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Supramolecular PEG−*co*-Oligo(*p*-benzamide)s Prepared on a Peptide Synthesizer

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Abstract: An automated synthesis protocol has been developed for the preparation of oligo(*p*-benzamide)s on solid support using a commercial peptide synthesizer employing a variation of standard Fmoc chemistry. Bis(trichloromethyl carbonate) in NMP was used to activate the aromatic carboxylic acids for acylation of secondary aromatic amines on solid support. N-Protected hepta(*p*-benzamide) was automatically prepared on solid support and manually converted to a solid supported block co-oligomer by attaching a poly(ethylene glycol) chain. Cleavage from the support could be achieved with minimal loss of the *p*-methoxybenzyl N-protective group. While the N-protected block co-oligomer was molecularly dissolved in nonpolar organic solvents, the N-deprotected block co-oligomer adopted a rod−coil conformation and showed strong aggregation as evidenced by gel permeation chromatography and transmission electron microscopy. Rigid rodlike aggregates could be observed in chloroform, toluene, as well as water.

Introduction

With the development of a broad range of living polymerization techniques, chemists have been able to design well-defined polymers as well as block copolymers and gain insight into their solution- and solid-state organization. However, discrete molecular weights and monomer sequence control typically observed for biomacromolecules cannot be achieved with these polymerization techniques. In recent years, the boundary between classical multistep organic synthesis and polymer chemistry has been crossed by many groups with the aim of creating new block copolymer architectures in which at least one block is precisely defined.[1] The growing interest in self-assembled solution- and solid-state structures with dimensions on the nanometer scale is a driving force for the development of multistep polymer syntheses employing the tools of synthetic organic chemistry.

One well-established way of preparing precisely defined oligomers and polymers is solid supported synthesis. The idea, which was first described by Merrifield,[2] has since been well-developed and automated for a variety of biologically relevant macromolecules. These include α- and β-peptides, glycopeptides, oligonucleotides, and oligosaccharides.[3] One of the first of very few examples where solid supported synthesis was employed for the preparation of materials were the liquid crystalline oligopeptides reported by Cormack et al.[4] Peptide/polymer hybrids prepared on solid support have also been shown by the Klok group[5] and Wooley et al.[6,7] Our group recently reported the solid supported synthesis of oligo(*p*-benzamide)s (OPBA) up to the decamer.[8] Other non-natural oligomers have also been prepared on solid support.[9]

The great advantage of solid supported syntheses is the potential for automation. This aspect has, however, only been addressed for non-natural oligomers in a few cases.[10−12] Commercial peptide synthesizers are typically laid out for the synthesis of Boc- or Fmoc-peptide synthesis (i.e., the synthesis of aliphatic amides with well-established coupling protocols). Repetitive syntheses of oligomers requiring reaction conditions drastically different from those preprogrammed into the peptide synthesizer are often not feasible to be carried out in these machines without major modifications. As a result, automation of oligomer syntheses, especially in the field of materials chemistry, is scarce.

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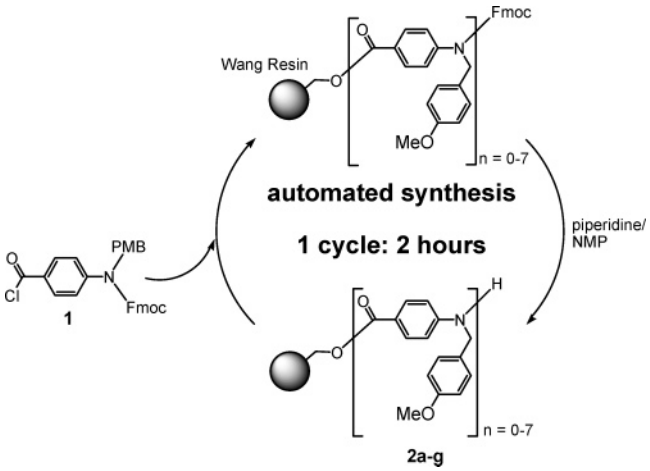
Scheme 1. Solid-Phase Synthesis of Oligo(*p*-benzamide)s **2a**−**g** Using a Modified Fmoc Peptide Coupling Protocol[8]



Here, we demonstrate the first example of an automated oligo(*p*-benzamide) synthesis carried out in a conventional peptide synthesizer employing a standard Fmoc compatible protocol. We also describe the linking of a polydisperse polymer to the solid supported OPBA, thereby preparing a rod−coil copolymer entirely on solid support.

