Tetrahedron Letters 52 (2011) 4021–4025

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

A convenient synthesis of new chromophoric tetracyanobutadiene-scaffolded peptides via a dipolar [2+2] cycloaddition–cycloreversion reaction ☆

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ARTICLE INFO

Article history:
Received 24 March 2011
Revised 19 May 2011
Accepted 27 May 2011
Available online 2 June 2011

Keywords:
Acetylenes
Cycloaddition
Donor–acceptor chromophores
π-Conjugated peptides
Peptides
Peptidomimetics

ABSTRACT

Herein, we report a novel approach for the synthesis of π-conjugated peptide-based donor–acceptor (D-π-A) chromophores, by reacting electron-rich alkynes with tetracyanoethylene. The desired tetracyanobutadiene-scaffolded peptides were obtained in good yields with various optical properties, λ_{max}: 321–492 nm, ε: 21,000–65,000 mol^{-1} dm^3 cm^{-1} depending on the substitution pattern of the cyanobutadiene scaffold.

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Peptides play a pivotal role in maintaining homeostasis in a living organism, among others, as hormones, signaling molecules, enzyme inhibitors. Functionalization of such peptides with reporter groups (fluorescent or chromophoric tags) is an important and often applied tool in chemical biology, to monitor the fate of the labeled peptide, thereby gaining information of its biological function, and also for studying the molecular basis of disease.[1] Such fluorescent/chromophoric labels are introduced post-synthetically, by featuring amino or thiol specific conjugation reactions.[2] However, in recent years, the development of novel chemoselective bioconjugation reactions, for example, native chemical ligation,[3] the Staudinger ligation,[4] oxime/hydrazone ligation,[5] and the Cu(I)-catalyzed cycloaddition between azides and alkynes,[6] has led to a renewed interest in biocompatible, site-specific introduction of fluorophores, chromophores, biotin, metal chelators and other biophysical probes. This renewed interest has resulted in many new peptide-derived constructs as tools in chemical biology for studying signal transduction and post-translational modification pathways in a living organism. Herein, we report on a novel approach for the synthesis of tetracyanobutadiene-scaffolded peptides that represent a new class of intense peptide-based chromophores (Fig. 1).

To access this new class of π-conjugated peptidic donor–acceptor (D-π-A) chromophores, the recently developed reaction between electron-rich alkynes with electron-deficient ethylenes has been applied.[7] This dipolar [2+2] cycloaddition-cycloreversion reaction leads, with a high degree of regio- and stereoselectivity, to a cyanobutadiene-scaffold, which is an intense chromophore and its optical properties can be fine-tuned by variation of the π-conjugation by the substituents.

The syntheses started with N-monoalkylation of aniline derivative **1** (Scheme 1). Despite the weak nucleophilicity of the amine, an efficient alkylation was obtained with one equivalent of ethyl bromoacetate in the presence of anhydrous Na_2CO_3 in dry DMF at 95 °C, and the N-monoalkylated product was obtained in 70% yield.[8] In the next step, N-methylation required at least three days of stirring in DMF/Na_2CO_3 at room temperature, since the low boiling point (41–43 °C) of iodomethane did not allow reaction at an elevated temperature. The N,N-dialkylated aniline **2** was obtained in an excellent yield of 98%. Saponification of ethyl ester **2** proceeded smoothly, and it turned out that during aqueous work-up the intermediate acid was stable in solution, but unstable as a solid since evaporation of the solvent resulted in the formation of an intractable black precipitate. Therefore, the transformation into the corresponding hydroxysuccinimide ester was performed directly in the solvent used for the aqueous extraction. Active ester **3** was obtained, as a stable solid, in good yield (77%) after

☆ Parts of this research have been presented at the 3rd EuCheMS Chemistry Congress (Nuremberg, Germany, August 2010) and at the 31st European Peptide Symposium (Copenhagen, Denmark, September 2010).

