Synthesis of Branched Oligonucleotides as Templates for the Assembly of Nanomaterials

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Branched oligonucleotides with symmetric arms **6**–**15**, which may contain biotin as a second recognition code, were prepared. These molecules are designed to be used for the directed assembly of nanomaterials. The branched structure of the desired oligonucleotides was confirmed by mass spectrometry on small branched oliognucleotides, by gel electrophoresis, and by hybridization with complementary oligonucleotide–nanoparticle conjugates, followed by visualization of the complexes by transmission electron microscopy.

Introduction. – Interest in branched nucleic acids has grown recently. Most of the work in this area was first focused on the study of branched oligoribonucleotides as splicing intermediates of eukaryotic mRNAs. [1][2]. Later, the complexity of the synthesis of the branching molecules and the low yields triggered the search for non-nucleoside branching molecules such as derivatives of 1,3-diaminopropan-2-ol [3] and pentaerythritol [4]. These branching molecules can be used to produce oligonucleotides carrying multiple labels to increase sensitivity in hybridization experiments [3][4]. Moreover, oligonucleotides with three arms are suitable building blocks for template-directed synthesis of nanostructures [5][6].

Among biological molecules, oligonucleotides have been used as template to assemble inorganic nanocrystals. The hybridization properties of the oligonucleotides permit assembly of gold nanoparticles at distances determined by the length of the oligonucleotides and also the formation of three-dimensional networks [7–10]. In addition, DNA can be metallized to form conducting wires between electrodes [11]. Single-walled carbon nanotubes have also been functionalized with DNA derivatives [12].

We describe here the preparation of branched oligonucleotides (*Scheme 1*), which consist of two or more sequences connected by a non-DNA material such as hexaethyleneglycol. This type of oligonucleotides may be used to assemble nanomaterials. Moreover, the presence of biotin in these oligonucleotides may direct a nanoparticle to the middle of the structure by using streptavidine carrying a nanoparticle [13][14]. Here, we describe the preparation of branched oligonucleotides **6**–**16** (*Table 1*) with two, three, and four arms of identical sequence. These molecules may allow the assembly of complex nanostructures by hybridization of a few simple elements [15].