Such rod−coil block copolymers are interesting building blocks for the formation of anisotropic supramolecular structures on the nanometer scale.[13−17] Automated synthesis provides rapid access to shape-persistent molecular nano-objects that could be conjugated with synthetic or biomacromolecules. To exemplify this potential, we chose an OPBA−PEG conjugate as our target structure. This also allowed us to compare the efficiency of the solid supported synthesis to a previously reported solution synthesis of similar materials.[18−20]

Results and Discussion

Automation of Oligomer Synthesis. The automated solid supported synthesis of an OPBA heptamer is shown in Scheme 1. Monomer **1** was synthesized from *p*-aminobenzoic acid via N-reductive alkylation with anisaldehyde followed by reaction with 9-fluorenylmethyl chloroformate (Fmoc-Cl).[8] The *N*-*p*-methoxybenzyl (PMB) protective group was introduced to prevent aggregation during oligomer synthesis.

We previously reported a carboxylic acid activation using thionyl chloride according to a method described most recently by the Ueda group[21] but initially described by Zollinger et al.[22] Here, we employed bis(trichloromethyl)carbonate (BTC, i.e., "triphosgene") in NMP[22,23] to generate the carboxylic acid chloride. This method avoids the generation of corrosive SO_2 gas and can therefore be safely carried out in a peptide synthesizer. Three different procedures for generating the activated amino acid **1** were examined for suitability in automated synthesis: (1) A stock solution of **1** in NMP was prepared outside the peptide synthesizer and subsequently filled into amino acid cartridges. (2) BTC and amino acid were filled as solids into a synthesizer cartridge and activated just prior to the coupling step by the automated addition of NMP. (3) 2-Chloro-*N*-methyl-1-pyrrolinium chloride[24] was isolated as a solid (see Supporting Information) and filled into an amino acid cartridge together with the amino acid. Automated addition of NMP resulted in activation of the amino acid. Oligomers could be synthesized automatically by all three methods; however, the pre-activation outside the peptide synthesizer proved to be advantageous as the solution could be freed of insoluble particles by syringe filtration, thereby avoiding potential clogging of the peptide synthesizer lines. Also, the reaction of BTC with NMP is very exothermic and generates CO_2. This can potentially result in contamination of the synthesizer lines and is therefore best carried out outside the peptide synthesizer.

An OPBA heptamer (**2g**) was prepared on the peptide synthesizer. The heptamer was chosen so we would be able to compare results from the solid supported synthesis to a previously reported solution synthesis that also yielded a hepta(*p*-benzamide).[18,20] RP-HPLC analysis of **2g** showed several peaks between 35 and 40 mL elution volume corresponding to the fully PMB-protected **2g** as well as mono PMB-deprotected isomers of **2g** as was shown by ESI mass spectroscopy. Compared to the solid supported synthesis carried out manually,[8] the automated synthesis has proved to be superior in several ways: (1) The yield of the oligomers was improved significantly. This is most likely because all chemical reactions as well as wash cycles were carried out under inert gas. (2) The mass recovery of functionalized resin was significantly higher as resin loss due to benchtop manipulations was avoided.

Rod−Coil Block *co*-Oligomer Synthesis. The hepta(*p*-benzamide) (**2g**) was reacted outside the peptide synthesizer with pentynoic acid chloride (**3**) to give compound **4** (Scheme 2). Azide functionalized monomethyl−PEG (**5**) was prepared from commercially available poly(ethylene glycol)monomethyl ether via tosylation[25] and reaction with sodium azide. **5** was attached to the alkyne functionalized **4** via [2 + 3]-cycloaddition using sodium ascorbate/$CuSO_4$[26] to yield solid supported block co-oligomer **6** linked via a 1,2,3-triazole unit. "Click Chemistry" was used for the coupling step as it provides high yielding reactions from very stable and chemically relatively inert coupling partners (i.e., the polymeric azide and the alkyne). The PEG−azide especially can be stored without notable decomposition for many weeks and used in coupling reactions without further activation steps. Additionally, the 1,2,3-triazole group provides a very robust linker that proved to be chemically inert under conditions for PMB deprotection.