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doi:10.1016/j.tetlet.2011.05.136

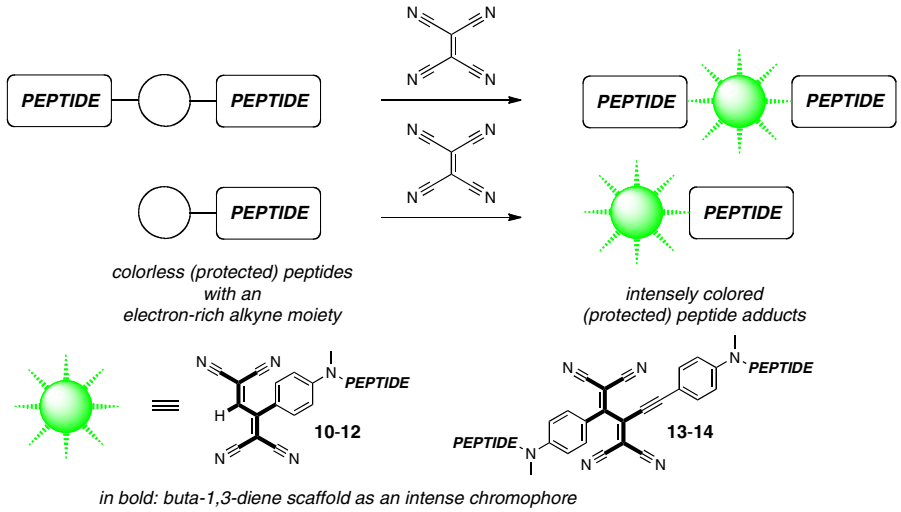
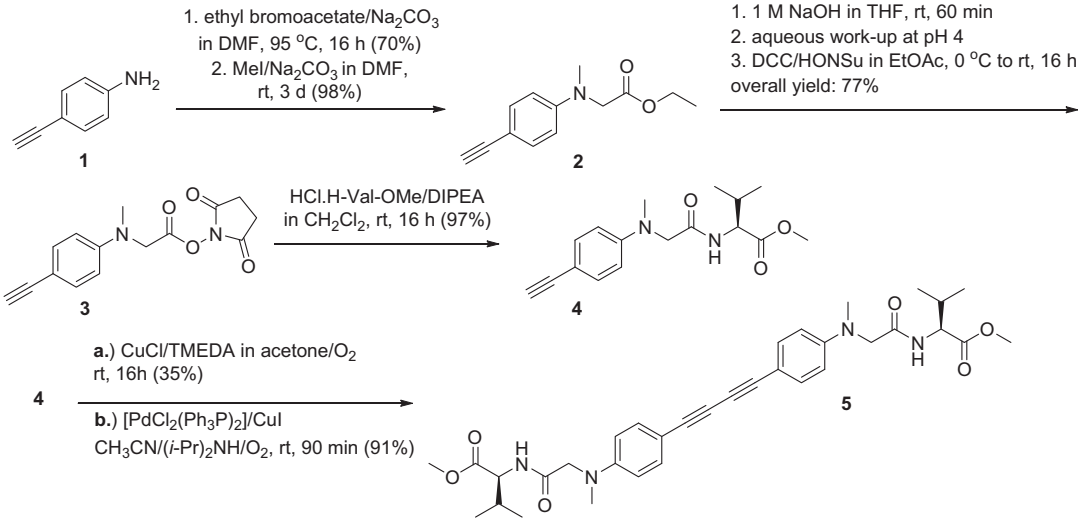


Figure 1. Reaction of electron-rich alkynes with TCNE results in intensely colored tetracyanobutadiene-scaffolded peptide-based chromophores.



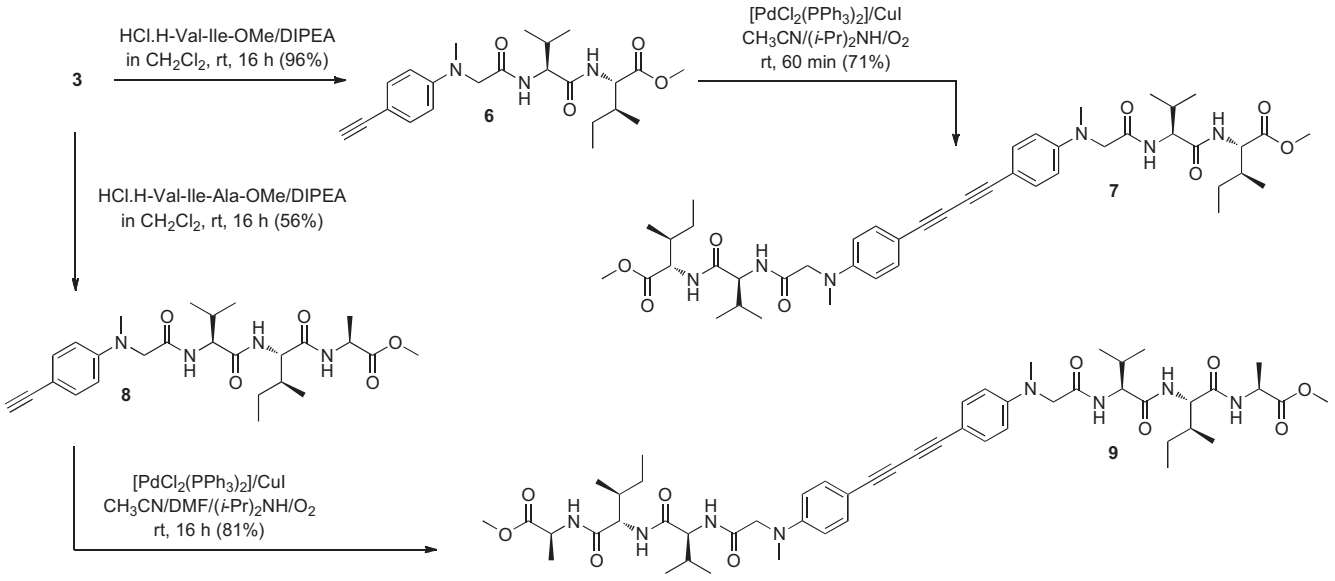
Scheme 1. Synthesis of alkyne **4**, and its corresponding dialkyne **5**.

