorescent beads observed during the library screening.

Conclusions

The synthesis of six new fluorophore-labelled α -C-nucleosides has been described. Ethynyl sugar **8** was used as a convenient, easily accessible key intermediate, which could be employed for Sonogashira cross-coupling with various dyes. The respective conjugates were obtained in high yield and could be directly converted into phosphoramidites. These monomer nucleosides could be used as fluorescent labels or probes in multiple DNA applications. In addition, the incorporation of those phosphoramidites into a combinatorial DNA-based polyfluorophore library is demonstrated. The library was screened for polyfluorophores with bright and diverse emission colours. The pyrene- and perylene-containing monomers Y and E were shown to undergo efficient excimer/excimer formation, if stacked adjacent to one another. The styryl-pyran dye K was successfully implemented into energy transfer cassettes, with Stokes shifts of up to 210 nm. The Nile red monomer N displayed emission above 600 nm when included into a polyfluorophore, albeit with low quantum yield in aqueous media. Finally, the current synthetic scheme is generally applicable and allows for a convenient and modular synthesis of unnatural α -C-nucleosides with various aromatic substituents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Notes and references

1. Wilson JN, Kool ET. *Org. Biomol. Chem.* 2006; 4:4265–4274. [PubMed: 17102869]
2. Boutorine AS, Novopashina DS, Krasheninina OA, Nozeret K, Venyaminova AG. *Molecules.* 2013; 18:15357–15397. [PubMed: 24335616]
3. Wagner C, Rist M, Mayer-Enthart E, Wagenknecht HA. *Org. Biomol. Chem.* 2005; 3:2062–2063. [PubMed: 15917887]