Polymer **6** could be cleaved from the solid support by treatment with TFA/DCM (50%) to give 800 mg (ca. 83% based

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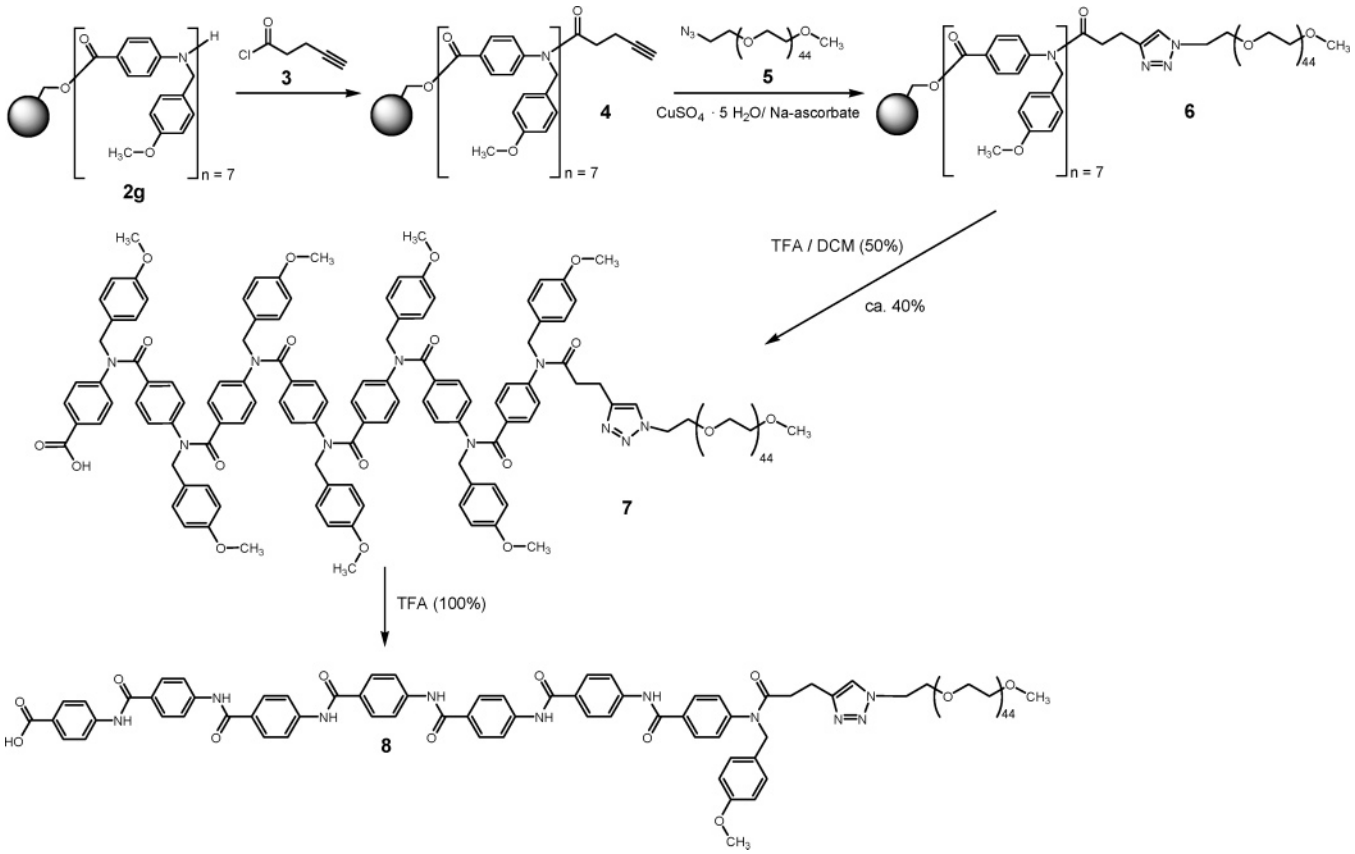
Scheme 2. Synthesis of the Hepta(*p*-benzamide)s-*b*−PEG Rod−Coil Copolymer **8** on Solid Support