recrystallization from 2-propanol. Subsequent N-acylation of H-Val-OMe gave the N^{α}-aryl dipeptide **4** [N^{α}-(4-ethynylphenyl)-N^{α}-(methyl)-Gly-Val-OMe][9] as an oil in an excellent yield of 97%.

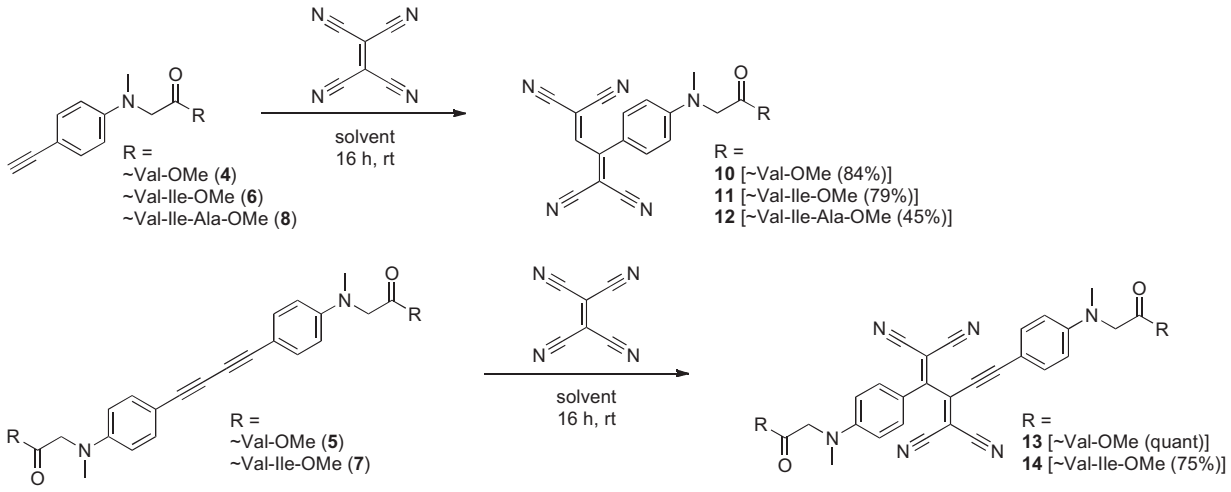
A versatile approach for oxidative acetylenic homocoupling was described by Hay in the early 1960s.[10,11a] In the presence of a complex formed by Cu(I)/*N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) as the catalyst and dioxygen, alkynes undergo smooth dimerization in nearly quantitative yield. However, in the case of alkyne **4**, a rather disappointing yield of 35% of the dimer **5** was achieved (Scheme 1). In this context, it should be mentioned that in an attempt to couple **4** to dimethyl 2,3-dibromofumarate with $[PdCl_2(PPh_3)_2]$/CuI under Sonogashira conditions, dialkyne **5** was isolated as a side product in 68% yield. This prompted us to attempt the Pd/Cu co-catalyzed homocoupling of terminal alkynes[11b] for the synthesis of **5**. Gratifyingly, alkyne **4** underwent smooth dimerization into **5**[12] in an excellent yield of 91% (Scheme 1).

