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A convenient [2+2] cycloaddition–cycloreversion reaction for the synthesis of 1,1-dicyanobuta-1,3-diene-scaffolded peptides as new imaging chromophores ☆

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ABSTRACT

We report on the chemoselective coupling between colorless peptide fragments functionalized with a mutually reactive electron-rich N^{α}-(4-ethynylphenyl)-N^{α}-(methyl)-glycyl- and an electron-deficient [4-(2,2-dicyanovinyl)]benzoyl moiety. The resulting donor-substituted 1,1-dicyanobuta-1,3-dienes represent a new class of orange-red colored (λ_{max} = 450–500 nm, with molar extinction coefficients (ε) above 5,000 mol^{-1} dm^3 cm^{-1}) peptide-based imaging chromophores.

Labeled peptides are important tools in chemical biology to obtain information on their biological function in, for example, cellular communication processes as well as to study the molecular basis of diseases.[1] The functionalization of peptides with biophysical reporter groups such as fluorophores, strongly absorbing chromophores, biotin, or spin-labels, is most often performed via post-synthetic chemoselective conjugation reactions, involving amino, thiol, or azide specific reactions.[2] An alternative approach for the installation of fluorescent or chromophoric organic molecules is the coupling of non-fluorescent/chromophoric precursor molecules to obtain fluorescence/chromophoric properties in the final product.[3] A versatile method to achieve this makes use of the Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC),[4] to enable modulation of the emission response of the newly synthesized fluorophores/chromophores.[5]

To extend the repertoire for installing a chromophoric property into non-chromophoric precursor molecules, we recently reported on a formal [2+2] cycloaddition–cycloreversion reaction between peptide-functionalized electron-rich alkynes and electron-deficient tetracyanoethylene (TCNE) to obtain 1,1,4,4-tetracyanobuta-1,3-diene-scaffolded peptides as a new class of π-conjugated peptidic donor–acceptor chromophores.[6,7] Here, we report on the use of N^{α}-[(4-(2,2-dicyanovinyl)]benzoyl moiety, as an alternative to TCNE, for N-terminal peptide modification, to modulate the optical properties, to increase peptide diversity, and to access 1,1-dicyanobuta-1,3-diene-scaffolded peptides as chromophores, in a single final assembly step, as shown schematically in Figure 1.

As a model compound, N^{α}-{[4-(2,2-dicyanovinyl)]benzoyl}-alanine ethyl ester (**3a**)[8] was synthesized (Scheme 1). 4-Formylbenzoic acid (**1**) was preactivated with benzotriazol-1-yloxy-tris-(dimethylamino)-phosphonium hexafluorophosphate/*N*,*N*-diisopropylethylamine (BOP/DIPEA) prior to the addition of HCl·H-Ala-OEt, to avoid imine formation between the aldehyde and the amine,[9] and amide **2a** was obtained in a high yield (93%). In the next step, an Al_2O_3-catalyzed Knoevenagel condensation[10] was performed in the presence of malononitrile in THF under reflux, which gave dicyanovinyl compound **3a** in good yield (76%) after purification by column chromatography. In a similar approach, valine derivative **3b**, and the dicyanovinyl compounds **3c** and **3d**, containing a dipeptide and a protected dipeptide, respectively, were synthesized in satisfactory yields ranging between 55% and 61% (Scheme 1).

In an initial attempt to perform the [2+2] cycloaddition–cycloreversion with these peptide congeners, *N*,*N*-dimethyl-4-ethynylaniline (**4**)[11] was reacted with dicyanovinyl derivative **3a** in

