"Synthesis of branched oligonucleotides as templates for the assembly of nanomaterials." Grimau, M.G., Iacopino, D., Aviñó, A., de la Torre, B.G., Ongaro, A., Fitzmaurice, D., Wessels, J., Eritja, R. Helv. Chim. Acta, 86(8), 2814-2826 (2003). doi: 10.1002/hlca.2003390232

Synthesis of Branched Oligonucleotides as Templates for the Assembly of Nanomaterials.

by **Marta G. Grimau[a]), Daniela Iacopino[b]), Anna Aviñó[a]), Beatriz G. de la Torre[a]), Andrea Ongaro [b]), Donald Fitzmaurice[b]), Jurina Wessels[c])** and **Ramon Eritja[*a]).**

[a]) Department of Structural Biology. Instituto de Biología Molecular de Barcelona. C.S.I.C., Jordi Girona 18-26, E-08034 Barcelona. Spain. (phone: +34(93)4006145; fax: +34(93)2045904; e-mail : recgma@cid.csic.es)

[b]) Department of Chemistry. University College of Dublin. Belfield, Dublin 4, Ireland.

[c]) Sony International (Europe) GmbH. Advanced Technology Center Stuttgart, Heinrich-Herth-Strasse 1, D-70327 Stuttgart, Germany.

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-diaminopropanol [3] and pentaerythriol [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 the 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 the preparation of branched oligonucleotides (*scheme 1*) consisting of two or more sequences connected by a non-DNA material such as hexaethyleneglycol. This type of oligonucleotide may be used to assemble nanomaterials. Moreover, the presence of biotin in these oligonucleotides may direct a nanoparticle to the middle of the structure using streptavidine carrying a nanoparticle [13, 14]. Here we describe the preparation of branched oligonucleotides with two, three and four arms of identical sequence. These molecules may allow the assembly of complex nanostructures by hybridisation of a few simple elements [15].

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 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 using reversed phosphoramidites.

Next, the synthesis of oligonucleotides **8** and **9** which have the opposite polarity to oligonucleotides **6** and **7** was undertaken. In this case, synthesis was begun in the middle of the molecule 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-Diamino-2-propanol 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 an excess of dimethoxytrityl chloride in pyridine [3]. Compound **2** was incorporated on controlled pore glass (CPG) using the hemisuccinate derivative (**3**) as described [16, 17].

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 ammonia and the resulting oligonucleotide **8** carrying two dimethoxytrityl [$(MeO)_2Tr$] groups was purified by HPLC. One single peak (purity 97%) was obtained, which had the expected molecular weight (M+H found 3333, expected 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 synthesis yield, judged 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 *bis*-[$(MeO)_2Tr$]-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 acetic acid 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**, **11** and **12**. First, a short

oligonucleotide (**10**) was prepared. Four thymidines were assembled using standard phosphoramidites. Thereafter, the symmetric branching phosphoramidite (**5**) was added to the sequence and, finally, four more thymidines were assembled using standard phosphoramidites and coupling times of 5 min. After ammonia treatment, the desired oligonucleotide **10** was obtained and characterized by mass spectrometry (M+H found 3936, expected 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 3*). First the 20 mer sequence was built in the 3´->5' direction 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'->3' direction using reversed phosphoramidites and the hexaethyleneglycol phosphoramidite. During the assembly of the second part, we observed the duplication of the amount of $(MeO)_2Tr$ 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 during 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 $(MeO)_2Tr$-containing oligonucleotides (*Figure 1*). We assigned the first peak to an oligonucleotide with two complete arms and a third one incomplete. This oligonucleotide contained a

single $(MeO)_2Tr$ group, and so it eluted first. The second peak corresponds to the desired sequence carrying two $(MeO)_2Tr$ 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) increase the coupling time to 300 s, b) increase the amount of phosphoramidite by using 0.2 M solutions instead of 0.1 M solutions and c) a cycle with a double coupling protocol. All 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**. Using 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 $(MeO)_2Tr$-containing oligonucleotides (*Figure 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, 8 M urea, *Figure 2*). As expected, the product with the desired length was in peak II. Peak I contained truncated sequences, mainly near the branching point (*Figure 2*).