Next, dialkynes **7** and **9** were synthesized (Scheme 2), essentially according to the same approach as described for dialkyne **5**. Since the peptide sequence was derived from the C-terminus (residues 40–42) of the highly amyloidogenic Alzheimer Aβ (1–42) peptide, the synthesis of **7**–**9** was rather challenging due to the restricted solubility, high intrinsic aggregation properties of these peptides.[13] The acylation of dipeptide H-Val-Ile-OMe by active ester **3** to afford alkyne **6** proceeded without any problems, a high yield was achieved. During the dimerization of **6** into **7**, a gel formed and the reduced solubility of dialkyne **7** was reflected in the lower isolated yield compared to compound **5**. Tripeptide HCl·H-Val-Ile-Ala-OMe was converted into alkyne **8** by reaction with active ester **3** and DIPEA as the base. During the acylation, the reaction mixture turned gradually into a gel, alkyne **8** was sparingly soluble in $CHCl_3$, prone to aggregation. After purification by column chromatography, and alkyne **8** was obtained in 56% yield. Next, the dimerization step required DMF as a co-solvent and dialkyne **9**[14] was obtained in a high yield (81%); this compound precipitated from the solution and was isolated by filtration. It turned out that dialkyne **9** was almost insoluble in common solvents such as MeOH, $CHCl_3$ or DMF, and only poorly soluble in DMSO.

A series of tetracyanobutadienes **10**–**14** was prepared by reacting tetracyanoethylene (TCNE) with a suitable alkyne (**4**, **6**, **8**) or dialkyne (**5**, **7**), as shown in Scheme 3. Donor-substituted, electron-rich alkynes reacted smoothly with electron-poor ethylenes in an atom-economic, one-step transformation in nearly quantitative yield to give the tetracyanobutadiene (TCBD) framework. This reaction is a formal [2+2] cycloaddition toward a cyclobutene



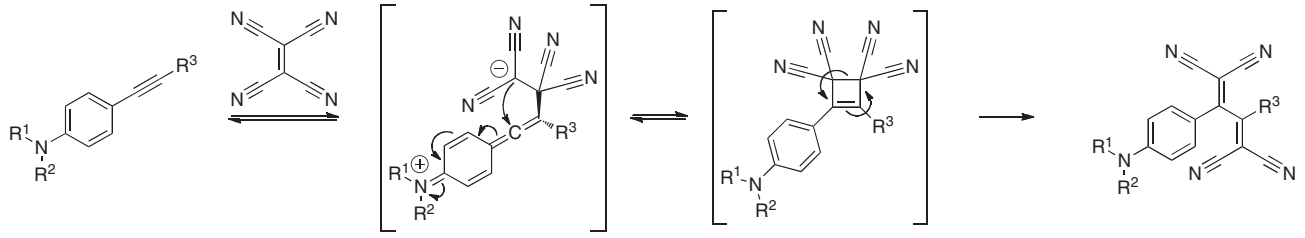
Scheme 2. Synthesis of alkynes **6** and **8**, and their corresponding dialkynes **7** and **9**.



Scheme 3. The [2+2] cycloaddition reaction of TCNE with peptide-derived alkynes.

intermediate, that undergoes an electrocyclic ring-opening to give the TCBDs (Scheme 4).[15]

Alkynes **4** and **6** were smoothly converted into their corresponding TCBDs which were isolated after purification by column chromatography in 84% and 79% yield for **10**[16] and **11**, respectively. The reaction of **8** with TCNE resulted in a precipitate from which TCBD **12** was isolated in a modest yield of 45%. To obtain more insight into the efficiency of this reaction, alkyne **8** was dissolved in $CDCl_3$, the course of the reaction was followed by ^{1}H NMR spectroscopy by monitoring the decrease of the $HC{\equiv}$ signal ($\delta = 2.99$), the increase of the $\sim CH{=}C(CN)_2$ signal ($\delta = 8.01$) relative to the OCH_3 signal ($\delta = 3.74$).[17] By means of this approach, a conversion of 77% after a 16 hour reaction time could be calculated, this value was in the range of the isolated yields of compounds **10** and **11**.



Scheme 4. Mechanism of the [2+2] cycloaddition–cycloreversion reaction.