on maximum resin loading) of the fully PMB-protected block co-oligomer **7**, which showed no aggregation in nonpolar solvents and could be purified by preparative gel permeation chromatography (GPC). Copolymer **8** was obtained by removal of the PMB protective groups with TFA (100%) and was characterized by ^{1}H NMR spectroscopy (Figure 1). Each transformation from compound **2g** to **7** was followed by RP-HPLC. Analytical amounts of oligomer were cleaved off the resin for this purpose. By attaching the highly polar monomethyl−PEG−azide (**5**) onto solid supported **4** to give **6**, the elution time was strongly decreased, as expected (see Supporting Information).

Successful attachment of the PEG block was confirmed by an ESI-mass spectrum of **7**, which shows only the fully PMB-protected product structure as a triple and quadruple charged mass distribution (see Supporting Information). All peaks in the mass spectrum could be assigned; the mass distribution of the sample is entirely due to the polydisperse PEG coil block.

After cleaving compound **7** from the resin and purifying it via preparative GPC in chloroform, we removed the PMB protection by stirring in TFA (100%) for 24 h at room temperature. ^{1}H NMR experiments revealed that deprotection was successful for all PMB protective groups except for the N-terminal one. Figure 1 (top) shows the ^{1}H NMR spectrum of an analytical sample of **4**, cleaved from the resin for NMR comparison. Figure 1 (middle) shows the ^{1}H NMR spectrum of PMB-protected compound **7** and PMB-deprotected polymer **8** (bottom). The ^{1}H NMR spectra for **4** and **7** are virtually identical as far as aromatic protons are concerned. The signal H2 (Figure 1) is the most upfield shifted PMB methylene signal. It was therefore assigned to the N-terminal PMB group. As can be seen in Figure 1 (bottom), all NMR signals due to the PMB groups have disappeared except for the N-terminal one. The aromatic signals for the N-terminal PMB group, H5 and H6 (Figure 1, bottom), can also be observed and have not shifted significantly compared to the spectrum of **7** (Figure 1, middle). Additionally, the appearance of signals at ca. 10.5 ppm due to the aromatic amide N−H groups proves the successful PMB deprotection.

The difficulty in removing the N-terminal PMB group is most likely due to the reduced conjugational stabilization of the O-protonated amide. As amide O-protonation is most likely the initial step in the mechanism of PMB cleavage, the N-terminal PMB group is more difficult to remove. PMB cleavage from aliphatic amides has been reported to require harsher conditions,[27] whereas aromatic amides have been deprotected at room temperature.[28] It is important to mention that failure to remove the N-terminal PMB group has no adverse effect on aggregation and superstructure formation.

The solid supported synthesis of oligo(*p*-benzamide) block copolymers has several advantages compared to the previously reported solution synthesis of analogous copolymers. In the previous case, the oligomer had to be built up in a polymer analogous reaction using soluble precursors. As the precursors themselves had to be synthesized in multistep reactions, the overall rod−coil copolymer was typically prepared on the time scale of several weeks. With the approach described here, the manual synthesis is reduced to very few steps and the PMB-

