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Biotin-functional oligo(*p*-phenylene vinylene)s synthesized using click chemistry

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ABSTRACT

Using Cu(1)-catalyzed [3+2] Huisgen 'click' cycloaddition, a rigid rod – like oligo(*p*-phenylene vinylene) (OPV) was functionalized at both ends with biotin ligands, combining the valuable electro-optical properties of conjugated organic molecules with the biological recognition capability of biotin. Biotin can be placed at variable distances from the oligomer via appropriate length of a hydrophilic spacer, which also serves to regulate the binding capabilities of the two terminal biotin units. To demonstrate this binding potential, networks were formed with streptavidin-coated quantum dots. The synthetic conditions are presented, together with representative optimizations of the key reactions. The organic compounds were analyzed by means of ATR/FTIR, ^{1}H NMR (200 or 600 MHz), ^{13}C NMR, 2D NMR (HMBC, HMQC experiments), MS (ESI or MALDI-TOF), and optical spectroscopy. Networks were imaged with TEM.

Oligo(*p*-phenylene vinylene)s (OPVs) and their substituted derivatives belong to the family of conjugated materials with valuable opto-electronic properties such as electroluminescence, photoconductivity, photo-luminescence, nonlinear optical response, and electrical conductivity after doping.[1–4] OPVs are well defined compared to higher molecular weight poly(phenylene vinylene)s, and have the advantage of being soluble and inherently easier to process, making them attractive electronic building blocks for organic functionalization. Biomolecule–polymer conjugates are widely used in medicine and biotechnology,[5–7] the biomolecule being either a macromolecule, for example, proteins, lipids, or ligands, such as sugar or biotin. Biotin binds the protein avidin with $K_a = 10^{15}$. As a consequence, the strong biotin–avidin interaction has been extensively utilized in the life sciences.[8–10] Biotinylated polymers and copolymers of poly(ethylene oxide) (PEO), poly(2-(diethylamino)ethyl methacrylate) (PDEAEMA), poly(ethylene glycol) (PEG), and poly(lactic acid) (PLA) have been studied for various biotechnological applications. PEG has been used as a variable spacer for biotin conjugates with avidin binding capability.[11–13]

The copper catalyzed azide-alkyne 1,2,3–triazole forming 'click' reaction[14–18] is a very popular coupling reaction that demonstrates the concept of click chemistry; the reaction has been used in a variety of fields, for example, drug discovery,[19] materials science,[20] and biology.[21] The chemoselective 'click' reaction is tolerant to the presence of a wide range of functional groups; it has been used to label cells,[22] synthesize dendrimers,[23] conducting polymers,[24–30] polymer modified viruses,[31] and gold nanoparticle–enzyme conjugates.[32] Recently, azide-functionalized biotinylated haptens and mannose-derivatized azide have been synthesized using a Kenner-type linker for the preparation of small-molecule-arrays.[33]

Herein, we report on using the copper catalyzed azide-alkyne 'click' cycloaddition reaction for the efficient functionalization of alkyne modified OPV oligomers with biotin–PEG–azides. The syntheses of the respective dialkyne functional OPVs and azide-terminated biotin–PEG derivatives are also presented. The luminescence properties of the resulting biotin–'clicked'–OPV conjugates were probed, and networks with streptavidin coated quantum dots (Qdots) were produced, and characterized using transmission electron microscopy (TEM).

The general synthetic strategy involves preparing two intermediates first, dialkyne functional oligomer **2** and azide functional biotin–PEG units **7** or **8** (Scheme 1), which then are subsequently combined by means of 'click'-chemistry. The approach incorporates flexibility: the conjugated oligomer can be chosen from a large library of oligomers or other molecules, depending on the desired electrooptical characteristics, or the targeted aggregation behavior. Compounds **7** and **8** represent a second platform for a library of potentially valuable reactions, involving a straightforward functionalization with biotin or other ligand segments of varying hydrophilicity via 'click'-chemistry; biotin may be placed at a defined distance from the conjugated oligomer by simply choosing a PEG segment of the desired chain length, depending on the specific requirements of the potential application. The chain length of the PEG segment also controls the biotin binding

