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Modular DNA-Programmed Assembly of Linear and Branched Conjugated Nanostructures

Kurt V. Gothelf,*,† Anne Thomsen,† Morten Nielsen,† Emiliano Cló,† and Raymond S. Brown‡

Center for Catalysis and Interdisciplinary Nanoscience Center (iNANO), Department of Chemistry, Langelandsgade 140, Aarhus University, 8000 Aarhus C, Denmark, and Department of Molecular Biology, Gustav Wieds Vej 10C, Aarhus University, 8000 Aarhus C, Denmark

Received September 4, 2003; E-mail: kvg@chem.au.dk

The ability to arrange and connect molecules is a major challenge in nanoscience today. Molecular electronic components have been made, for example,[1−8] but we are currently unable to assemble them into specifically designed circuits. There is a universal need to find a material that can be widely used as a platform for building nanodevices and machines. In a bottom up approach, nanostructures are built atom by atom or molecule by molecule. Because it is not possible to efficiently fabricate nanosized devices by scanning-probe techniques, an alternative is to design molecular components capable of self-assembly.[9] It has been shown that complex artificial supramolecular structures can be produced in laboratories.[10,11] However, an ideal system requires the design of a set of molecular building blocks, which can be encoded to self-assemble into a variety of stable and well-defined nanostructures. Currently, this principle has been demonstrated only on the micrometer scale when three-dimensional electronically functional networks were formed via patterned polyhedra by capillary forces.[12]

We have developed a bottom up approach using molecular modules that self-assemble and couple covalently into predetermined nanomatrix. Complementary sequences attached to linear and three-way branched organic modules direct assembly without the need for any additional DNA templates. The modules are encoded as components that can be programmed to make a variety of structures. The macromolecular structures are with their conjugated backbone and metal links potential conductors and could be the basis for molecular electronic circuits.

It has previously been reported that organic compounds and materials linked to short DNA sequences can be selected and juxtaposed as a consequence of their DNA base pairing.[13−17] Our goal is to develop a set of molecular modules to build predesigned covalently linked structures. Czlapinski and Sheppard have shown that metal−salen formation between two salicylaldehydes attached to short oligonucleotides can be controlled by a DNA template.[18] In a similar way, other custom designed reactions between compounds attached to DNA can be directed.[19−26]

In our molecular engineering strategy, two or three salicylaldehyde groups are contained within the same compound, enabling the assembly and covalent coupling of multiple modules. Furthermore, no additional DNA template is needed because the oligonucleotides attached on either side of the salicylaldehyde groups act as clamps to hold the organic modules in a predetermined arrangement (Figure 1). We have synthesized linear oligonucleotide-functionalized modules (LOMs) and tripoidal oligonucleotide-functionalized modules (TOMs) (Figure 1A). The organic backbone of the modules is synthesized by the coupling of 5-iodosalicylaldehyde derivatives to 1,4-di- and 1,3,5-triethynylbenzene.[27−29]