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). *Figure 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 began at 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 the symmetric branching molecule (**5**). In this way four hydroxyl groups were generated, on which the desired oligonucleotide sequence was assembled. First, a short oligonucleotide (**13**) was prepared. Four thymidines were assembled using standard phosphoramidites with increased coupling times. After ammonia treatment, the desired oligonucleotide **13** was obtained and characterized by mass spectrometry (M+H found 5832, expected 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 judged 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, *Figure 4*). Lanes 1, 2 and 5 contain synthetic linear oligonucleotides of 100, 100 and 33 bases. Lanes 3 and 4 contain the crude

material from the synthesis of oligonucleotides **14** (44 bases) and **15** (80 bases). The 4-branched oligonucleotides ran faster than expected (especially **15**). A broad band appeared on both oligonucleotides **14** and **15**. The 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 hybridisation properties of oligonucleotides is a promising approach [7-12]. In this paper branched oligonucleotides with identical arms which may carry biotin (*scheme 1*) were prepared. 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-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 using 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 shown 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 ref [5]).

This study was supported by the Commission of the European Unions as part of the Information Societies Technology Programme (IST-1999-11974), by the Dirección General de Investigación Científica (BQU2000-0649) and The Generalitat de Catalunya (2001-SGR-0049).

Experimental Part

General. Phosphoramidites and ancillary reagents used during 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'-Bis(4-hydroxybutyryl)-1,3-diamido-2-propanol (**1**) and its *bis*[$(MeO)_2$Tr]-protected derivative (**2**) were synthesized as described elsewhere [12]. *Instrumentation.* Oligonucleotide sequences were synthesized on a *Applied Biosystems* DNA synthesizer model 392 (*Applied Biosystems,* USA). Mass spectra (matrix-assisted laser desorption ionization time-of-flight, MALDI-TOF) were provided by the Mass spectrometry service at the University of Barcelona. UV-Visible spectra were recorded on a *Shimadzu* UV-2101PC and a HP8452A diode array spectrophotometer. 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 N,N-dimethylaminopyridine in dichloromethane 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) using Ph_3P and 2,2'-dithio-*bis*-(5-nitropyridine) [17] for condensation. Coupling time was increased from 5 min to 15 min obtaining a loading of 18 $\mu mol \cdot g^{-1}$.

Oligonucleotide Synthesis. Oligonucleotide sequences were prepared 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 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) increase of coupling time from 30 s to 300 s, b) increase the phosphoramidite concentration using 0.2M soln. instead of 0.1M soln. of phosphoramidite 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 using the standard 1-μmol synthesis cycle. Oligonucleotides **13**, **14** and **15** were prepared using a 200-nmol synthesis cycle with an increased coupling time (480 s).

Oligonucleotide carrying a thiol group at 5'end (**16**) was prepared on 1-μmol scale 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 1ml of concentrated ammonia (overnight, 55ºC). Oligonucleotide support carrying a thiol group at the 5'-end was treated overnight with 1ml of 50 mM dithio-DL-threitol (DTT) in concentrated ammonia at 55ºC. The excess of DTT was eliminated with a Sephadex G-25 column (NAP-10, *Pharmacia*, Sweden) just prior to conjugation with gold nanoparticles.

Purification of oligonucleotides. The products resulting from ammonia treatment were dissolved in water and purified by HPLC. Column : PRP-1, 10 μm (*Hamilton*, USA) (305 x 7mm), flow rate 3 ml/min. A 20min linear gradient from 15 to 50% acetonitrile over 100mM aqueous triethylammonium acetate was used for oligonucleotides carrying the $(MeO)_2$-Tr group. After removal of the $(MeO)_2$-Tr group with 80% acetic acid (30min) the resulting oligonucleotides were purified on the same column using a 20min linear gradient from 5 to 25% acetonitrile over 100 mM aqueous 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** (3x200 nmols): 45 OD units. **7** (2x200 nmols): 26 OD units. **8** (1-μmol): 17 OD units. **9** (1-μmol): 8 OD units. **10**, (200 nmols) 5 OD units. **11**, (200 nmols) 7 OD units. **12**, (2x 200 nmols) 19 OD units. **13**, (200 nmols) 5 OD units**. 14** (200 nmols): 8 OD units after Sephadex before electrophoresis, 1.5 OD units after electrophoresis. **15** (200 nmols): 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 gold salt as described in detail in [19]. 3,3',3''-Phosphinidene-tris(benzenesulfonic acid) trisodium salt (phosphine) was added to gold solution until final phosphine concentration of 0.5M. The mixture was stirred for 10 h. Solid NaCl was added to the solution until it turned from deep burgundy to purple color. The solution was centrifuged at 8,000 rpm for 20 min, the supernatant was discarded and the precipitated was redispersed in 0.5mM phosphine. Solution was precipitated again with NaCl, centrifuged and redispersed in 0.5× TBE buffer (final gold nanoparticle concentration 0.8μM).