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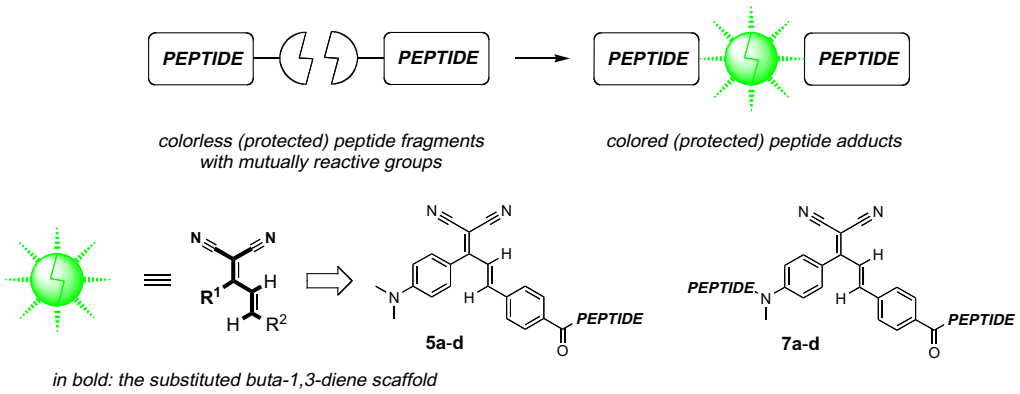
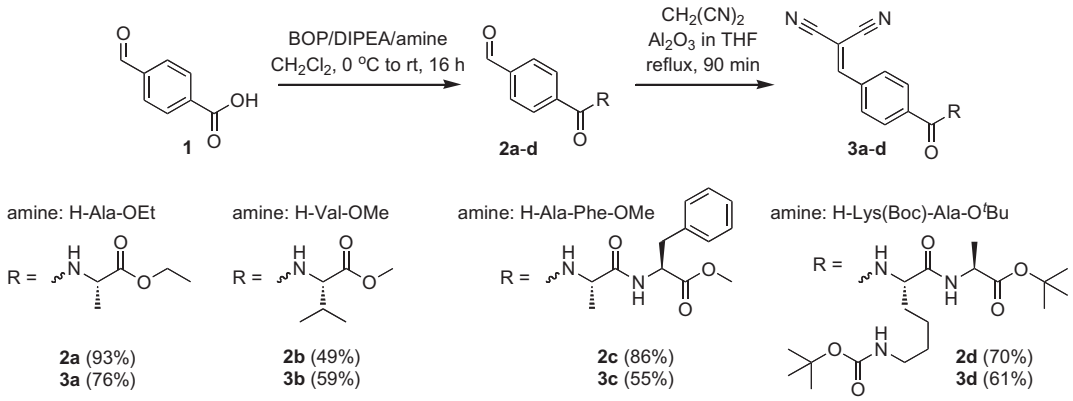


Figure 1. Mutually reactive groups result in strongly absorbing 1,1-cyanobuta-1,3-diene-scaffolded peptide-based chromophores.



Scheme 1. Synthesis of the building blocks to obtain dicyanovinyl derivatives **3a**–**d**.

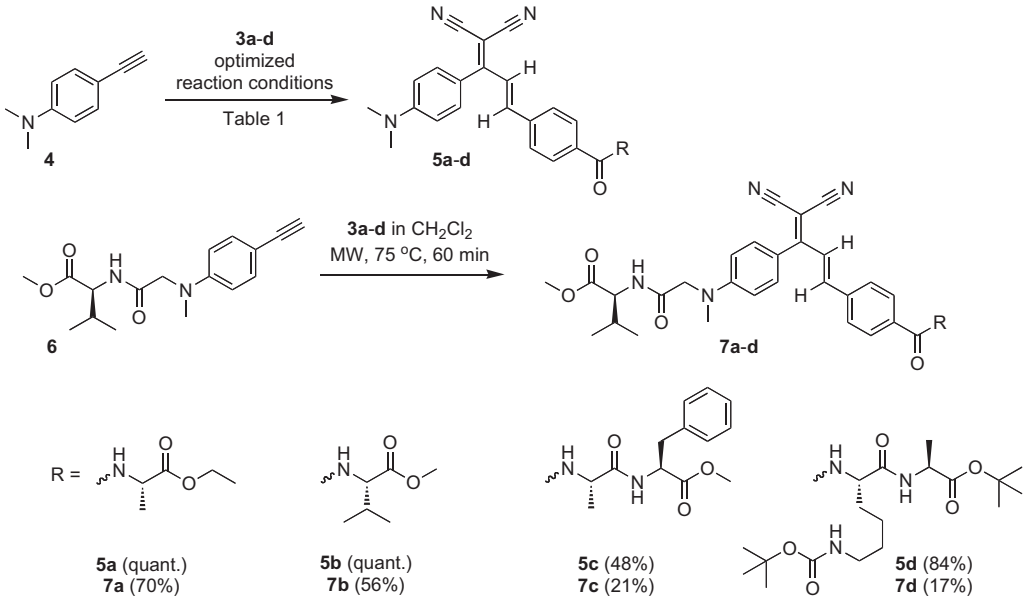
Table 1
Reaction optimization between alkyne **4** and dicyanovinyl derivative **3a** to yield dicyanobutadiene **5a**