Scheme 1. *Schematic Representation of Oligonucleotides Described in this Paper*

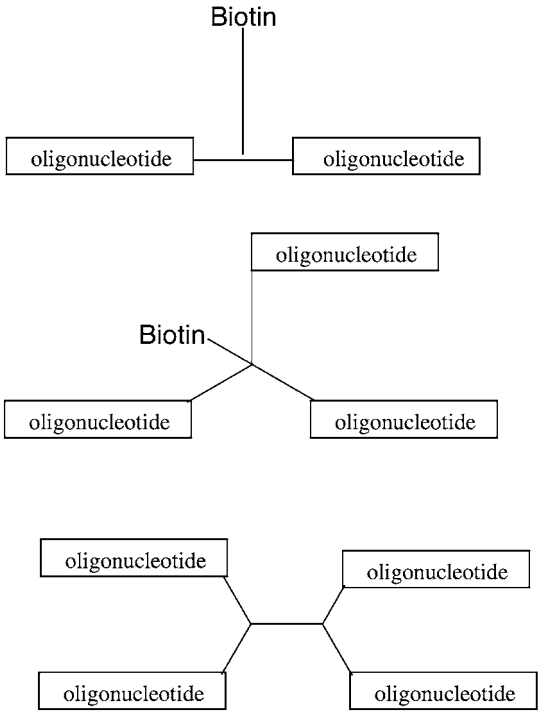


Table 1. *Oligonucleotide Sequences Prepared*

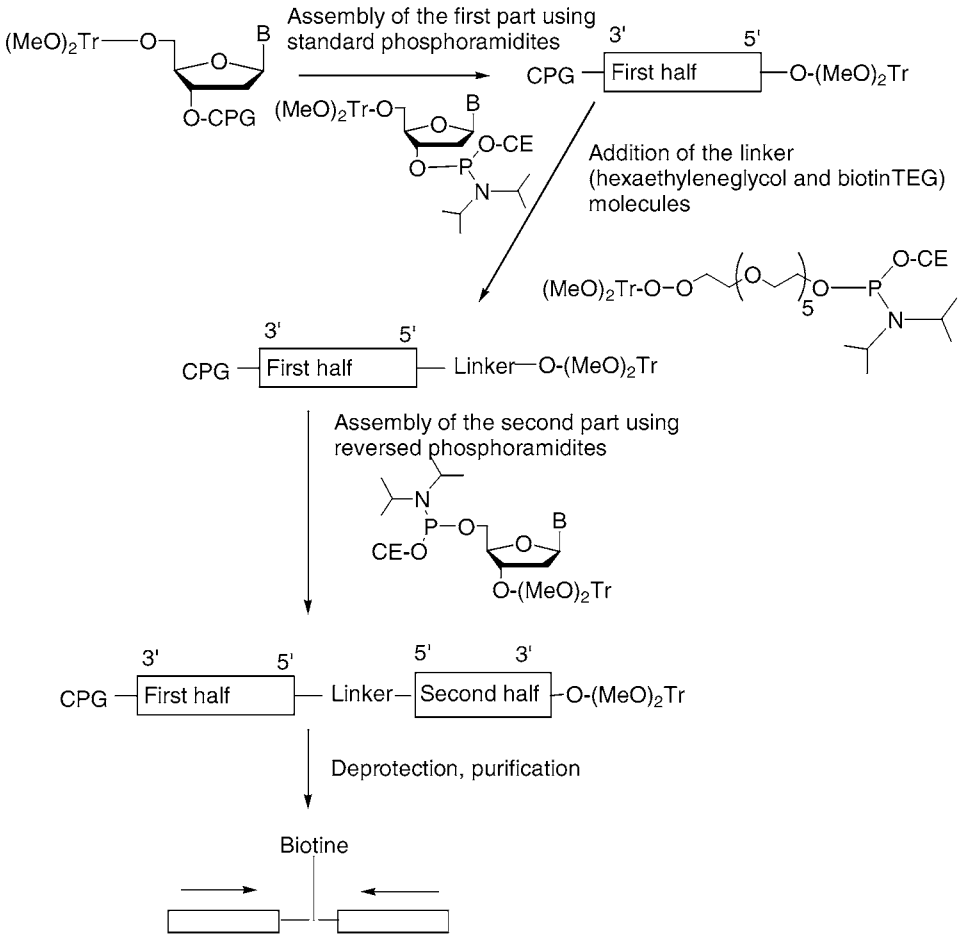
No.	Sequence[a])
6	3′-CGTAACTCGCTACGTCCGTC-heg-CTGCCTGCATCGCTCAATGC-3′
7	3′-CGTAACTCGCTACGTCCGTC-heg-bio-heg-CTGCCTGCATCGCTCAATGC-3′
8	5′-TTTT-bps-TTTT-5′
9	5′-CTGCCTGCATCGCTCAATGC-heg-bps-heg-CGTAACTCGCTACGTCCGTC-5′
10	3′-TTTT-bpp-(TTTT-5′)$_2$
11	3′-CGTAACTCGCTACGTCCGTC-heg-bpp-(-heg-CTGCCTGCATCGCTCAATGC3′)$_2$
12	3′-CGTAACTCGCTACGTCCGTC-heg-bio-bpp-(-heg-CTGCCTGCATCGCTCAATGC-3′)$_2$
13	(5′-TTTT)$_2$-bpp-bps-bpp-(TTTT-5′)$_2$
14	(5′-TCTCCTCCTTC-heg)$_2$-bpp-bps-bpp-(heg-CTTCCTCCTCT-5′)$_2$
15	(5′-CTGCCTGCATCGCTCAATGC-heg)$_2$-bpp-bps-bpp-(heg-CGTAACTCGCTACGTCCGTC-5′)$_2$
16	5′-thiol-hexyl-phosphate-AAAAAAGCATTGAGCGATGCAGGCAG-3′

[a]) heg: hexaethyleneglycol, bio: biotin–tetraethyleneglycol, bps: [–$PO_3O(CH_2)_3CONHCH_2$]$_2$CHOH, bpp: [–$PO_3O(CH_2)_4CONHCH_2$]$_2$CHOPO$_3$–.

Results. – 1. *Synthesis of Branched Oligonucleotides Carrying Two Identical Arms.* Oligonucleotides **6** and **7**, with two identical arms, were prepared by sequential addition of phosphoramidites (*Scheme 2*). Starting from the 3′-end, the first half of the sequence was assembled by using standard phosphoramidites. Subsequently, hexaethyleneglycol

(oligonucleotides **6** and **7**) and biotin–tetraethyleneglycol (oligonucleotide **7**) phosphoramidites were added. Finally, the second half of the molecule was assembled with reversed phosphoramidites.

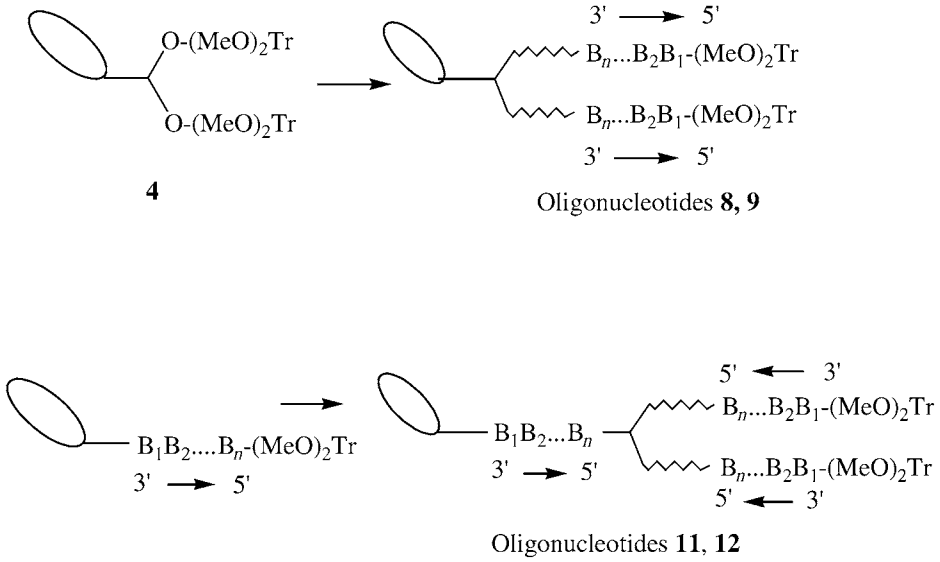
Scheme 2. *Outline of the Synthesis of Oligonucleotides* **6** *and* **7** *with Two Identical Arms*



Next, the synthesis of oligonucleotides **8** and **9**, which have the opposite polarity to oligonucleotides **6** and **7**, was undertaken. In this case, synthesis started in the middle of the molecule by using a solid support containing a branching molecule, and both strands were synthesized simultaneously (*Scheme 3*). Although this strategy required a special solid support, there was no need for reversed phosphoramidites.