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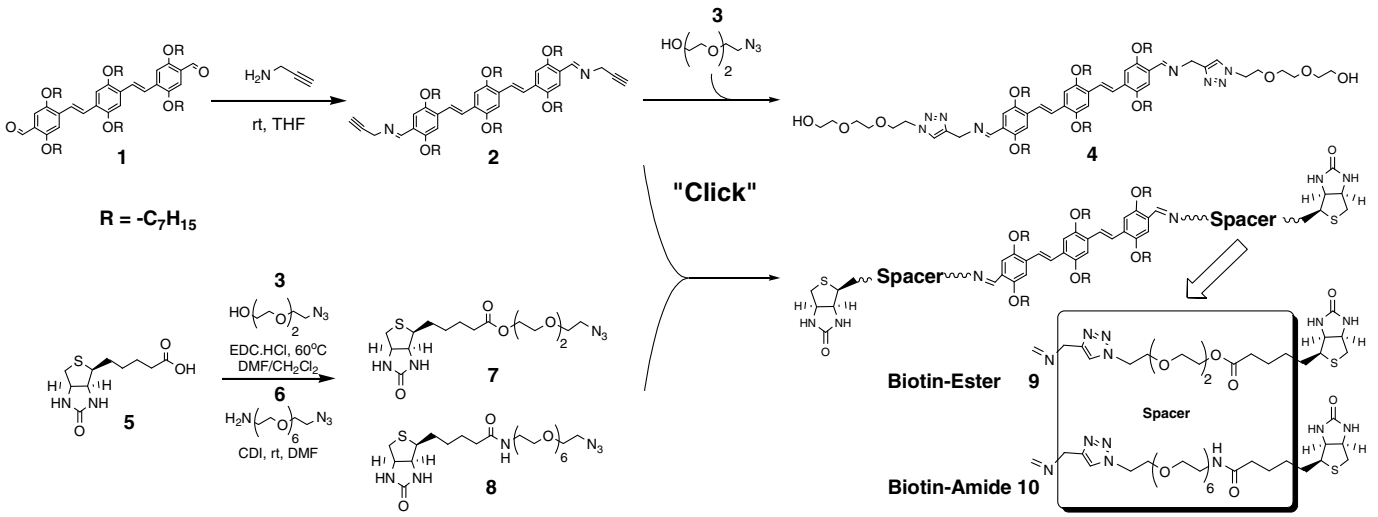

Scheme 1. 'Click' functionalization of oligo(*p*-phenylene vinylene).

capability as well as the overall hydrophobic (conjugated oligomer)/hydrophilic (biotin–PEG spacer) balance of the building blocks.

Specifically, the conjugated oligomer OPV, trimer dialdehyde **1**, was synthesized and characterized in analogy to literature,[34,35] and subsequently reacted with propargylamine under Schiff base formation conditions to produce dialkyne functionalized trimer OPV **2** in quantitative yields (Scheme 1).

In a next step, we synthesized azide functional biotin–PEG conjugates **7** and **8** (biotin–PEG–N3) with different PEG spacers using either 1,3-dicyclohexylcarbodiimide (DCC), carbonyldiimidazole (CDI), or 1-[3-(dimethylamino) propyl]-3-ethylcarbodiimide hydrochloride (EDC·HCl) mediated coupling. Two coupling reactions, that is, esterification and amidation, were employed. In order to vary the distance of biotin from the conjugated oligomer and overall hydrophobic/ hydrophilic balance (see above), we used PEG segments of different lengths for the esterification and amidation, the latter using four additional repeat units in the PEG segment. Representative optimization reactions are summarized in Table 1 and show satisfactory yields.

Using DCC in DMF or DMSO/CH_2Cl_2 resulted in no reaction or trace yields of the biotin–ester **7** after about 20 h at room temperature, as did attempts using EDC.HCl in DMF or CDI in DMSO under similar conditions (not shown in Table 1). The reaction worked when CDI or EDC.HCl together with 4-dimethylaminopyridine (DMAP) in DMF was employed; modest yields of **7** could be achieved after 18 h at ambient conditions (Table 1, entries 5 and 1, respectively). The coupling reagent EDC·HCl was selected because its urea byproduct is water-soluble, hence enabling more facile purification of the target ester. The reaction conditions were further optimized: increased yields could be obtained at higher reaction temperatures and longer reaction times, and/or with the solvent mixture DMF/CH_2Cl_2 (Table 1, entries 2, 3, and 7).

The amidation proved easier than the esterification and proceeded smoothly at ambient temperatures to give **8** with significantly higher yields than ester **7** under analogous conditions (Table 1, entries 4 and 6), using a commercial NH_2-functional PEG azide, and making further optimizations unnecessary. It should be noted that the commercial NH_2-functional PEG azide (~90% purity) was used without any further purification.