Entry	Solvent	*T* (°C)	Time	Isolated yield (%)
1	CH_2Cl_2	25	4 d	19
2	DMF	100	16 h	79
3	DMA	100	16 h	67
4	DMA	130	16 h	Intractable material
5	CH_2Cl_2	60 (MW)	20 min	40
6	CH_2Cl_2	75 (MW)	60 min	Quant.

CH_2Cl_2 at room temperature. Under these conditions, product formation was rather slow, and after 4 days of stirring, compound **5a**[12] was isolated in 19% yield as an intense purple-red solid (entry 1, Table 1, Scheme 2). Apparently, dicyanovinyl derivatives are less reactive toward electron-rich alkynes than TCNE, and this reaction needed to be optimized. The results are shown in Table 1. Running the reaction in DMF at 100 °C[7d] for 16 h improved the efficiency and **5a** was isolated in 79% yield (entry 2). To avoid the presence of amines due to any decomposition of DMF at elevated temperatures, *N*,*N*-dimethylacetamide (DMA) was used as an alternative (entries 3 and 4). Unfortunately, no further improvements were observed; moreover, running the reaction at 130 °C led to an intractable reaction mixture. This made us try microwave irradiation as an alternative way of heating, based on our positive experience with the Cu(I)-catalyzed cycloaddition between peptide-based azides and alkynes.[13] Therefore, the reaction was performed in CH_2Cl_2 at 60 °C under microwave irradiation (entry 5) over 20 min and **5a** was isolated in 40% yield, a remarkable increase in yield in a significantly shorter reaction time compared to entry 1. Finally, a quantitative yield of **5a** was obtained by running the reaction in CH_2Cl_2 at 75 °C under microwave irradiation (entry 6) for 60 min using excess of the alkyne (1.2 equiv) to facilitate purification by column chromatography.

These optimized reaction conditions were used for the synthesis of 1,1-dicyanobuta-1,3-diene-scaffolded peptides **5b**–**d** (alkyne **4** + dicyanovinyl derivatives **3b**–**d**) and **7a**[14]–**d** (alkyne **6**[15] + dicyanovinyl derivatives **3a**–**d**), as shown in Scheme 2. The highest yields were obtained with alkyne **4** (48–100%), while the cycloaddition products of alkyne **6** were obtained in yields ranging from 17% to 70%, partly due to the increasing bulkiness of the peptide moieties and the low solubility and rather difficult purification of the obtained dicyanobutadienes. Figure 2 shows the aromatic region of the ^{1}H NMR spectrum of **5b**[16] indicating the *E*-selectivity of the cycloaddition–cycloreversion reaction, resulting from the torquoselectivity of the latter step,[7d] since ^3J-values of 15.7 Hz for both olefinic protons were observed.

The UV–Vis spectra (in $CHCl_3$) of compounds **5a** and **7a** are shown in Figure 3A. Dicyanobutadiene **5a** showed an absorption maximum at $\lambda = 358$ nm with a high molar extinction coefficient (ε) of 25,000 mol^{-1} dm^3 cm^{-1}, and a second, less intense maximum, at $\lambda = 475$ nm (ε 8,000 mol^{-1} dm^3 cm^{-1}). A solution of compound **5a** was orange-red in color while as a solid, this class of



Scheme 2. The [2+2] cycloaddition reaction of dicyanovinyl compounds with alkynes.