1,3-Diaminopropan-2-ol was reacted with γ-butyrolactone to yield the desired triol **1** (*Scheme 4*) [3]. The two primary alcohols were protected with the $(MeO)_2Tr$ group by reaction of triol **1** with excess $(MeO)_2TrCl$ in pyridine [3]. Compound **2** was incorporated on controlled-pore glass (CPG) by using the hemisuccinate derivative **3** as described in [16][17].

Scheme 3. *Outline of the Synthesis of Branched Oligonucleotides* **8**, **9**, **11**, *and* **12** *with Two and Three Identical Arms*

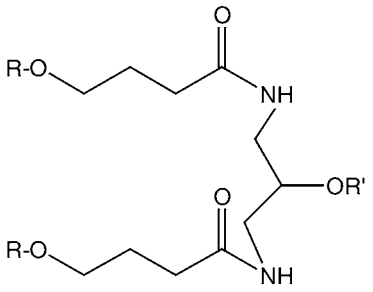


The resulting support carrying the symmetric branching molecule **4** was tested by the preparation of a short oligonucleotide sequence **8**. After the assembly of four thymidines, the solid support was treated with NH_3, and the resulting oligonucleotide **8** carrying two $(MeO)_2Tr$ groups was purified by HPLC. One single peak (purity 97%) was obtained, which had the expected molecular weight ($[M+H]^+$: found 3333; calc. for $C_{133}H_{162}N_{18}O_{65}P_8$: 3300).

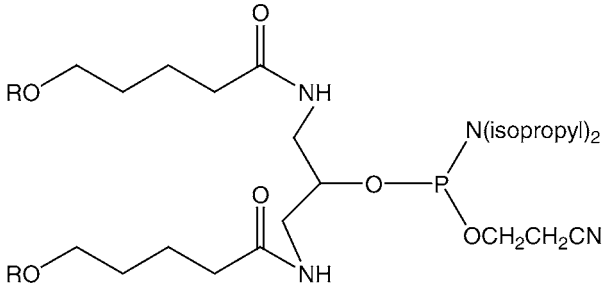
Branched oligonucleotide **9**, which has two identical arms of 20 bases, was prepared. The standard 1-μmol synthesis cycle was used. Overall yield, determined by the absorbance of the $(MeO)_2Tr$ group released on each detritylation, was 60% (98% step yield). In agreement with trityl values, analysis of the $[(MeO)_2Tr]_2$-oligonucleotide **9** by HPLC gave a major peak in the area of elution of tritylated oligonucleotides, which was 54% of the total absorbance. The sample that produced this peak was treated with AcOH and analyzed by 15% polyacrylamide, 8M urea gel electrophoresis. A single band with the mobility of a 40-base oligonucleotide was observed (data not shown).

2. *Synthesis of Branched Oligonucleotides Carrying Three Identical Arms.* The use of the symmetric branching molecule to prepare oligonucleotides carrying three branches was examined on oligonucleotides **10**–**12**. First, a short oligonucleotide **10** was prepared. Four thymidines were assembled by using standard phosphoramidites. Thereafter, the symmetric branching phosphoramidite **5** was added to the sequence, and, finally, four more thymidines were assembled by using standard phosphoramidites and with coupling times of 5 min. After NH_3 treatment, the desired oligonucleotide **10** was obtained and characterized by mass spectrometry ($[M+H]^+$: found: 3936; calc. for $C_{133}H_{180}N_{26}O_{89}P_{12}$: 3938). Subsequently, the synthesis of oligonucleotides **11** and **12** was undertaken. The method was similar to the synthesis of oligonucleotides **8** and **9**

Scheme 4. *Structures of the Reagents Used for the Introduction of the Symmetric Branching Molecule into Oligonucleotides*



1 R=R'= H
2 R=$(MeO)_2$Tr, R'=H
3 R=$(MeO)_2$Tr, R'=$COCH_2CH_2COOH$
4 R=$(MeO)_2$Tr, R'=$COCH_2CH_2CONH$-LCAA-CPG



5 R=$(MeO)_2$Tr

(*Scheme 3*). First the 20mer sequence was built in the $3' \rightarrow 5'$ direction by using standard phosphoramidites and the hexaethyleneglycol phosphoramidite. In oligonucleotide sequence **12**, the biotin–tetraethyleneglycol was added at this point. Thereafter, the symmetric branching phosphoramidite **5** was added to the sequence, and, finally, the rest of the desired sequence was assembled in the $5' \rightarrow 3'$ direction by using reversed phosphoramidites and the hexaethyleneglycol phosphoramidite. During the assembly of the second part, we observed the duplication of the amount of $(MeO)_2$Tr released during detritylation. This is in agreement with the duplication of the second part of the sequence. Coupling yields for the assembly of the second half were lower than the yields for the assembly of the first part (20% overall yield compared with 80%). This may be due to the use of reversed phosphoramidites and hexaethyleneglycol phosphoramidite, and steric hindrance at the branching point. HPLC Analysis revealed two products in the area of elution of $(MeO)_2$Tr-containing oligonucleotides (*Fig. 1*). We assigned the first peak to an oligonucleotide with two complete arms and a third one incomplete. This oligonucleotide contained a single $(MeO)_2$Tr group, and so