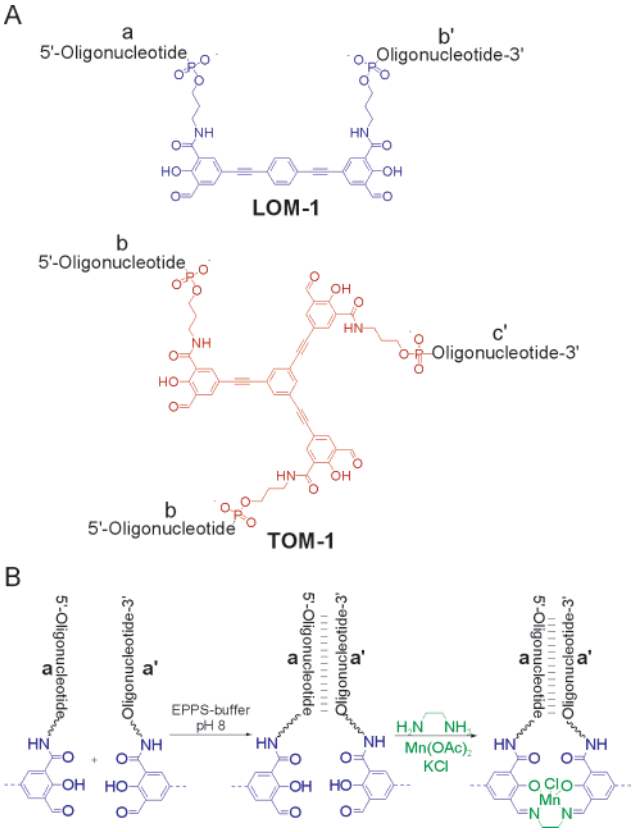

Figure 1. Chemical structures of LOM and TOM. (A) LOM-1 contains two 15-nt DNA sequences a and b′ (see Table 1). TOM-1 contains three 15-nt DNA sequences, one c′ and two b chains. (B) DNA-controlled formation of a covalent manganese−salen link between two salicylaldehyde termini linked to complementary DNA sequences.

Modules have 15 nucleotides (nt) at each terminus, which can link up others containing complementary sequences.

The salicylaldehyde groups of two modules are brought in close proximity when their complementary DNA sequences are annealed together. This enables us to control coupling of the two functional groups to give a manganese−salen link between two modules by reaction with ethylenediamine and a manganese salt. Because this is a pseudo intramolecular reaction, it is considerably faster than the nondirected reaction. In fact, no products of the latter reaction were actually observed in our experiments.

The oligo(phenylene ethynylene)-based backbones of the linear and tripoidal modules are rigid linear and planar structures in which the geometrical freedom is limited to rotation around acetylene bond axes (Figure 1A). The metal−salen forming coupling reaction was deliberately chosen, because the linkages between the individual headgroups of the modules will be essentially linear due to the

† Department of Chemistry.
‡ Department of Molecular Biology.

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Table 1. DNA Sequences for the LOM and TOM Modules

module		DNA sequences
LOM-1	a-LM-b′	5′-ATTGATCTAGTTGAT-LM-TGTACATCTACACTT-3′
LOM-2	b-LM-c′	5′-AAGTGTAGATGTACA-LM-ACTTCAGTTGGTCGT-3′
LOM-3	c-LM-d′	5′-ACGACCAACTGAAGT-LM-CTGTAGACATATGTT-3′
LOM-4	e-LM-a′	5′-ACAACTAGGACCTAA-LM-ATCAACTAGATCAAT-3′
TOM-1[a]	b_2-TM-c′	(5′-AAGTGTAGATGTACA)$_2$-TM-ACTTCAGTTGGTCGT-3′
TOM-2[b]	c-TM-a′	5′-ACGACCAACTGAAGT-TM-ATCAACTAGATCAAT-3′
TOM-3[a]	f_2-TM-b′	(5′-ATAGAGCTCGTGTTC)$_2$-TM-TGTACATCTACACTT-3′

[a] Two similar sequences have been synthesized from the two DMTr sites of a TM. [b] The third hydroxy functionality of the TM contains no oligonucleotide.

stereochemistry of the manganese−salen complex formed (Figure 1B). The salen ligand adopts a locked coplanar geometry by chelating a manganese ion.[30] Nanomatrix based on our module components can therefore possess high structural order.

LOMs are bifunctional and TOMs are trifunctional, and they can be combined to form a variety of linear and branched predefined manganese−salen-linked structures. Encoding of each module is easily achieved by placing the organic part at the center of the chosen nucleotide sequences by automated DNA synthesis. In this manner, four encoded LOMs and three encoded TOMs were prepared, and the DNA sequences are given in Table 1.

Self-assembly and coupling of up to four differently encoded linear modules at 25 °C are described in Figure 2A−C. Reaction products, salen-linked dimers, trimers, and tetramers (**S-1**, **S-2**, and **S-3**), were analyzed by denaturing polyacrylamide gel electrophoresis in 8 M urea (Figure 2D). The dimer **S-1** is formed by coupling of **LOM-1** with **LOM-2**, and the reaction is essentially complete under these conditions. The coupling of three linear modules produces only very small amounts of dimers (Figure 2, lane 4). This clearly demonstrates the bifunctional reactivity of the central module. The mass of the products **S-1** and **S-2** was confirmed by MALDI-TOF. It is also possible to efficiently assemble four linear modules (Figure 2D, lane 5). Under denaturing conditions, minor gel bands are seen in Figure 2, lanes 4 and 5. However, in all reactions, the expected product corresponds to the major gel band.