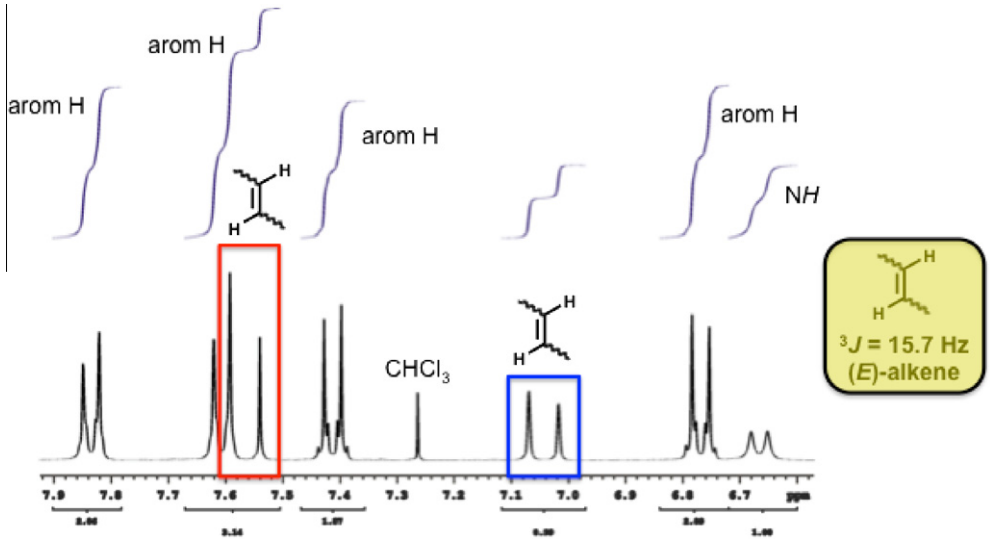


Figure 2. Aromatic region of the ^{1}H NMR spectrum of **5b** (see Ref. 12 for a complete assignment) indicating the *E*-selectivity of the cycloaddition–cycloreversion reaction.

peptide chromophores was found to have a purple-black color with a typical metallic luster.[17] The orange-red color originates from the longer-wavelength band for which charge-transfer character has been previously established.[7c,d] This charge-transfer character is supported by a reversible quenching experiment: protonation of the anilino donor with trifluoroacetic acid (TFA) eliminates this absorption band, whereas it is fully restored upon neutralization with triethylamine (Et_3N) as is shown in Figure 3B. The absorption spectrum of **7a** indicated a maximum at $\lambda = 358$ nm with a molar extinction coefficient of 17,000 mol^{-1} dm^3 cm^{-1}, while the second maximum was hypsochromically shifted, as compared to **5a**, by 40 nm to $\lambda = 435$ nm (ε 5,100 mol^{-1} dm^3 cm^{-1}) (Fig. 3A), which is also apparent from the bright-yellow color of the solution. This relatively large hypsochromic shift (blue shift) maybe explained by the σ-acceptor character of the α-carbonyl moiety, which tempers the electron-donating properties of the aniline nitrogen.

In conclusion, the synthesis of a new class of peptide-based chromophores has been described via the [2+2] cycloaddition–cycloreversion reaction between an electron-rich alkyne and an electron-deficient ethylene species, resulting in colored peptide scaffolds, ranging from yellow to orange-red, with high molar extinction coefficients. In principle, building blocks such as N^{α}-(4-ethynylphenyl)-N^{α}-(methyl)-glycine or N^{α}-[4-(2,2-dicyanovinyl)benzoyl]-glycine would be ideally suited for peptide modification to access this novel class of π-conjugated peptides as donor–acceptor chromophores. Finally, this chemistry can be considered as a model study for a novel bioconjugation approach to biologically relevant peptides in which the imaging chromophores are installed during bioconjugation as the final reaction step.