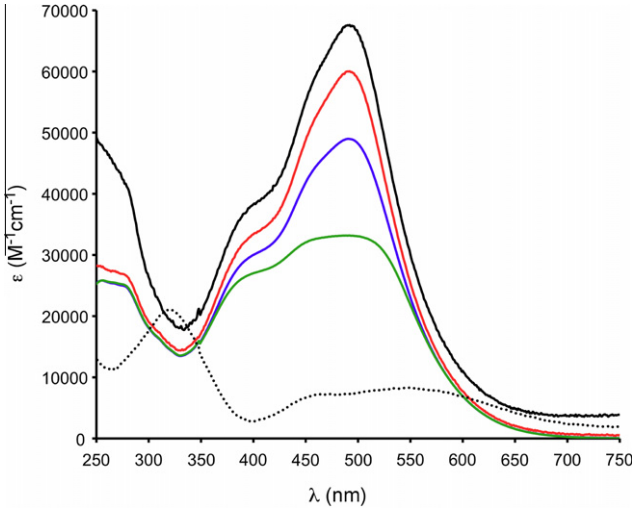


Figure 2. UV–vis spectra (in $CHCl_3$ at $T = 298$ K) of **10** (black round dotted line: 1 μM); **13** (solid black line: 1 μM, red line: 2 μM, blue line: 20 μM, green line: 45 μM).

Tetracyanobutadienes **10**–**12** were obtained as purple-black solids with a metallic luster, and were stable in air at ambient temperatures.

The addition of TCNE to a solution of dialkynes **5** and **7** (in CH_2Cl_2, and $CHCl_3$, respectively) resulted immediately in a color change of the reaction mixture, and compounds **13**[18] and **14** were isolated as dark-red colored solids in quantitative and 75% yields, respectively. Since dialkyne **9** was not soluble in solvents that were inert toward TCNE its corresponding TCBD could not be obtained.

The UV–vis spectra of compounds **10** and **13** are shown in Figure 2. An absorption maximum at $\lambda = 321$ nm (ε 21,000 mol^{-1} dm^3 cm^{-1}) was observed for tetracyanobutadiene **10**, while tetracyanobutadiene **13** showed a maximum at $\lambda = 492$ nm with a high molar extinction coefficient of 65,000 mol^{-1} dm^3 cm^{-1}.[19] The molar extinction coefficient was found to be highly concentration dependent, as shown for compound **13**. At $c = 1$ μM, a molar extinction coefficient of 65,000 mol^{-1} dm^3 cm^{-1} at $\lambda_{max} = 492$ nm was found, while at $c = 45$ μM, ε had dropped to 30,000 mol^{-1} dm^3 cm^{-1}. This concentration dependency was a strong indication of (peptide) aggregation, and this effect was more pronounced by elongation of the peptide sequence, from ~Gly-Val-OMe, to ~Gly-Val-Ile-OMe and ~Gly-Val-Ile-Ala-OMe (data not shown).

In conclusion, a new class of peptide-based chromophores has been described featuring the [2+2] cycloaddition–cycloreversion reaction between an electron-rich alkyne and tetracyanoethylene, resulting in intensely-colored peptide constructs with high molar extinction coefficients. This chemistry can be considered as a model study for bioorthogonal modification of peptides with imaging chromophores possessing tunable optical properties. Since the cyanobutadiene scaffold has been functionalized with peptides that have a strong β-sheet propensity, these newly designed peptide chromophores may lead to the development of imaging probes for amyloid deposits.

Acknowledgements

D.T.S.R. gratefully acknowledges the ETH Zürich Research Council for financial support, and Utrecht University for facilitating a sabbatical leave at the ETHZ. Dr. Milan Kivala (Friedrich-Alexander-Universität, Erlangen-Nürnberg, Germany) is acknowledged for his interest and contributions to the scientific discussions with respect to this work. Dr. Johan Kemmink (Utrecht University) is thanked for measuring the ^{13}C HSQC of dialkyne **9**. Professor Rob M. J. Liskamp (Utrecht University) is acknowledged for critically reading the manuscript and for his suggestions, and enthusiastic support.