A series of control experiments are shown in Figure 2D, lanes 6−10. Three LOMs were first annealed in the absence of the coupling reagents, but their short DNA double helices are completely disrupted in 8 M urea on the gel (Figure 2D, lane 6) to the original single-stranded monomers. In another experiment, pairs of linear modules with noncomplementary DNA sequences are mixed and treated with the coupling reagents. Only unreacted monomers are seen (Figure 2D, lanes 7 and 8). Covalently linked linear modules are not formed if either one of the coupling agents, ethylenediamine and $Mn(OAc)_2$, is absent (Figure 2D, lanes 9 and 10).

More complex structures can be built by combining symmetric and asymmetric tripoidal modules with linear modules as shown in Figure 3A−H. The products of coupling one, two, or three linear modules with a single tripoidal module (Figure 3I, lanes 3, 4, and 5) are as expected. Two, three, or four tripoidal modules can also be linked together (Figure 3I, lanes 6, 9, and 10), containing 75, 135, and 165 nt, respectively, in high yields.

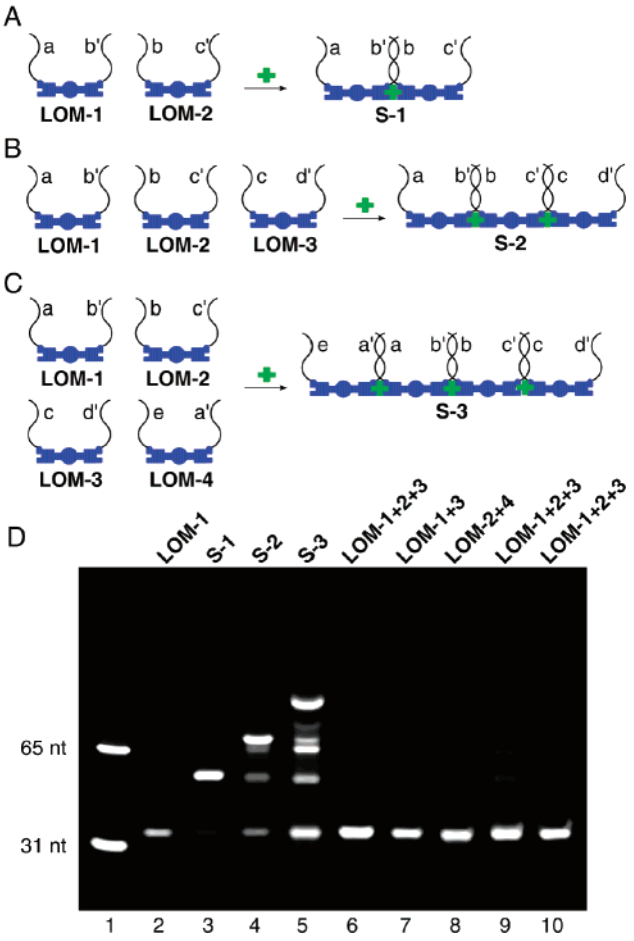

Figure 2. Analysis of linear module (LOM) coupling reactions on denaturing polyacrylamide gels. (A−C) Pictographic coupling scheme of LOMs to form rigid linear salen oligomers **S-1**, **S-2**, and **S-3**. The 1,4-diethynylbenzene-linked salicylaldehydes in the linear modules (blue) are assembled by the oligonucleotides below (black curved lines) and coupled by ethylenediamine (EDA) and $Mn(OAc)_2$ (green cross). (D) Electrophoresis in 8 M urea shows that the salen products are covalently linked in the presence of 1.0 mM EDA and 0.5 mM $Mn(OAc)_2$. The uppermost gel bands are **S-1** (60 nt), **S-2** (90 nt), and **S-3** (120 nt). Controls are shown in lane 6 (**LOM-1**, **LOM-2**, and **LOM-3**, no EDA, no $Mn(OAc)_2$); lane 7 (**LOM-1** and **LOM-3**); lane 8 (**LOM-2** and **LOM-4**); lane 9 (**LOM-1**, **LOM-2**, and **LOM-3**, no $Mn(OAc)_2$); and lane 10 (**LOM-1**, **LOM-2**, and **LOM-3**, no EDA).

DNA−melting point curves were measured and compared for unreacted and salen-linked LOM−LOM, LOM−TOM, and TOM−TOM dimers. The melting temperatures (T_m) are consistent with those of double-stranded DNA helices. The presence of a salen-link in the dimers results in a T_m increase of 17−26 °C.