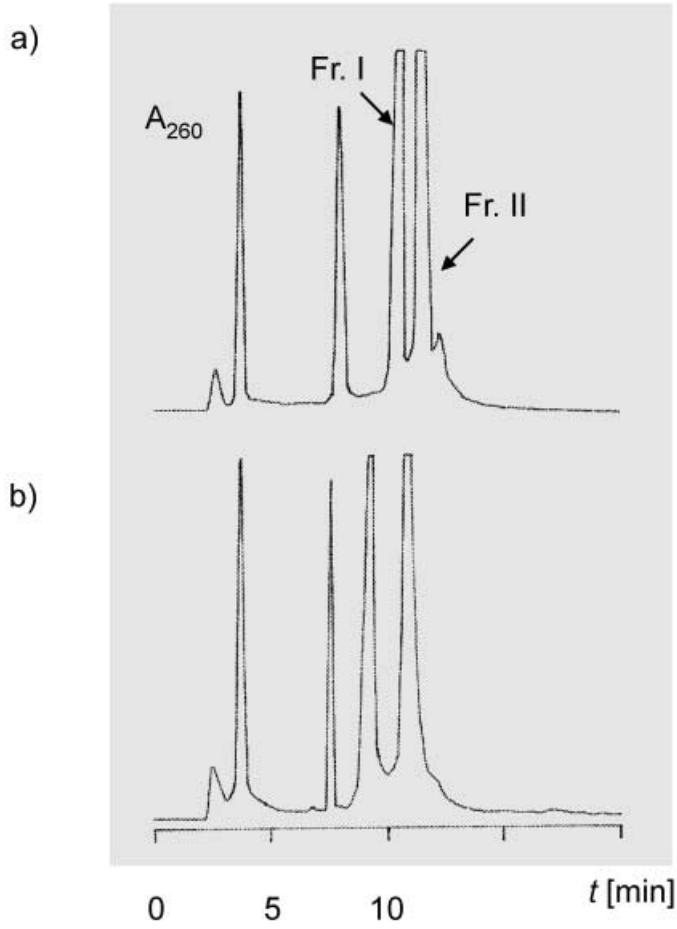


Fig. 1. *HPLC Profiles of $(MeO)_2$Tr-containing oligonucleotides* **11** (a) *and* **12** (b) *with three arms.* Truncated sequences without $(MeO)_2$Tr groups had a retention time of less than 5 min. Benzamide eluted at 7–8 min. *Fr. I* contained oligonucleotides with one $(MeO)_2$Tr group. *Fr. II* contained the desired sequence with two $(MeO)_2$Tr groups.

it eluted first. The second peak corresponds to the desired sequence carrying two $(MeO)_2$Tr groups and, thus, eluted later.

A range of conditions were examined in an attempt to increase the yields of branched oligonucleotides. These changes include: *a*) increasing the coupling time to 300 s, *b*) increasing the amount of phosphoramidite by using 0.2M solutions instead of 0.1M solutions, and *c*) a cycle with a double coupling protocol. All of the changes improved the elongation of the second half of the oligonucleotide, the most effective being the increase of the coupling time and the double-coupling protocol. The optimized cycle with increased coupling time and double-coupling protocol was used for the preparation of oligonucleotides **11** and **12**. By the new cycle the overall yield rose from 20 to 80%, similar to the yields that were obtained without the introduction

of the branching point. HPLC Analysis gave two peaks in the area corresponding to $(MeO)_2$Tr-containing oligonucleotides (*Fig. 1*). This time, the second peak assigned to the desired sequences was larger (area ratio in the second synthesis 2:3, area ratio in the first synthesis 3:1) than the first peak assigned to truncated sequences. A similar result was obtained during the preparation of sequence **11**. The products were analyzed by denaturing polyacrylamide gel electrophoresis (PAGE) (20% polyacrylamide, 8M urea; *Fig. 2*). As expected, the product with the desired length was in peak *II*. Peak *I* contained truncated sequences, mainly near the branching point (*Fig. 1*).