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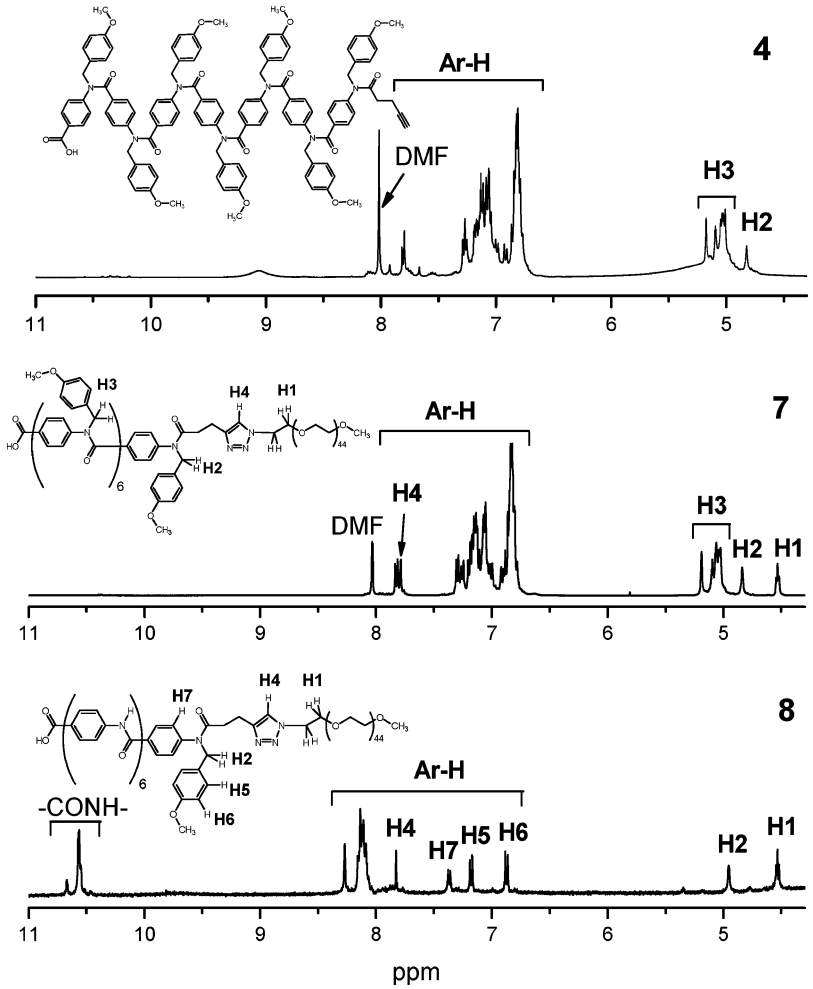

Figure 1. ^{1}H NMR (DMF-d_7). Top: Analytical sample of **4**, cleaved from the resin. Middle: End group analysis of purified block co-oligomer **7** and **8** (bottom). Only the oligo(*p*-benzamide) end groups are shown. The PEG signal is omitted for clarity. The DMF peak at $\delta = 8.03$ ppm in the bottom spectrum was suppressed experimentally. This allowed the recording of the low-intensity peaks H4−H7. The aromatic signals between 8 and 8.3 ppm in the bottom spectrum are therefore artificially reduced in intensity.

deprotected final copolymer can be prepared on the time scale of several days. In addition, the solid supported synthesis using a capping step in between monomer couplings ensures that only the product structure is eventually coupled to the polymeric coil block. In the solution synthesis of copolymers, complete reaction of every monomer unit has to be ensured before adding the next monomer, as purification and separation from faulty couplings are generally not possible.

The time advantage in the preparation of these copolymers on solid support is very important as it will allow the rapid synthesis of many structurally diverse oligomers and their corresponding block copolymers. The structural diversity of supramolecular polymers is very important to gain insight into the molecular structure−super structure relationship.

We expect that the solid supported synthesis described here will be feasible for the preparation of copolymers on the multigram scale. We believe that the combination of a solid supported synthesis that allows the rapid evaluation of structurally diverse architectures in combination with a solution synthesis that can produce materials on the multi-10 g scale will allow for an efficient screening process in the search for new supramolecular materials.

Aggregation Studies. In addition, the removal of the PMB protective groups was indirectly confirmed by examining a chloroform solution of **8** by GPC (see Supporting Information). Block co-oligomer **7** adopted a random coil-like structure that is molecularly dissolved in chloroform. By removing the *N*-PMB protective groups, the thermodynamically favored trans-conformation[29] of the OPBA (with respect to the phenyl rings) is adopted. This resulted in a rigidification of the OPBA block and thereby formation of a rod−coil block co-oligomer. Because of the formation of hydrogen bonds among the rodlike OPBA, strong aggregation could be observed in nonpolar solvents such as chloroform (see Supporting Information).