The results illustrate the versatility and flexibility of our system achieved by mixing selected modules possessing complementary DNA sequences. We have constructed linear products, angled branch points, three-way junctions, and five-way junctions. These are examples of the basic components required for building a molecular wire or a two-dimensional nanomatrix.

The DNA-programmed assembly of organic modules presented here is a proof of principle for a fundamentally new way to synthesize organic oligomers. We have successfully demonstrated the method with both linear and branched types of organic modules. A series of complex oligomers are easily formed by DNA-directed self-assembly and covalent coupling of up to four modules. The phenylacetylene backbones are potential conducting wires,[3,4] and future theoretical and experimental studies will reveal the electronic

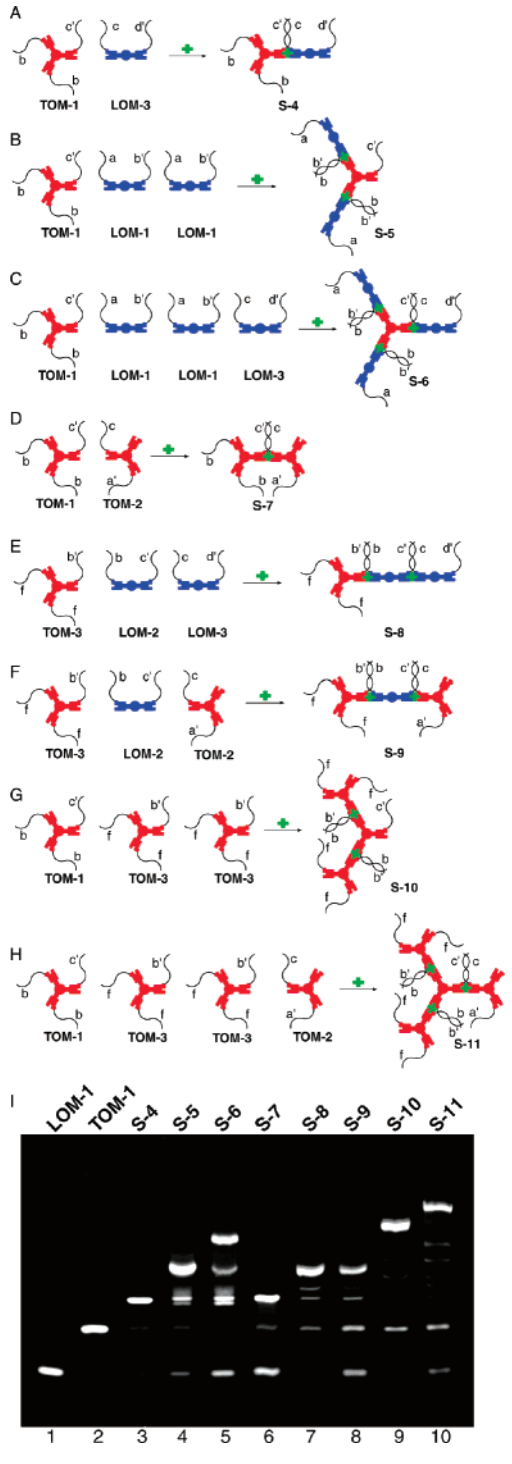

Figure 3. Analysis of LOM + TOM reactions on denaturing polyacrylamide gels. (A−H) Coupling scheme of LOMs (blue) + TOMs (red) to form products **S-4**−**S-11**. (I) Electrophoresis in 8 M urea showing various constructs (lanes 3−10) ranging in size from **S-4** (75 nt) to **S-11** (165 nt). **LOM-1** (30 nt) and **TOM-1** (45 nt) markers (lanes 1 and 2).

properties of these metal-linked oligomers. Obviously, the method can be extended to other organic compounds by the incorporation of salicylaldehyde headgroups. Introduction of cleavable linkers[23] such as disulfides between the organic backbone and the oligonucleotides will enable the complete removal of DNA from the matrix. Future studies will show if this modular approach can be extended to connect and fabricate complex nanodevices.