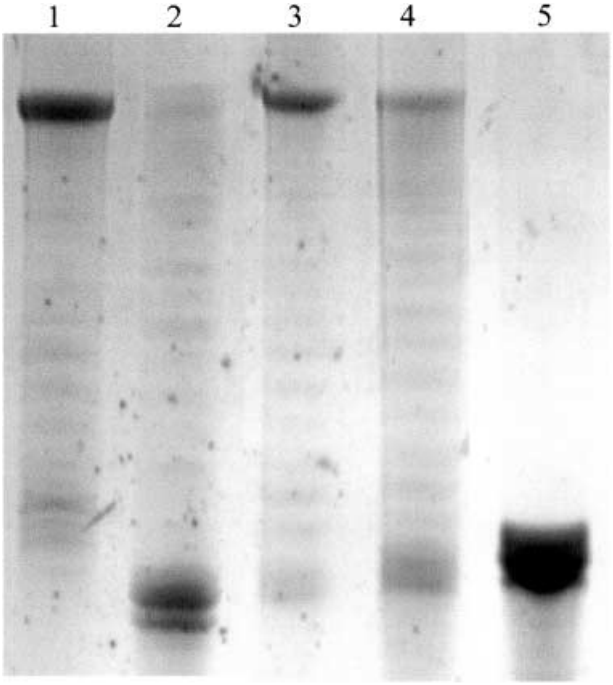


Fig. 2. *20% Polyacrylamide, 8M urea gel electrophoresis of HPLC* Fractions I *and* II *of oligonucleotides* **11** *and* **12** *with three arms.* The gel was stained with STAINS-ALL. *Lane 1: Fr. II*, oligonucleotide **11**; *Lane 2: Fr. I*, oligonucleotide **11**; *Lane 3: Fr. II*, oligonucleotide **12**; *Lane 4: Fr. I*, oligonucleotide **12**; *Lane 5:* oligonucleotide **7**. The double band of oligonucleotide **7** is due to the diastereoisomers of biotin–tetraethyleneglycol molecule.

The trimeric structure of oligonucleotide **11** was confirmed by hybridization with complementary oligonucleotide carrying gold nanoparticles, followed by visualization of the assemblies by transmission electron microscopy (TEM). *Fig. 3* shows groups of three particles obtained by TEM of a sample resulting from hybridization of oligonucleotide **11** with its complementary sequence **16**, carrying gold nanoparticles.

3. *Synthesis of Branched Oligonucleotides Carrying Four Identical Arms.* The synthesis of oligonucleotides **13**, **14**, and **15** carrying four equal arms was undertaken. The synthetic strategy is shown in *Scheme 5*. The synthesis started in the middle of the molecule. The first branching point was generated with the solid support having the symmetric branching molecule **4**, followed by the addition of the phosphoramidite of

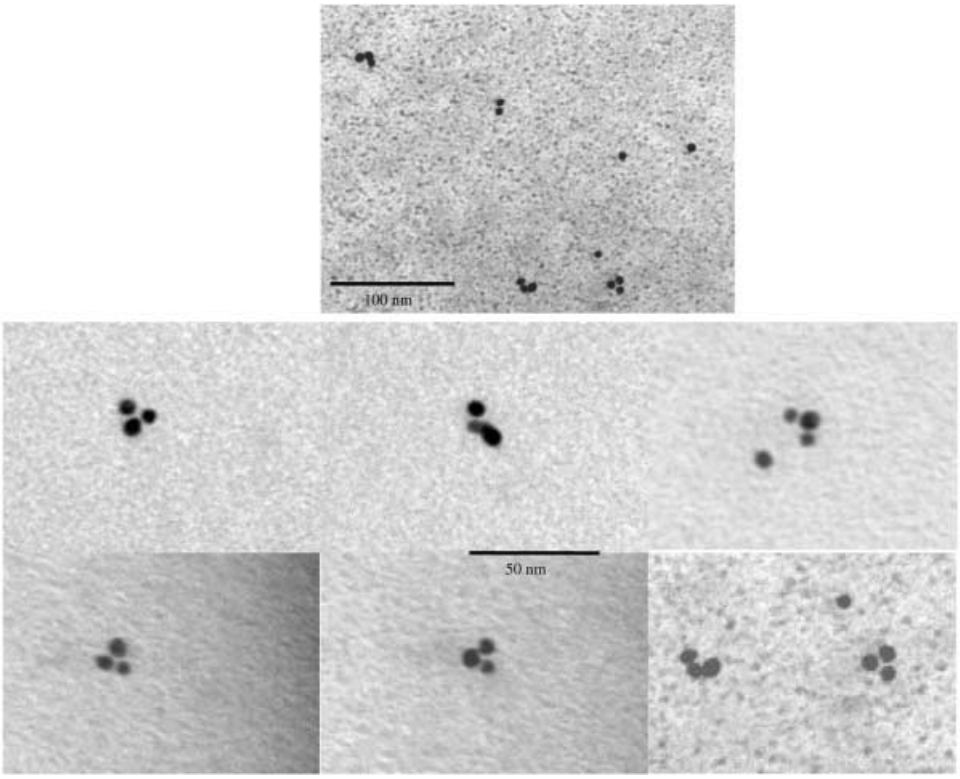


Fig. 3. *Representative TEM images of trimeric DNA–gold nanoparticle assemblies*

the symmetric branching molecule **5**. In this way, four OH groups were generated, on which the desired oligonucleotide sequence was assembled. First, a short oligonucleotide **13** was prepared. Four thymidines were assembled by using standard phosphoramidites with increased coupling times. After NH_3 treatment, the desired oligonucleotide **13** was obtained and characterized by mass spectrometry ($[M+H]^+$: found: 5832; calc. for $C_{197}H_{276}N_{38}O_{131}P_{18}$: 5829). Afterwards, the synthesis of oligonucleotides **14** and **15** was performed. A tetraethyleneglycol molecule was added between the branching points and the oligonucleotide sequences. The addition of 11 bases generated the 44mer oligonucleotide **13**, and the addition of 20 bases generated the 80mer oligonucleotide **14**. For the assembly of the sequences, standard phosphoramidites were used on a 200-nmol synthesis cycle with increased coupling time. The assembly of the sequences was similar to standard synthesis, as determined by the absorbance of the $(MeO)_2Tr$ group (step coupling yield 98%). The resulting products were analyzed and purified by gel electrophoresis (10% polyacrylamide, 8M urea; *Fig. 4*). Lanes *1*, *2*, and *5* contain synthetic linear oligonucleotides of 100, 100, and 34 bases, respectively. Lanes *3* and *4* contain the crude material from the synthesis of oligonucleotides **14** (44 bases) and **15** (80 bases). The four-branched oligonucleotides ran faster than expected (especially **15**). A broad band appeared on both oligonucleotides **14** and **15**. The