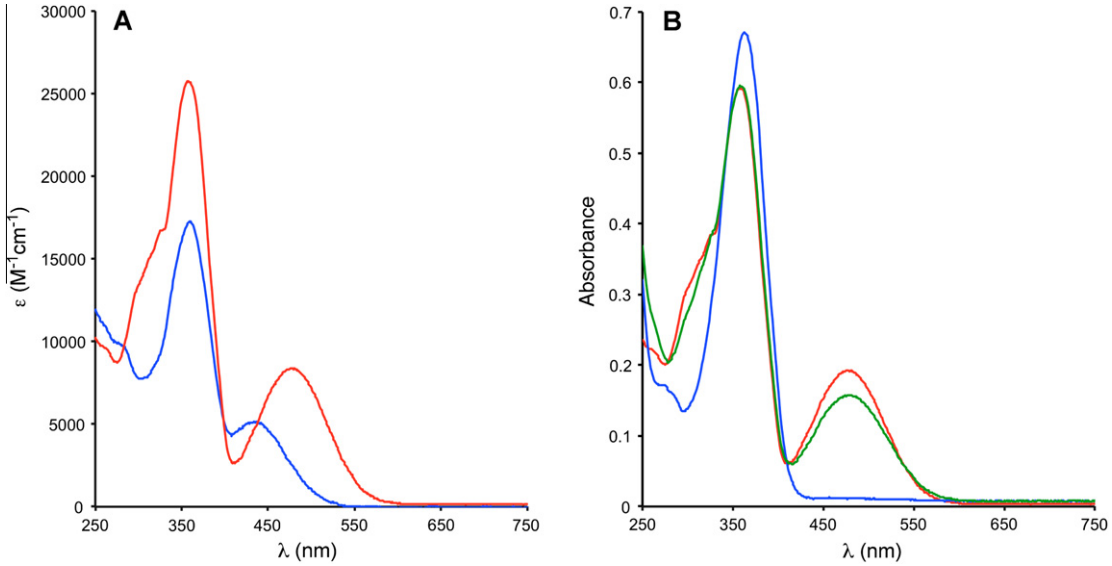


Figure 3. (A) UV–Vis spectra (in $CHCl_3$ at $T = 298$ K) of **5a** (red line: 23 μM) and **7a** (blue line: 15 μM); (B) Reversible charge-transfer quenching of **5a**: (red line: 23 μM in $CHCl_3$), after the addition of TFA (10 μL) (blue line), after neutralization with Et_3N (10 μL) (green line).