References and notes

1. (a) Tung, C.-H. *Biopolymers (Peptide Science)* **2004**, *76*, 391; (b) Rao, J.; Dragulescu-Andrasi, A.; Yao, H. *Curr. Opin. Biotechnol.* **2007**, *18*, 17; (c) Lee, S.; Xie, J.; Chen, X. *Chem. Rev.* **2010**, *110*, 3087.
2. Dent, A. H.; Aslam, M. *Bioconjugation, Protein Coupling Techniques for the Biomedical Sciences*; Macmillan: London, 2000. Chapter 2.
3. Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. H. *Science* **1994**, *266*, 776.
4. Saxon, E.; Bertozzi, C. R. *Science* **2000**, *287*, 2007.
5. Gaertner, H. F.; Rose, K.; Cotton, R.; Timms, D.; Camble, R.; Offord, R. E. *Bioconjugate Chem.* **1992**, *3*, 262.
6. (a) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057; (b) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596.
7. (a) Michinobu, T.; May, J. C.; Lim, J. H.; Boudon, C.; Gisselbrecht, J.-P.; Seiler, P.; Gross, M.; Biaggio, I.; Diederich, F. *Chem. Commun.* **2005**, 737; (b) Michinobu, T.; Boudon, C.; Gisselbrecht, J.-P.; Seiler, P.; Frank, B.; Moonen, N. P.; Gross, M.; Diederich, F. *Chem. Eur. J.* **2006**, *12*, 1889; (c) Jarowski, P. D.; Wu, Y.-L.; Boudon, C.; Gisselbrecht, J.-P.; Gross, M.; Schweizer, W. B.; Diederich, F. *Org. Biomol. Chem.* **2009**, *7*, 1312; (d) Wu, Y.-L.; Jarowski, P. D.; Schweizer, W. B.; Diederich, F. *Chem. Eur. J.* **2010**, *16*, 202.
8. Kruijtzer, J. A. W.; Hofmeyer, L. J. F.; Heerma, W.; Versluis, C.; Liskamp, R. M. J. *Chem. Eur. J.* **1998**, *4*, 1570.
9. *N^{α}-(4-Ethynylphenyl)-N^{α}-(methyl)-glycyl-valine methyl ester* (**4**): $R_f = 0.33$ (Et_2O); $[\alpha]_D^{20}$ +51.8 ($c = 0.49$ in $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 0.73$ (d, $^3J(H,H) = 6.9$ Hz, 3H; γCH_3 Val), 0.86 (d, $^3J(H,H) = 6.9$ Hz, 3H; γ′CH_3 Val), 2.12 (m, 1H; βCH Val), 2.99 (s, 1H; HC≡), 3.08 (s, 3H; NCH_3), 3.69 (s, 3H; OCH_3), 3.83 (d, $^3J(H,H) = 18$ Hz, 1H; CH_2 Gly), 3.96 (d, $^3J(H,H) = 38.4$ Hz, 1H; CH_2 Gly), 4.55 (dd, $^3J(H,H) = 4.8$ Hz, $^3J(H,H) = 9$ Hz, 1H; αCH Val), 6.66 (d, $^3J(H,H) = 9$ Hz, 2H; arom H), 6.75 (d, $^3J(H,H) = 9$ Hz, 1H; amide NH), 7.39 (d, $^3J(H,H) = 9$ Hz, 2H; arom H); ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): $\delta = 171.8$, 169.5, 148.5, 133.3, 112.6, 111.4, 84.0, 75.6, 58.1, 56.7, 52.2, 39.7, 31.1, 19.1, 17.6; UV–vis ($CHCl_3$): λ_{max} (ε) = 282 nm (36,750 mol^{-1} dm^3 cm^{-1}); ESMS calcd for $C_{17}H_{22}N_2O_3$: 302.16, found: *m/z* 303.25 $[M+H]^+$, 321.30 $[(M+H_2O)+H]^+$.
10. Hay, A. S. *J. Org. Chem.* **1962**, *27*, 3320.
11. For a review on acetylenic (homo)coupling, see: (a) Siemsen, P.; Livingston, R. C.; Diederich, F. *Angew. Chem., Int. Ed.* **2000**, *39*, 2632; We used the following approach: (b) Batsanov, A. S.; Collings, J. C.; Fairlamb, I. J. S.; Holland, J. P.; Howard, J. A. K.; Lin, Z.; Marder, T. B.; Parsons, A. C.; Ward, R. M.; Zhu, J. *J. Org. Chem.* **2005**, *70*, 703.