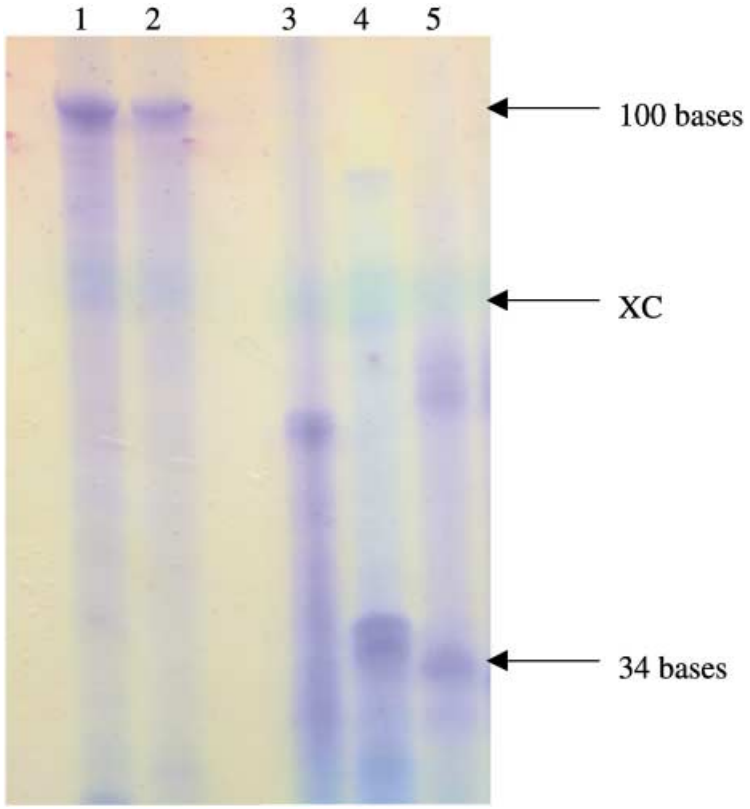


Fig. 4. *10% Polyacrylamide, 8*M *urea gel electrophoresis of crude oligonucleotides* **14** *and* **15** *with four arms.* The gel was stained with STAINS-ALL. *Lanes 1* and *2:* synthetic oligonucleotides of 100 bases; *Lane 3:* branched oligonucleotide **15** (80 bases); *Lane 4:* branched oligonucleotide **14** (44 bases); *Lane 5:* synthetic oligonucleotide of 34 bases. Under these conditions the xylene cyanol (XC) dye ran as a 70-base oligonucleotide.

anomalous mobility is attributed to the structure of the branched oligonucleotide. The broad band is due to the isomers of the branching units.

Melting experiments performed with duplexes of oligonucleotides **14** and **15** with their complementary oligonucleotides gave the same melting temperatures when compared with their linear analogues (*Table 2*).

Discussion. – The ability to control the assembly of nanomaterials from well-defined units is a key step that is expected to allow the exploitation of the technological potential of these materials. The use of the hybridization properties of oligonucleotides is a promising approach [7–12]. In this paper, branched oligonucleotides with identical arms, which may carry biotin (*Scheme 1*), were reported. These oligonucleotides are designed to be used as templates for the controlled assembly of nanomaterials.

Two synthetic strategies were used for the preparation of two-armed oligonucleotides. The first approach consists of the sequential addition of the nucleoside and non-

Scheme 5. *Outline of the Synthesis of Branched Oligonucleotides* **14** *and* **15** *with Four Arms with Identical Sequence*