4. Guo J, Xu N, Li Z, Zhang S, Wu J, Kim DH, Marma MS, Meng Q, Cao H, Li X, Shi S, Yu L, Kalachikov S, Russo JJ, Turro NJ, Ju J. *Proc. Natl. Acad. Sci. USA*. 2008; 105:9145–9150. [PubMed: 18591653]
5. Ma D-L, He H-Z, Chan DS-H, Leung C-H. *Chem. Sci*. 2013; 4:3366–3380.
6. Cannon BL, Kellis DL, Davis PH, Lee J, Kuang W, Hughes WL, Graugnard E, Yurke B, Knowlton WB. *ACS Photonics*. 2015; 2:398–404. [PubMed: 25839049]
7. Tam DY, Dai Z, Chan MS, Liu LS, Cheung MC, Bolze F, Tin C, Lo PK. *Angew. Chem. Int. Ed*. 2016; 55:164–168.
8. Jungmann R, Steinhauer C, Scheible M, Kuzyk A, Tinnefeld P, Simmel FC. *Nano Lett*. 2010; 10:4756–4761. [PubMed: 20957983]
9. Jungmann R, Avendano MS, Woehrstein JB, Dai M, Shih WM, Yin P. *Nat. Methods*. 2014; 11:313–U292. [PubMed: 24487583]
10. Teo YN, Kool ET. *Chem. Rev*. 2012; 112:4221–4245. [PubMed: 22424059]
11. Ensslen P, Wagenknecht H-A. *Acc. Chem. Res*. 2015; 48:2724–2733. [PubMed: 26411920]
12. Garo F, Häner R. *Angew. Chem. Int. Ed*. 2012; 51:916–919.
13. Ensslen P, Gärtner S, Glaser K, Colsmann A, Wagenknecht H-A. *Angew. Chem. Int. Ed*. 2016; 55:1904–1908.
14. Teo YN, Wilson JN, Kool ET. *J Am. Chem. Soc*. 2009; 131:3923–3933. [PubMed: 19254023]
15. Guo J, Wang S, Dai N, Teo YN, Kool ET. *Proc. Natl. Acad. USA*. 2011; 108:3493–3498.
16. Dai N, Kool ET. *Chem. Soc. Rev*. 2011; 40:5756–5770. [PubMed: 21290032]
17. Samain F, Ghosh S, Teo YN, Kool ET. *Angew. Chem. Int. Ed*. 2010; 49:7025–7029.
18. Kwon H, Samain F, Kool ET. *Chem. Sci*. 2012; 3:2542–2549.
19. Yuen LH, Franzini RM, Wang S, Crisalli P, Singh V, Jiang W, Kool ET. *Angew. Chem. Int. Ed*. 2014; 53:5361–5365.
20. Kwon H, Jiang W, Kool ET. *Chem. Sci*. 2015; 6:2575–2583. [PubMed: 26146537]
21. Sinkeldam RW, Greco NJ, Tor Y. *Chem. Rev*. 2010; 110:2579–2619. [PubMed: 20205430]
22. Crisalli, P.; Kool, ET. *Fluorescent Analogs of Biomolecular Building Blocks: Design and Applications*. 1. Wilhelmsson, M.; Tor, Y., editors. Vol. ch. 14. John Wiley & Sons; 2016. p. 320–355.
23. Hoffer M. *Chem. Ber*. 1960; 93:2777–2781.
24. Mahalingam SM, Manjunath BN, Aidhen IS. *Proc. Indian Natn. Sci. Acad*. 2005; 71:267–282.
25. Ren RXF, Chaudhuri NC, Paris PL, Rumney S, Kool ET. *J Am. Chem. Soc*. 1996; 118:7671–7678. [PubMed: 20865136]
26. Bobula T, Hocek M, Kotora M. *Tetrahedron*. 2010; 66:530–536.
27. Ne as D, Hidasová D, Hocek M, Kotora M. *Org. Biomol. Chem*. 2011; 9:5934–5937. [PubMed: 21785793]
28. Chiba J, Takeshima S, Mishima K, Maeda H, Nanai Y, Mizuno K, Inouye M. *Chem. Eur. J*. 2007; 13:8124–8130. [PubMed: 17607793]
29. Gao JM, Watanabe S, Kool ET. *J Am. Chem. Soc*. 2004; 126:12748–12749. [PubMed: 15469249]
30. Wang S, Guo J, Ono T, Kool ET. *Angew. Chem. Int. Ed*. 2012; 51:7176–7180.
31. Okamoto A, Tainaka K, Fujiwara Y. *J Org. Chem*. 2006; 71:3592–3598. [PubMed: 16626146]
32. Varghese R, Gajula PK, Chakraborty TK, Wagenknecht H-A. *Synlett*. 2009:3252–3257.
33. Ensslen P, Fritz Y, Wagenknecht H-A. *Org. Biomol. Chem*. 2015; 13:487–492. [PubMed: 25374296]
34. Zhang X, Xiao Y, Qi J, Qu J, Kim B, Yue X, Belfield KD. *J Org. Chem*. 2013; 78:9153–9160. [PubMed: 23984818]
35. Ziessel R, Ulrich G, Harriman A, Alamiry MAH, Stewart B, Retailleau P. *Chem. Eur. J*. 2009; 15:1359–1369. [PubMed: 19115286]
36. Stambasky J, Hocek M, Kocovsky P. *Chem. Rev*. 2009; 109:6729–6764. [PubMed: 19761208]
37. Wellington KW, Benner SA. *Nucleos. Nucleot. Nucl*. 2006; 25:1309–1333.

38. Nakahara M, Kuboyama T, Izawa A, Hari Y, Imanishi T, Obika S. *Bioorg. Med. Chem. Lett.* 2009; 19:3316–3319. [PubMed: 19419865]
39. Adamo MFA, Pergoli R. *Org. Lett.* 2007; 9:4443–4446. [PubMed: 17910462]
40. Ludek OR, Marquez VE. *Tetrahedron.* 2009; 65:8461–8467. [PubMed: 20625519]
41. Adamo MFA, Pergoli R, Moccia M. *Tetrahedron.* 2010; 66:9242–9251.
42. Roy S, Caruthers M. *Molecules.* 2013; 18:14268–14284. [PubMed: 24252996]
43. Nestler HP, Bartlett PA, Still WC. *J Org. Chem.* 1994; 59:4723–4724.
44. Cicchi S, Fabbriizzi P, Ghini G, Brandi A, Foggi P, Marcelli A, Righini R, Botta C. *Chem. Eur. J.* 2009; 15:754–764. [PubMed: 19053090]
45. Birks JB, Christophorou LG. *Nature.* 1962; 194:442–444.
46. Paris PL, Langenhan JM, Kool ET. *Nucl. Acids Res.* 1998; 26:3789–3793. [PubMed: 9685497]
47. Teo YN, Kool ET. *Bioconjugate Chem.* 2009; 20:2371–2380.
48. Kashida H, Takatsu T, Sekiguchi K, Asanuma H. *Chem. Eur. J.* 2010; 16:2479–2486. [PubMed: 20066689]
49. Jose J, Burgess K. *J Org. Chem.* 2006; 71:7835–7839. [PubMed: 16995693]
50. Mukherjee S, Raghuraman H, Chattopadhyay A. *Biochim. Biophys. Acta.* 2007; 1768:59–66. [PubMed: 16934217]
51. Hu X, Zhang J, Yu Z, Xie Y, He H, Qi J, Dong X, Lu Y, Zhao W, Wu W. *Nanomed. Nanotech. Biol. Med.* 2015; 11:1939–1948.