Finally, in the key step, intermediate azide functional PEG–biotins **7** and **8** were 'clicked' onto the dialkyne functional OPV **2**, yielding the target conjugates **9** and **10** (Scheme 1). Representative synthesis results are summarized (Table 2).

Initial 'clicking' attempts using $CuSO_4{\cdot}5H_2O$/sodium ascorbate mixtures were unsuccessful in various solvent mixtures (not shown in Table 2), resulting in the degradation of the two imine functions in **2** and the re-formation of the corresponding aldehyde functional **1**. Model reactions of alkyne **2** and PEG–azide **3** via [3+2] Huisgen cycloaddition in the presence of commercially available $Cu(CH_3CN)_4PF_6$ resulted in very good yields of the corresponding PEG-functional OPV **4** (Scheme 1) (Table 2, entries 1–3). Incidentally, **4** represents a potentially valuable material in its own rights.

We then proceeded to 'click' the dialkyne-functional OPV **2** and the azide-terminated biotin conjugates **7** and **8** under similar mild reaction conditions. The reactions were performed in CH_2Cl_2 using 0.2–0.5 M equiv of Cu(I) catalyst to give the 1,4-triazoles in good to very good yields (Table 2, entries 4–7). The main challenge consisted in finding conditions, under which the formation of the byproduct OPV (di)aldehyde (see above) was minimized, as it

Table 1
Representative synthesis results of biotin–PEG–N_3's **7** and **8**[b]

Entry	Coupling reagent/catalyst	Solvent	*T* (°C)/*t* (h)	Yield[a] (%)
1	EDC·HCl[c]/DMAP	DMF	rt/18	20
2		DMF/CH_2Cl_2	60/24	65
3			rt/48	60
4				80[b]
5	CDI[c]	DMF	rt/18	30
6				65[b]
7			80/24	40

[a] Isolated yield.
[b] Isolated yield of **8**.
[c] EDC·HCl = 1-(3-(dimethyl amino) propyl)-3-ethylcarbodiimide hydrochloride, CDI = carbonyl diimidazole; conditions: [Biotin] = 80 mM; [PEG–N_3] = 100 mM; [EDC·HCl] = 100 mM; [CDI] = 234 mM; rt = ambient temperature.

Table 2
Representative click reactions of OPV dialkyne **2**[a]

Entry	Azide	Product	$Cu(CH_3CN)_4PF_6$ (mol equiv)	*T* (h)	Yield[b] (%)
1	**3**	**4**	1	8	80
2	**3**	**4**	0.2	10	90
3	**3**	**4**	0.2	18	95
4	**7**	**9**	0.5	18	70
5	**7**	**9**	0.2	18	85
6	**8**	**10**	0.5	18	85
7	**8**	**10**	0.2	18	95

[a] Conditions: [**2**] = 5.7 mM; [**3**], [**7**], and [**8**] = 13.8 mM; ambient temperature; solvent CH_2Cl_2.
[b] Yields based on ^1H NMR.

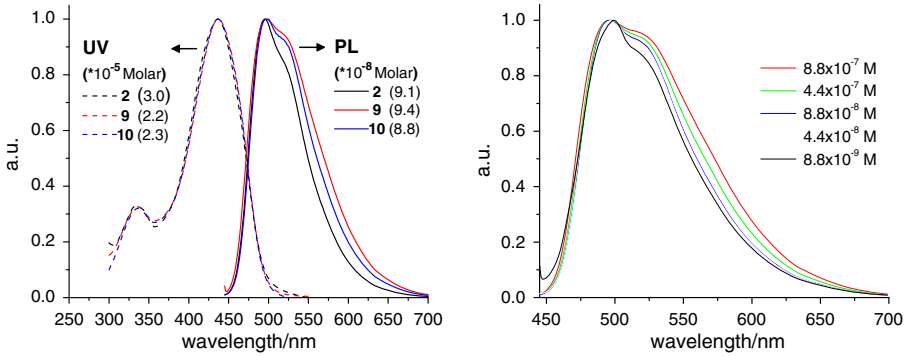

Figure 1. UV–vis absorption and emission (normalized) of OPVs **2**, **9**, and **10** (left), concentration-dependent emission of **10**; solutions in DMF, excitation at 438 nm.