A chloroform solution of **8** was drop cast onto carbon-coated copper grids and investigated by TEM, STEM, and cryo-TEM (see Supporting Information). Figure 2 (left) shows an STEM as well as TEM image (inset) in which rodlike micellar aggregates of **8** extending over several hundreds of nanometers can be seen. The TEM images show only the stacks of aromatic

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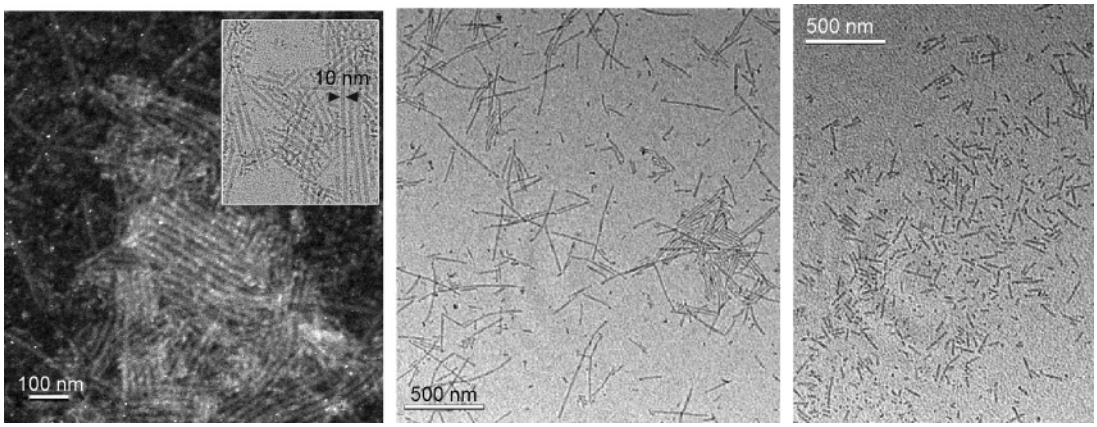

Figure 2. Left: scanning TEM image of **8** deposited from chloroform solution ($c = 0.5$ g L^{-1}) onto carbon-coated copper grids. The inset (left) shows a TEM image of **8** with the same magnification ($c = 0.5$ g L^{-1}). Middle: TEM image of **8** deposited from toluene solution ($c = 0.5$ g L^{-1}) onto carbon-coated copper grids. Right: TEM image of **8** deposited from water solution ($c = 0.5$ g L^{-1}) onto carbon-coated copper grids. Rodlike micelles can be seen in all three images.

oligomers; the PEG coils are not visible but most likely the reason for the spacing of the rodlike micelles. A width of ca. 10 nm was measured for the micelle cores (see Figure 2, left, inset). This value is in good agreement with the hockey puck micelle model proposed previously.[20] TEM measurements from toluene and water solution of **8** were also carried out (Figure 2, middle and right). The same rodlike micellar aggregates with identical dimensions were observed as for the chloroform solution. This clearly shows that the strongly aggregating oligo(*p*-benzamide) drives the aggregation process irrespective of the solvatization of the PEG coil block. This interesting phenomenon as well as the influence of coil blocks other than PEG will be studied in more detail in the future.

In conclusion, we have described the first automated solid supported synthesis of oligo(*p*-benzamide)s using a conventional peptide synthesizer with a standard Fmoc chemistry compatible synthesis protocol. This opens the way for the potential to synthesize strongly aggregating nano-object copolymers with varying sequence-defined molecular geometries. It also reduces the labor-intensive synthesis of these strongly aggregating supramolecular synthons to a few manual steps. The compatibility with the automated synthesis of peptides opens the path for potential hybrid structures such as rod−coil peptide amphiphiles. To exemplify the synthesis of hybrid structures, the supported oligomers were linked to a poly(ethylene glycol) coil block to give a block co-oligomer carrying the precisely defined oligomer. The oligomer was N-deprotected to adopt the thermodynamically preferred rodlike (all-trans) and strongly aggregating conformation. The resulting rod−coil block co-oligomer was analyzed by GPC, giving evidence for strong solution aggregation of the block co-oligomer. TEM imaging revealed rigid rodlike micelles stretching over several hundred nanometers. The ability to use solid support in the synthesis of shape-persistent nanoscale molecular objects, such as OPBAs, provides a combinatorial way for the development of a wide range of diverse supramolecular architectures. The synthesis protocol is compatible with commercial peptide synthesizers and gives access to the rapid preparation of shape-persistent nanoscale molecular objects. These objects could further serve as scaffolds for the positioning of functional groups at precisely defined distances with respect to each other, thus opening new possibilities for catalysis.

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Supporting Information Available: Experimental section describing the syntheses as well as characterization of all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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