12. *Dialkyne* (**5**): $R_f = 0.40$ (EtOAc/Et_2O 1:1); mp 131–132 °C; $[\alpha]_D^{20}$ +110.5 ($c = 0.41$ in $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 0.73$ (d, $^3J(H,H) = 6.9$ Hz, 6H; γCH_3 Val), 0.86 (d, $^3J(H,H) = 6.9$ Hz, 6H; γ′CH_3 Val), 2.14 (m, 2H; βCH Val), 3.09 (s, 6H; NCH_3), 3.69 (s, 6H; OCH_3), 3.85 (d, $^3J(H,H) = 18$ Hz, 1H; CH_2 Gly), 3.98 (d, $^3J(H,H) = 39$ Hz, 1H; CH_2 Gly), 4.55 (dd, $^3J(H,H) = 4.8$ Hz, $^3J(H,H) = 9$ Hz, 2H; αCH Val), 6.66 (d, $^3J(H,H) = 9$ Hz, 4H; arom H), 6.71 (d (overlapping signal), 2H; amide NH), 7.40 (d, $^3J(H,H) = 9$ Hz, 4H; arom H); ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): $\delta = 171.8$, 169.3, 148.9, 133.7, 112.6, 111.1, 81.7, 73.0, 58.0, 56.7, 52.2, 39.7, 31.1, 19.1, 17.6; UV–vis ($CHCl_3$): λ_{max} (ε) = 368 (59,000), 343 (77,000), 322 nm (73,500 mol^{-1} dm^3 cm^{-1}); ESMS calcd for $C_{34}H_{42}N_4O_6$: 602.31, found: *m/z* 603.50 $[M+H]^+$.
13. Peptide synthesis was performed in solution according to: Ray, S.; Das, A. K.; Drew, M. G. B.; Banerjee, A. *Chem. Commun.* **2006**, 4230.
14. *Dialkyne* (**9**): Mp 266 °C (dec); ^1H NMR (300 MHz, DMSO-d_6, 25 °C): $\delta = 0.77$–0.84 (m, 24H; γCH_3 Val/γ′CH_3 Val (4 × 3H)/γCH_3 Ile (2 × 3H)/δCH_3 Ile (2 × 3H)), 1.05 (m, 2H; γ′CH_2 Ile), 1.24 (d, $^3J(H,H) = 7.2$ Hz, 6H; βCH_3 Ala), 1.40 (m, 2H; γ′CH_2 Ile), 1.68 (m, 2H; βCH Ile), 1.94 (m, 2H; βCH Val), 3.01 (s, 6H; NCH_3), 3.58 (s, 6H; OCH_3), 4.06 (m, 4H; CH_2 Gly), 4.22 (m, 6H; αCH Val (2 × 1H)/αCH Ile (2 × 1H)/αCH Ala (2 × 1H)), 6.60 (d, $^3J(H,H) = 9$ Hz, 4H; arom H), 7.28 (d, $^3J(H,H) = 9$ Hz, 4H; arom H), 7.84 (d, $^3J(H,H) = 8.7$ Hz, 2H; amide NH), 7.98 (d, $^3J(H,H) = 9$ Hz, 2H; amide NH), 8.38 (d, $^3J(H,H) = 6.6$ Hz, 2H; amide NH); ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C): $\delta = 133.9$, 112.5, 57.7, 56.6, 55.0, 51.7, 47.8, 39.6, 37.0, 30.7, 24.3, 19.3, 18.1, 16.8, 15.1, 11.1 (16 lines out of 24 based on an HSQC); ESMS calcd for $C_{52}H_{74}N_8O_{10}$: 970.55, found: *m/z* 971.80 $[M+H]^+$, 993.55 $[M+Na]^+$.
15. This reaction is another example of 'click chemistry', see: Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004.
16. *N^{α}-[4-(1,1,4,4-Tetracyanobuta-1,3-dien-2-yl)phenyl]-N^{α}-(methyl)-glycyl-valine methyl ester* (**10**):[20] $R_f = 0.38$ (EtOAc/Et_2O 1:1 v/v), $R_f = 0.42$ ($CHCl_3$/MeOH 95:5 v/v), $R_f = 0.58$ (CH_2Cl_2/EtOAc 1:1 v/v); mp 140–141 °C; ^1H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 0.76$ (d, $^3J(H,H) = 6.9$ Hz, 3H; γCH_3 Val), 0.87 (d, $^3J(H,H) = 6.9$ Hz, 3H; γ′CH_3 Val), 2.15 (m, 1H; βCH Val), 3.24 (s, 3H; NCH_3), 3.71 (s, 3H; OCH_3), 4.03 (d, $^3J(H,H) = 18$ Hz, 1H; CH_2 Gly), 4.14 (d, $^3J(H,H) = 34.2$ Hz, 1H; CH_2 Gly), 4.55 (dd, $^3J(H,H) = 4.8$ Hz, $^3J(H,H) = 8.7$ Hz, 1H; αCH Val), 6.40 (d, $^3J(H,H) = 8.7$ Hz, 1H; amide NH), 6.82 (d, $^3J(H,H) = 9$ Hz, 2H; arom H), 7.48 (d, $^3J(H,H) = 9$ Hz, 2H; arom H), 8.03 (s, 1H; HC=C(CN)$_2$); ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): $\delta = 171.7$, 167.8, 159.8, 155.2, 153.2, 131.8, 119.4, 112.8, 112.7, 111.5, 108.7, 97.6, 82.9, 57.0, 56.8, 52.4, 39.9, 31.1, 19.1, 17.7; UV–vis ($CHCl_3$): λ_{max} (ε) = 553 (6800), 463 (6000), 321 nm (21,000 mol^{-1} dm^3 cm^{-1}); ESMS calcd for $C_{23}H_{22}N_6O_3$: 430.18, found: *m/z* 463.55 $[(M+CH_3OH)+H]^+$, 485.55 $[(M+CH_3OH)+Na]^+$.
17. The residual solvent peak $CHCl_3$ (7.26 ppm) at $T = 298$ K was used as reference.