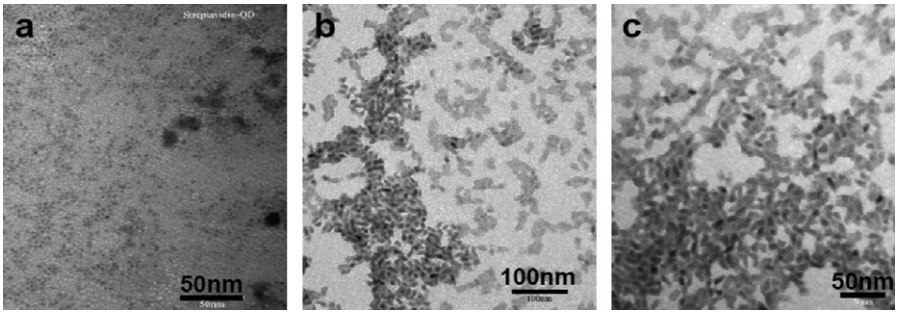

Figure 2. TEM images of (a) streptavidin Qdot control, (b) network of streptavidin Qdots and 18 μM biotin–OPV **10**, and (c) network of streptavidin Qdots and 1 μM biotin–OPV **10**.

could not be avoided entirely (~5% by ^{1}H NMR). When larger amount of Cu catalyst was employed more aldehyde byproduct was produced (Table 2, entries 1, 4, and 6 vs the respective other entries). Also, the ester **7** produced more aldehyde than the amide **8**, resulting in overall lower yields in **9** vs **10**. After workup, the compound purity was 95%; this level of purity is quite reasonable if one takes into account the fact that these molecules are oligomers with molecular weights over 1500 D. Attempts to purify the samples further via column chromatography lead to no additional improvement. The best and most consistent results were obtained after 18 h using 0.2 equiv of catalyst (Table 2, entries 2, 5, and 7).

The compounds synthesized were analyzed by means of ATR/FTIR, ^{1}H NMR (200 or 600 MHz), ^{13}C NMR, and MS (ESI or MALDI-TOF). The Cu-catalyzed cycloaddition typically leads to exclusively 1,4 product, heteronuclear NMR-coupling experiments confirmed this in the cases of **4**, **9,** and **10** (see Supplementary data). The electro-optical properties of the OPV are retained after the attachment of the biotin–spacer elements, as can be observed from the very similar absorption and photoluminescence characteristics of **2**, **9**, and **10** in DMF solution (Fig. 1, left). Due to the high fluorescence quantum efficiency of the trimeric OPV moiety,[36] the biotin OPV conjugates show fluorescence even at very low concentrations. Together with a high molar extinction coefficient,[36] the optical properties make these materials attractive candidates for imaging/biosensing applications. A broadening of the fluorescence in the longer wavelengths in the cases of **9** and **10** is most probably a consequence of enhanced aggregation of the fluorophore moieties in the polar solvent DMF, and supported by concentration-dependent emission spectra (Fig. 1, right).

One objective for synthesizing soluble biotin–OPV was to see if they could be used as 'adaptors' to connect streptavidin coated quantum dots (Qdots). Preliminary studies were performed to evaluate the abilities of **9** and **10** to bind the biotin binding sites on commercially available streptavidin Qdots 655 nm by incubating the (biotin–OPV–biotin)s **9** and **10** with the Qdots. The Qdots polyvalently display biotin binding sites; we anticipated the addition of bivalent biotin–OPV would result in a network of Qdots connected by OPV linkers. The network formation was observed using transmission electron microscopy (TEM) (Fig. 2). The Qdot control sample (Fig. 2a) shows no aggregation. Samples with combined streptavidin-Qdots and biotin–OPV–biotin **10** clearly show network formation (Fig. 2b and c). In contrast, multiple attempts with biotin–OPV–biotin **9** (not shown) yielded inconclusive results. A tentative explanation could be that the longer PEG spacer in the case of **10** is necessary for the biotin moieties to access the streptavidin binding sites.

The electro-optical properties of **9**, **10**, as well as the networks with two interacting electronic systems are currently under further investigation and will be presented elsewhere. Also, the fabrication of biotin–OPV monolayers on silica chips is underway for arraying Qdots in two dimensions for bio-sensing applications.

In conclusion, we have developed a highly efficient, novel approach to combine the electro-optical properties of conjugated organic molecules, such as OPVs, with the biotechnological functionalities of biotin, using the Cu(I)-catalyzed [3+2] Huisgen cycloaddition. This click chemistry route for synthesizing conducting/conjugated oligomer-based biomolecules described here will be useful for the design of a variety of biologically active molecules, biosensors, and biomedical device coatings.

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Supplementary data

Supplementary data (experimental and analytical details) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.08.080.

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