Preparation of oligonucleotide-gold conjugates.

Thiolated oligonucleotide (**16**) complementary to oligonucleotides **11** and **15** was combined (50μM, 0.5× TBE buffer) with gold nanoparticle solution in 1:0.9 Au:DNA molar ratio mixing well. The resulting solution was brought to 50mM NaCl (from 1M NaCl) and incubated overnight.

Gold nanoparticles functionalized with thiol-DNA **16** were mixed with branched oligonucleotide **11** carrying 3 copies of complementary oligonucleotide in stoichiometric amounts. The resulting solutions 50mM 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 copper 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, 0.05M Tris·HCl buffer (pH 7.5). Duplexes were annealed by slow cooling from 90 ˚C to room temperature. UV Absorption spectra and melting curves (absorbance vs temperature) were recorded in 1-cm path-length cells using a Shimadzu UV2101PC UV/VIS spectrophotometer (*Shimadzu*, Japan) with a temp. controller with a programmed temp. increase of 0.5˚/min. Melting curves were obtained monitoring the absorbance at 260 nm on duplex concentrations of approx. 2μM. Melting temperatures were measured at the maximum of the first derivative of the melting curve. Results: see *Table 2*.

REFERENCES

[1] M.J. Damha, K. Ganeshan, R.H.E. Hudson, V. Zabarylo, V. *Nucleic Acids Res.* **1992**, *20*, 6565.

[2] M. Grotli, R. Eritja, B. Sproat, *Tetrahedron* **1997**, *53*, 11317

[3] V.A. Korshun, N.B. Pestov, E.V. Nozhevnikova, I.A. Prokhorenko, S.V. Gontarev, Y.A. Berlin, Y.A., *Synthetic Comm.* **1996**, *26*, 2531.

[4] M.S. Shchepinov, I.A. Udalova, A.J. Bridgman, E.M. Southern, *Nucleic Acids Res.* **1997**, *25*, 4447.

[5] M. Scheffler, A. Dorenbeck, S. Jordan, M. Wüstefeld, G. von Kiedrowski, *Angew. Chem. Int. Ed.* **1999**, *38*, 3312.

[6] L.H. Eckardt, K. Naumann, W.M. Pankau, M. Rein, M. Schweitzer, N. Winhab, G. von Kiedrowski, *Nature* **2002**, *420*, 286.

[7] C.A. Mirkin, R.L. Letsinger, R.C. Mucic, J.J. Storhoff, *Nature* **1996**, *382*, 607.

[8] A.P. Alivisatos, K.P. Johnsson, X. Peng, T.E. Wilson, C.J. Loweth, M.P. Bruchez, P.G. Schultz, *Nature* **1996**, *382*, 609-611.

[9] C.J. Loweth, W.B. Caldwell, X. Peng, A.P. Alivisatos, P.G. Schultz, *Angew. Chem. Int. Ed.* **1999**, *38*, 1808.

[10] J.J. Storhoff, C.A. Mirkin, *Chem. Rev.* **1999**, *99*, 1849.

[11] E. Braun, Y. Eichen, U. Sivan, G. Ben-Joseph, *Nature* **1998**, *391*, 775.

[12] K.A. Williams, P.T.M. Veenhuizen, B.G. de la Torre, R. Eritja, C. Dekker, *Nature* **2002**, *420*, 761.

[13] C.M. Niemeyer *Angew. Chem. Int. Ed.* **2001**, *40*, 4128.

[14] S. Connolly, D. Fitzmaurice, *Adv. Mater.* **1999**, *11*, 1202.