Fig. 1.
Molecular structures and one letter abbreviation of the ODF monomers (left side) and a representative ODF (right side).

Fig. 2. Normalized absorption and fluorescence spectra of *C*-nucleosides **9a-f**. The spectra of *C*-nucleosides **9a-d** and **9e-f** were recorded in MeOH and EtOAc, respectively. Blue: **9a**; light green: **9b**; orange: **9c**; red: **9d**; dark green: **9e**; black: **9f**.

Fig. 3.

Normalized absorption and fluorescence spectra of selected ODF sequences. The spectra were recorded in PBS, if not indicated otherwise. Blue: PYYSSP; light green: PSSYEP; orange: PKYYYYP; red: PSKKKP; dark green: PYYKNP; black: PYYKNP in MeOH.

Scheme 1.

Synthesis of alkyne **8**. Reagents and conditions: (i) AcCl, MeOH, rt, 1 h; (ii) BnBr, NaH, tetra-*n*-butylammonium iodide, THF, rt, overnight; (iii) AcCl, H₂O, dioxane, rt, overnight; (iv) ethynylmagnesium bromide, THF, rt, 2 d; (v) TsCl, KOH, H₂O, dioxane, rt, 13 h; (vi) BCl₃, CH₂Cl₂, -78 °C, 1.5 h; (vii) DMTCl, DMAP, pyridine, rt, 2 h. DMT = 4,4'-dimethoxytrityl.

Scheme 2.

Synthesis of phosphoramidites **10a-f**. Reagents and conditions: (i) R-X (X = Br for **9a** and **9b**, X = I for **9c**, **9e** and **9f**, X = OTf for **9d**), Pd(PPh₃)₄, CuI, NEt₃, DMF, 80 °C, 2.5 h; (ii) 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, DIPEA, CH₂Cl₂, RT, 1.5 h. DMT = 4,4'-dimethoxytrityl.

Table 1Spectral properties of *C*-nucleosides **9a–f**. All spectra were measured in MeOH, if not indicated otherwise

Dye	$\lambda_{\text{max,abs}}^a$ [nm]	ϵ_{max} [M ⁻¹ cm ⁻¹]	$\lambda_{\text{max,em}}^b$ [nm]	Φ_{F}
9a	359	61000 ± 3000	383	0.186 ± 0.010
9b	454	37000 ± 3000	458	0.704 ± 0.016
9c	438	34000 ± 3000	562	0.111 ± 0.002
9d	564	52000 ± 3000	640	0.41 ± 0.04
9e^c	669	133000 ± 10000	686	1.00 ± 0.11
9f^c	693	101000 ± 8000	729	0.24 ± 0.03

^aAbsorption maxima have a ±1 nm imprecision.^bFluorescence maxima are reproducible within a ±2 nm range.^cMeasured in EtOAc.

Table 2

Spectral properties of selected ODFs. All spectra were measured in PBS, if not indicated otherwise

Sequence	λ_{abs}^a [nm]	λ_{em}^b [nm]	Φ_F^c
PYYSSP	364	503	0.30 ± 0.03
	346	404	
	283	370	
	274		
PSSYEP	462	506	0.413 ± 0.008
	435	402	
	364	371	
	348		
	285		
	274		
PKYYYYP	457	579	0.043 ± 0.008
	367	403	
	348	384	
	285		
PSKKKP	424	597	0.0218 ± 0.0007
	345		
	283		
PYYKNP	602	665	n.d.
	465		
	367		
	348		
	284		
	276		
PYYKNP ^d	579	638	0.0097 ± 0.0002
	466	404	
	360	383	
	343		
	282		

^a Absorption maxima have a ± 1 nm imprecision.^b Fluorescence maxima are reproducible within a ± 2 nm range.^c Excitation around 325 nm.^d Measured in MeOH.