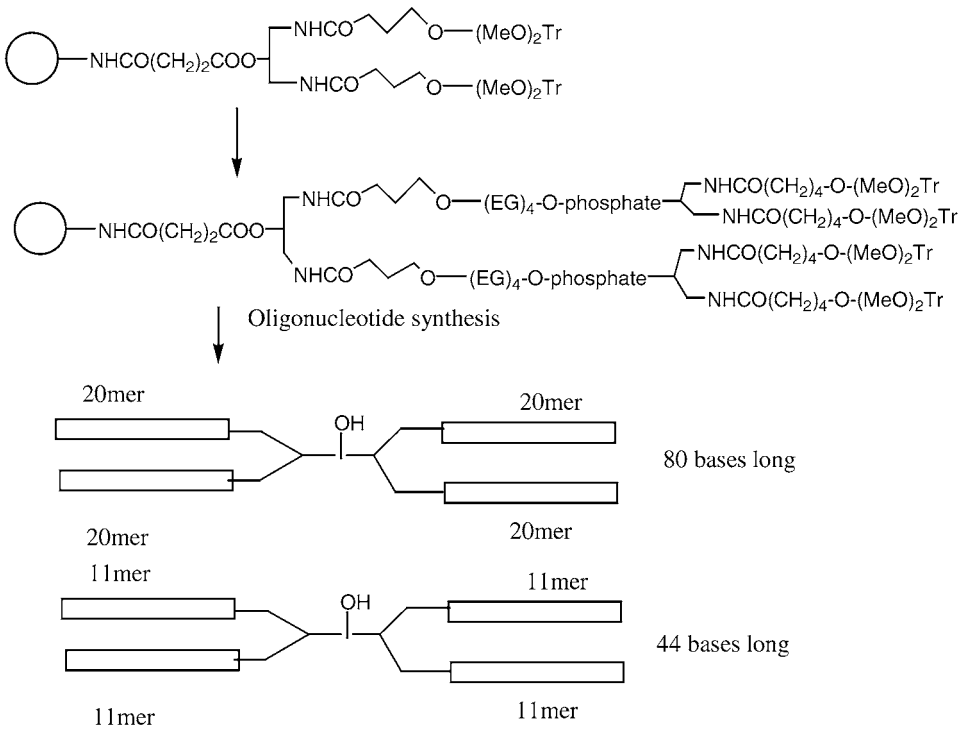


Table 2. *Melting Temperatures of Duplexes Formed by Oligonucleotides* **14** *and* **15**, *and the Linear Control Sequences*

Oligonucleotide	T_m [°C][a])
14	45
5′-TCTCCTCCTTC-3′	46
15	72
5′-CTGCCTGCATCGCTCAATGC-3′	72

[a]) Complementary sequences: 3′-AGAGGAGGAAG-5′ and 3′-GACGGACGTAGCGAGTTACG-5′, 0.050M *Tris*·HCl, 0.15M NaCl, pH 7.5, error in T_m is $\pm 1°$.

nucleoside units from one to the other end. The second approach consists of the parallel extension of the two arms from a central branching molecule. Both approaches gave good results but the second was the best solution for the synthesis of the 3′–3′ linked oligonucleotides such as **9** because there is no need to use reversed phosphoramidites.

The preparation of three-armed oligonucleotides was performed by a combination of both methods. The first arm was prepared by sequential addition of monomers, and the second and third arm were assembled in parallel. The coupling reaction was recalcitrant after the addition of the branching in the middle of the molecule. These

difficulties were overcome by modifying the synthesis cycle by increasing coupling time and the concentration of monomers, and by double coupling. Similar results were obtained during the synthesis of branched RNA [2], and these may be due to steric hindrance. We showed that three-armed oligonucleotides can easily be purified from truncated sequences by reversed-phase HPLC as developed for standard oligonucleotides.

Four-armed oligonucleotides were only obtained by the simultaneous assembly of the four strands. In addition to the results presented in this paper, we tried unsuccessfully to use asymmetric branching molecules, and combine sequential and parallel additions (data not shown). Although the results obtained in the synthesis of three- and four-armed oligonucleotides are not directly comparable, we believe that parallel synthesis of more than one chain is more efficient near the solid support than in the middle of a DNA sequence (see also [5]).

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Experimental Part

General. Phosphoramidites and ancillary reagents used in the oligonucleotide synthesis were from *Applied Biosystems* (*PE Biosystems Hispania S.A.,* Spain), *Cruachem* (*Cruachem Ltd.*, Scotland), and *Glen Research* (*Glen Research Inc.*, USA). The rest of the chemicals were purchased from *Aldrich, Sigma*, or *Fluka* (*Sigma-Aldrich Química S.A.*, Spain). Long-chain amino controlled pore glass (LCAA-CPG) was purchased from *CPG* (*CPG, Inc.*, New Jersey, USA). Solvents were from *S.D.S.* (*S.D.S.*, France). *NAP-10* columns (*Sephadex G-25*) were purchased from *Pharmacia Biotech*. N,N′*-(2-Hydroxypropane-1,3-diyl)bis[4-hydroxybutanamide]* (**1**) and its bis[$(MeO)_2$Tr]-protected derivative **2** were synthesized as described in [12]. Oligonucleotide sequences were synthesized on an *Applied Biosystems* DNA synthesizer model *392*. UV/VIS spectra were recorded on a *Shimadzu UV-2101PC* and a *Hewlett-Packard 8452A* diode-array spectrophotometer. Mass spectra (matrix-assisted laser desorption ionization time-of-flight, MALDI-TOF) were provided by the mass spectrometry service at the University of Barcelona. Transmission electron microscopy was performed on a *Jeol 2000 FX TEMSCAN*.

Preparation of Solid Supports. Compound **2** [3] was reacted with succinic anhydride in the presence of 4-(dimethylamino)pyridine in CH_2Cl_2 according to the literature [16]. Solid-support carrying the symmetric branching molecule was prepared by reacting the 3′-hemisuccinate derivative of the symmetric branching molecule **3** with amino controlled-pore glass (long-chain amino alkyl-CPG) with Ph_3P and 2,2′-dithiobis(5-nitropyridine) [17] for condensation. Coupling time was increased from 5 to 15 min obtaining a loading of 18 μmol g^{-1}.

Oligonucleotide Synthesis. Oligonucleotide sequences were prepared by using standard (benzoyl- or isobutyryl-protected) 3′-[(2′-cyanoethyl)phosphoramidites] and reversed (benzoyl- or isobutyryl-protected) 5′-[(2′-cyanoethyl)phosphoramidites]. Supports were obtained from commercial sources or prepared as described above. The symmetric branching phosphoramidite **5**, the hexaethyleneglycol phosphoramidite, and the biotin-tetraethyleneglycol phosphoramidite were from commercial sources (*Cruachem*, Scotland, and *Glen Research*, USA). The tetraethyleneglycol phosphoramidite was prepared according to the literature [18].