18. *1,1,4,4-Tetracyanobutadiene* (**13**):[20] R_f = 0.44 (CH_2Cl_2/MeOH 95:5 v/v); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 0.76 (d, ^3J(H,H) = 6.9 Hz, 3H; CH_3 Val), 0.77 (d, ^3J(H,H) = 6.9 Hz, 3H; CH_3 Val), 0.87 (d, ^3J(H,H) = 6.9 Hz, 6H; γCH_3/γ′CH_3 Val), 2.14 (m, 2H; βCH Val), 3.20 (s, 3H; NCH_3), 3.24 (s, 3H; NCH_3), 3.71 (s, 6H; OCH_3), 3.97 (d, ^3J(H,H) = 18.3 Hz, 1H; CH_2 Gly), 4.04 (d, ^3J(H,H) = 18 Hz, 1H; CH_2 Gly), 4.09 (d, ^3J(H,H) = 33.3 Hz, 1H; CH_2 Gly), 4.20 (d, ^3J(H,H) = 32.1 Hz, 1H; CH_2 Gly), 4.53 (ddd, ^3J(H,H) = 0.9 Hz, ^3J(H,H) = 4.8 Hz, ^3J(H,H) = 9.0 Hz, 2H; αCH Val), 6.38 (d, ^3J(H,H) = 9.0 Hz, 1H; amide NH), 6.44 (d, ^3J(H,H) = 9.0 Hz, 1H; amide NH), 6.70 (d, ^3J(H,H) = 9 Hz, 2H; arom H), 6.79 (d, ^3J(H,H) = 9.3 Hz, 2H; arom H), 7.51 (d, ^3J(H,H) = 9 Hz, 2H; arom H), 7.78 (d, ^3J(H,H) = 9.3 Hz, 2H; arom H); ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): δ = 171.7, 168.1, 167.6, 161.4, 153.4, 151.9, 149.3, 135.9, 132.2, 123.0, 119.5, 113.5, 112.6, 112.5, 112.0, 110.8, 107.5, 89.8, 88.6, 77.7, 77.2, 57.0, 56.8, 56.6, 52.4, 39.9, 39.8, 31.1, 19.1, 17.7, 17.6 (31 lines out of 36); UV–vis ($CHCl_3$): λ_{max} (ε) = 492 (65,000), 400 nm (33,700 mol^{-1} dm^3 cm^{-1}); ESMS calcd for $C_{40}H_{42}N_8O_6$: 730.81, found: *m*/*z* 731.50 $[M+H]^+$, 753.45 $[M+Na]^+$, 1462.45 $[2M+H]^+$.
19. In comparison with commonly used fluorophores, among others, 5-carboxyfluorescein: ε = 74,000 mol^{-1} dm^3 cm^{-1}, λ_{max} = 495 nm; 4-chloro-7-nitrobenz-2-oxa-1,3-diazole (NBD-Cl): ε = 9800 mol^{-1} dm^3 cm^{-1}, λ_{max} = 336 nm.
See also: www.invitrogen.com/site/us/en/home/References/Molecular-Probes-The-Handbook.html; last visited on March 7, 2011.
20. Due to the intense color of the TCBDs an accurate $[\alpha]_D^{20}$ value could not be measured.