Acknowledgments

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8. *N^{α}-[4-(2,2-Dicyanovinyl)benzoyl]-alanine ethyl ester* (**3a**): $R_f = 0.68$ (CH_2Cl_2/MeOH, 95:5 v/v), $R_f = 0.33$ (CH_2Cl_2/MeOH, 98:2 v/v); mp 112–117 °C; $[\alpha]_D^{20} = +59.1$ ($c = 0.33$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 1.30$ (t, $^3J(H,H) = 7.2$ Hz, 3H; CH_3), 1.51 (d, $^3J(H,H) = 7.2$ Hz, 3H; βCH$_3$ Ala), 4.23 (q, $^3J(H,H) = 7.2$ Hz, 2H; OCH_2), 4.75 (quin, $^3J(H,H) = 7.2$ Hz, 1H; αCH Ala), 7.03 (d, $^3J(H,H) = 7.2$ Hz, 1H; amide NH), 7.83 (s, 1H; =CH), 7.92 (m, 4H; arom H); ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): $\delta = 172.7$, 164.7, 158.4 (2 lines), 138.5, 133.1, 130.5, 128.0, 113.1, 112.1, 84.7, 61.8, 48.8, 18.4, 14.1; UV–Vis ($CHCl_3$): λ_{max} (ε) = 314 nm (28,000 mol^{-1} dm^3 cm^{-1}); ESMS calcd for $C_{16}H_{15}N_3O_3$: 297.31, found m/z 298.65 $[M+H]^+$.
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12. *N^{α}-{4-[(1E)-4,4-dicyano-3-[4-(dimethylamino)phenyl]buta-1,3-dien-1-yl]benzoyl}-alanine ethyl ester* (**5a**):[18] $R_f = 0.62$ (CH_2Cl_2/MeOH, 95:5 v/v), $R_f = 0.66$ (EtOAc/Et_2O, 1:1 v/v), $R_f = 0.37$ (Et_2O); $R_t = 38.8$ min;[19] mp 136–140 °C; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 1.32$ (t, $^3J(H,H) = 7.2$ Hz, 3H; CH_3), 1.53 (d, $^3J(H,H) = 7.2$ Hz, 3H; βCH$_3$ Ala), 3.10 (s, 6H; $N(CH_3)_2$), 4.22 (q, $^3J(H,H) = 7.2$ Hz, 2H; OCH_2), 4.78 (quin, $^3J(H,H) = 7.2$ Hz, 1H; αCH Ala), 6.75 (d, $^3J(H,H) = 9$ Hz, 2H; arom H), 6.79 (overlapping signal, 1H; amide NH), 7.01 (d, $^3J(H,H) = 15.6$ Hz, 1H; =CH), 7.40 (d, $^3J(H,H) = 9$ Hz, 2H; arom H), 7.54 (d, $^3J(H,H) = 15.6$ Hz, 1H; =CH), 7.60 (d, $^3J(H,H) = 8.4$ Hz, 2H; arom H), 7.82 (d, $^3J(H,H) = 8.4$ Hz, 2H; arom H); ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): $\delta = 172.9$, 170.0, 165.5, 152.7, 146.2, 137.6, 135.6, 131.6, 128.4, 127.7, 126.9, 119.5, 114.8, 114.0, 111.3, 78.2, 77.2, 61.8, 48.7, 40.1, 18.8, 14.3; UV–Vis ($CHCl_3$): λ_{max} (ε) = 475 (8,350), 358 nm (25,750 mol^{-1} dm^3 cm^{-1}); ESMS calcd for $C_{26}H_{26}N_4O_3$: 442.20, found m/z 443.40 $[M+H]^+$.
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14. *Compound* **7a**: $R_f = 0.58$ (CH_2Cl_2/MeOH, 9:1 v/v), $R_f = 0.26$ (CH_2Cl_2/MeOH, 95:5 v/v), $R_f = 0.07$ (EtOAc/hexane, 1:1 v/v); $R_t = 38.5$ min;[19] mp 149–153 °C; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 0.81$ (d, $^3J(H,H) = 6.6$ Hz, 3H; γ CH_3 Val), 0.92 (d, $^3J(H,H) = 6.6$ Hz, 3H; γ′CH_3 Val), 1.32 (t, $^3J(H,H) = 7.2$ Hz, 3H; CH_3), 1.55 (d, $^3J(H,H) = 7.2$ Hz, 3H; βCH$_3$ Ala), 2.17 (m, 1H; βCH Val), 3.20 (s, 3H; NCH_3), 3.73 (s, 3H; OCH_3), 3.97 (d, $^3J(H,H) = 18$ Hz, 1H; CH_2 Gly), 4.09 (d, $^3J(H,H) = 35.1$ Hz, 1H; CH_2 Gly), 4.27 (q, $^3J(H,H) = 7.2$ Hz, 2H; OCH_2), 