Oligonucleotides **6**, **7**, **10**, **11**, and **12** were prepared on 200-nmol scale by using polystyrene supports (*LV200*, *Applied Biosystems*). The coupling time of the biotin and the hexaethyleneglycol phosphoramidites was increased from 30 to 480 s. During the synthesis of the second half of oligonucleotides **11** and **12**, yields were unsatisfactory. The following changes on the synthesis cycle were studied: *a*) increasing the coupling time from 30 to 300 s, *b*) increasing the phosphoramidite concentration to 0.2M instead of 0.1M soln., and *c*) implementation a double-coupling protocol. A new cycle with increased coupling time and double-coupling protocol was used. The rest of the oligonucleotides were prepared on 1-μmol scale. Oligonucleotides **8** and **9** were prepared by the standard 1-μmol synthesis cycle. Oligonucleotides **13**, **14**, and **15** were prepared by a 200-nmol synthesis cycle with an increased coupling time (480 s).

The oligonucleotide carrying a SH group at the 5′-end, **16**, was prepared on 1-μmol scale by using standard phosphoramidites and the phosphoramidite of $(MeO)_2$Tr-protected 6-hydroxyhexyl disulfide (*Glen Research*, USA).

Deprotection of Oligonucleotides. The resulting supports were treated with 1 ml of conc. NH_3 (overnight, 55°). Oligonucleotide support carrying a SH group at the 5′-end was treated overnight with 1 ml of 50 mM dithio-DL-threitol (DTT) in conc. NH_3 at 55°. The excess DTT was eliminated on a *Sephadex G-25* column (*NAP-10*, *Pharmacia*, Sweden) just prior to conjugation with gold nanoparticles.

Purification of Oligonucleotides. The products resulting from NH_3 treatment were dissolved in H_2O and purified by HPLC (column: *PRP-1*, 10 μm (*Hamilton*, USA; 305 × 7 mm), flow rate 3 ml/min). A 20-min linear gradient from 15 to 50% MeCN over 100 mM aq. triethylammonium acetate was used for oligonucleotides carrying the $(MeO)_2$Tr group. After removal of the $(MeO)_2$Tr group with 80% AcOH (30 min), the resulting oligonucleotides were purified on the same column with a 20-min linear gradient from 5 to 25% MeCN over 100 mM aq. triethylammonium acetate. Oligonucleotides **14** and **15** were first desalted with a *Sephadex G-25* (*NAP-10*) column and, then, purified by gel electrophoresis.

Yields after purification: **6** (3 × 200 nmol): 45 OD units; **7** (2 × 200 nmol): 26 OD units; **8** (1 μmol): 17 OD units; **9** (1 μmol): 8 OD units; **10** (200 nmol): 5 OD units; **11** (200 nmol): 7 OD units; **12** (2 × 200 nmol): 19 OD units; **13** (200 nmol): 5 OD units; **14** (200 nmol): 8 OD units after *Sephadex* before electrophoresis, 1.5 OD units after electrophoresis; **15** (200 nmol): 12 OD units after *Sephadex* before electrophoresis, 1 OD units after electrophoresis.

Preparation of Gold Nanoparticles. Gold nanoparticles with an average diameter of 5 nm were prepared by tannic reduction of a Au salt as described in detail in [19]. 3,3′,3″-Phosphinidenetris(benzenesulfonic acid) trisodium salt (phosphine) was added to the Au soln. until final phosphine concentration of 0.5M. The mixture was stirred for 10 h. Solid NaCl was added to the soln. until it turned from deep burgundy to purple color. The soln. was centrifuged at 8000 rpm for 20 min, the supernatant was discarded, and the precipitate was redispersed in 0.5 mM phosphine. The soln. was precipitated again with NaCl, centrifuged, and redispersed in 0.5 × TBE buffer (final gold nanoparticle concentration 0.8 μM).

Preparation of Oligonucleotide–Gold Conjugates. Thiolated oligonucleotide **16** complementary to oligonucleotides **11** and **15** was combined (50 μM, 0.5 × TBE buffer) with Au nanoparticle soln. in a 1:0.9 Au/DNA molar ratio by mixing well. The resulting soln. was brought to 50 mM NaCl (from 1M NaCl) and incubated overnight.

Gold nanoparticles functionalized with thiol-DNA **16** were mixed with branched oligonucleotide **11** carrying three copies of complementary oligonucleotide in stoichiometric amounts. The resulting solns. of 50 mM in NaCl were incubated overnight prior to TEM characterization.

Transmission Electron Microscopy. A *Jeol 2000 FX TEMSCAN* was used at an acceleration voltage of 80 keV to image the samples. A drop (10 μl) of the dispersion to be imaged was deposited for 1 min on carbon-coated Cu grid (400 mesh).

Melting Experiments. Melting experiments were performed by mixing equimolar amounts of two strands dissolved in a soln. that contained 0.15M NaCl and 0.05M *Tris* · HCl buffer (pH 7.5). Duplexes were annealed by slow cooling from 90° to r.t. UV Absorption spectra and melting curves (absorbance *vs.* temp.) were recorded in 1-cm path-length cells with a *Shimadzu UV2101PC* UV/VIS spectrophotometer with a temp. controller and a programmed temp. increase of 0.5°/min. Melting curves were obtained monitoring the absorbance at 260 nm on duplex concentrations of *ca.* 2 μM. Melting temps. were measured at the maximum of the first derivative of the melting curve. For results, see *Table 2*.

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