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Supporting Information Available: Experimental procedures for oligonucleotide synthesis, purification, coupling reactions, DNA−melting point curves, and MALDI-TOF spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Joachim, C.; Gimzewski, J. K.; Aviram, A. *Nature* **2000**, *408*, 541.
(2) Service, R. F. *Science* **2002**, *295*, 2398.
(3) Tour, J. M. *Acc. Chem. Res.* **2000**, *33*, 791.
(4) Reed, M. A.; Chen, J.; Rawlett, A. M.; Price, D. W.; Tour, J. M. *Appl. Phys. Lett.* **2001**, *78*, 3735.
(5) Collier, C. P.; Mattersteig, G.; Wong, E. W.; Luo, Y.; Beverly, K.; Sampaio, J.; Raymo, F. M.; Stoddart, J. F.; Heath, J. R. *Science* **2000**, *289*, 1172.
(6) Park, J.; Pasupathy, A. N.; Goldsmith, J. I.; Chang, C.; Yaish, Y.; Petta, J. R.; Rinkoski, M.; Sethna, J. P.; Abruna, H. D.; McEuen, P. L.; Ralph, D. C. *Nature* **2002**, *417*, 722.
(7) Liang, W.; Shores, M. P.; Bockrath, M.; Long, J. R.; Park, H. *Nature* **2002**, *417*, 725.
(8) Mitchell, R. H.; Ward, T. R.; Wang, Y.; Dibble, P. W. *J. Am. Chem. Soc.* **1999**, *121*, 2601.
(9) Whitesides, G. M.; Grzybowski, B. *Science* **2002**, *295*, 2418.
(10) Fujita, M., Ed. *Molecular Self-Assembly, Organic Versus Inorganic Approaches*; Springer: New York, 2000.
(11) Reinhoudt, D. N.; Crego-Calama, M. *Science* **2002**, *295*, 2403.
(12) Gracias, D.; Tien, J.; Breen, T. L.; Hsu, C.; Whitesides, G. M. *Science* **2000**, *289*, 1170.
(13) Storhoff, J. J.; Mirkin, C. A. *Chem. Rev.* **1999**, *99*, 1849.
(14) Waybright, S. M.; Singleton, C. P.; Wachter, K.; Murphy, C. J.; Bunz, U. H. *J. Am. Chem. Soc.* **2001**, *123*, 1828.
(15) Niemeyer, C. M. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 585.
(16) An, Y.-Z.; Chen, C.-H. B.; Andersen, J. L.; Sigman, D. S.; Foote, C. S.; Rubin, Y. *Tetrahedron* **1996**, *52*, 5179.
(17) LaBean, T. H.; Yan, H.; Kopatch, J.; Liu, F.; Winfree, E.; Reif, J. H.; Seeman, N. C. *J. Am. Chem. Soc.* **2000**, *122*, 1848.
(18) Czlapinski, J. L.; Sheppard, T. L. *J. Am. Chem. Soc.* **2001**, *123*, 8618.
(19) Gartner, Z. J.; Liu, D. R. *J. Am. Chem. Soc.* **2001**, *123*, 6961.
(20) Gartner, Z. J.; Kanan, M. W.; Liu, D. R. *Angew. Chem., Int. Ed.* **2002**, *41*, 1796.
(21) Gartner, Z. J.; Kanan, M. W.; Liu, D. R. *J. Am. Chem. Soc.* **2002**, *124*, 10304.
(22) Calderone, C. T.; Puckett, J. W.; Gartner, Z. J.; Liu, D. R. *Angew. Chem., Int. Ed.* **2002**, *41*, 4104.
(23) Gartner, Z.; Grubina, R.; Calderone, C. T.; Liu, D. R. *Angew. Chem., Int. Ed.* **2003**, *42*, 1370.
(24) Li, X.; Liu, D. R. *J. Am. Chem. Soc.* **2003**, *125*, 10188.
(25) Summerer, D.; Marx, A. *Angew. Chem., Int. Ed.* **2002**, *41*, 89.
(26) Eckardt, L. H.; Naumann, K.; Pankau, W. M.; Rein, M.; Schweitzer, M.; Windhab, N.; Kiedrowski, G. v. *Nature* **2002**, *420*, 286.
(27) Nielsen, M.; Thomsen, A. H.; Cló, E.; Kirpekar, F.; Gothelf, K. V., submitted.
(28) Nielsen, M.; Gothelf, K. V. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2440.
(29) Nielsen, M.; Larsen, N. B.; Gothelf, K. V. *Langmuir* **2002**, *18*, 2795.
(30) Pospisil, P. J.; Carsten, D. H.; Jacobsen, E. N. *Chem.-Eur. J.* **1996**, *2*, 974.

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