4.59 (dd, $^3J(H,H) = 4.5$ Hz, $^3J(H,H) = 9$ Hz, 1H; αCH Val), 4.78 (quin, $^3J(H,H) = 7.2$ Hz, 1H; αCH Ala), 6.64 (d, $^3J(H,H) = 9.3$ Hz, 1H; amide NH Val), 6.80 (d, $^3J(H,H) = 7.2$ Hz, 1H; amide NH Ala), 6.85 (d, $^3J(H,H) = 9.3$ Hz, 2H; arom H), 6.96 (d, $^3J(H,H) = 15.6$ Hz, 1H; =CH), 7.40 (d, $^3J(H,H) = 9.3$ Hz, 2H; arom H), 7.57 (d, $^3J(H,H) = 15.6$ Hz, 1H; =CH), 7.61 (d, $^3J(H,H) = 8.1$ Hz, 2H; arom H), 7.82 (d, $^3J(H,H) = 8.1$ Hz, 2H; arom H); ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): $\delta = 173.1$, 172.1, 170.0, 169.0, 165.6, 151.5, 146.8, 137.5, 136.0, 131.4, 128.6, 127.9, 126.7, 122.1, 114.2, 113.4, 112.6, 61.8, 57.6, 56.8, 52.3, 48.7, 39.7, 31.2, 22.7, 19.1, 18.7, 17.6, 14.1; UV–Vis ($CHCl_3$): λ_{max} (ε) = 435 (5,100), 358 nm (17,000 mol^{-1} dm^3 cm^{-1}); ESMS calcd for $C_{33}H_{37}N_5O_6$: 599.68, found m/z 600.40 $[M+H]^+$, 622.70 $[M+Na]^+$.
15. The synthesis of alkyne **6** is described in Ref. 6
16. *N^{α}-{4-[(1E)-4,4-dicyano-3-[4-(dimethylamino)phenyl]buta-1,3-dien-1-yl]benzoyl}-valine methyl ester* (**5b**): $R_f = 0.08$ (CH_2Cl_2/MeOH, 98:2 v/v), $R_f = 0.14$ (EtOAc/hexane, 2:1 v/v), $R_f = 0.30$ (EtOAc/hexane, 1:1 v/v); $R_t = 38.8$ min;[19] mp 163–165 °C; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 1.03$ (t, $^3J(H,H) = 5.8$ Hz, 6H; CH_3 Val), 2.32 (m, 1H; βCH Val), 3.10 (s, 6H; $N(CH_3)_2$), 3.79 (s, 3H; OCH_3), 4.80 (m, 1H; αCH Val), 6.66 (d, $^3J(H,H) = 8.5$ Hz, 1H; amide NH), 6.76 (d, $^3J(H,H) = 9$ Hz, 2H; arom H), 7.02 (d, $^3J(H,H) = 15.7$ Hz, 1H; =CH), 7.39 (d, $^3J(H,H) = 9$ Hz, 2H; arom H), 7.54 (d, $^3J(H,H) = 15.7$ Hz, 1H; =CH), 7.59 (d, $^3J(H,H) = 8.5$ Hz, 2H; arom H), 7.81 (d, $^3J(H,H) = 8.5$ Hz, 2H; arom H); ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): $\delta = 172.5$, 170.0, 166.2, 152.8, 146.2, 135.8, 131.6, 128.4, 127.8, 127.0, 119.5, 114.0, 111.3, 57.5, 52.3, 40.0, 31.6, 18.9, 17.9 (19 lines out of 22); UV–Vis ($CHCl_3$): λ_{max} (ε) = 475 (9,800), 358 nm (31,000 mol^{-1} dm^3 cm^{-1}); ESMS calcd for $C_{27}H_{28}N_4O_3$: 456.54, found m/z 457.45 $[M+H]^+$.
17. *In comparison with commonly used fluorophores such as 4-chloro-7-nitrobenz-2-oxa-1,3-diazole (NBD-Cl)*: $\varepsilon = 9{,}800$ mol^{-1} dm^3 cm^{-1}, $\lambda_{max} = 336$ nm.

See also: www.invitrogen.com/site/us/en/home/References/Molecular-Probes-The-Handbook.html; last visited on September 29, 2011.

18. Due to the deeply colored solution of dicyanobutadienes, an accurate $[\alpha]_D^{20}$ value could not be measured.
19. HPLC analysis was performed on a Phenomenex Luna C8 column (particle size: 5 μm, pore size 100 Å, 250 × 4.6 mm) using a linear gradient starting from buffer A (0.1% TFA in CH_3CN/H_2O, 5:95 v/v) to buffer B (0.1% TFA in CH_3CN/H_2O, 95:5 v/v) over 60 min at a flow rate of 1 mL/min.