[15] D. Iacopino, A. Ongaro, L. Nagle, R. Eritja, D. Fitzmaurice, *Nanotechnology* **2003**, *14*, 447.

[16] E. Ferrer, G. Neubauer, M. Mann, R. Eritja, *J. Chem. Soc., Perkin Trans. 1* **1997**, 2051.

[17] K.C. Gupta, P. Kumar, D. Bhatia, A.K. Sharma, *Nucleosides & Nucleotides* **1995**, *14*, 829.

[18] S. Rumney, E.T. Kool, *J. Am. Chem. Soc.* **1995**, *117*, 5635.

[19] J.W Slot, H.J. Geuze, *Eur. J. Cell Biol*. **1985**, *38*, 87.

Table 1: Oligonucleotide sequences prepared

Number	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-CTGCCTGCATCGCTCAATGC-3’)$_2$
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_3-$O(CH_2)_3$-$CONH$-CH_2]$_2$-$CHOH$, bpp: [-PO_3-$O(CH_2)_4$-$CONH$-CH_2]$_2$-$CHOPO_3$-

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'-AGA GGA GGA AG-5' and 3'-GAC GGA CGT AGC GAG TTA CG-5', 0.050M Tris·HCl, 0.15M NaCl, pH 7.5, error in T_m is ±1 °.

LEGENDS

Scheme 1: Schematic representation of oligonucleotides described in this paper.

Scheme 2. Outline of the synthesis of oligonucleotides **6** and **7** with two identical arms.

Scheme 3. Outline of the synthesis of branched oligonucleotides **8**, **9**, **11** and **12** with two and three identical arms.

Scheme 4. Structure of the reagents used for the introduction of the symmetric branching molecule into oligonucleotides.

Scheme 5. Outline of the synthesis of branched oligonucleotides **14** and **15** with four arms with identical sequence.

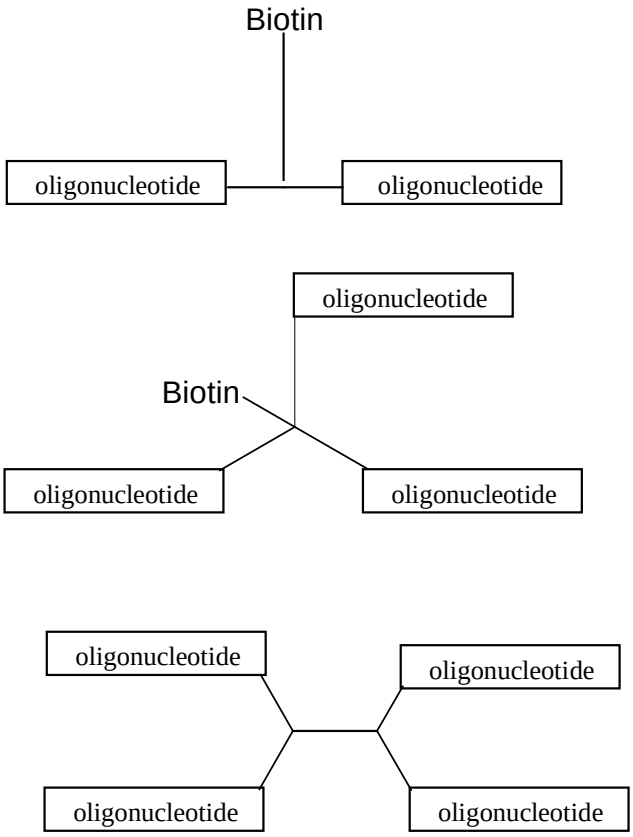
Figure 1: HPLC profiles of $(MeO)_2Tr$-containing oligonucleotides **11** (a) and **12** (b) with three arms. Truncated sequences without $(MeO)_2Tr$ groups had a retention time of less than 5 minutes. Benzamide eluted at 7-8 minutes. Fraction I contained oligonucleotides with one single $(MeO)_2Tr$ group. Fraction II contained the desired sequence with two $(MeO)_2Tr$ groups.

Figure 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: Fraction II, oligonucleotide **11**. Lane 2: Fraction I, oligonucleotide **11**. Lane 3: Fraction II, oligonucleotide **12**. Lane 4: Fraction I,

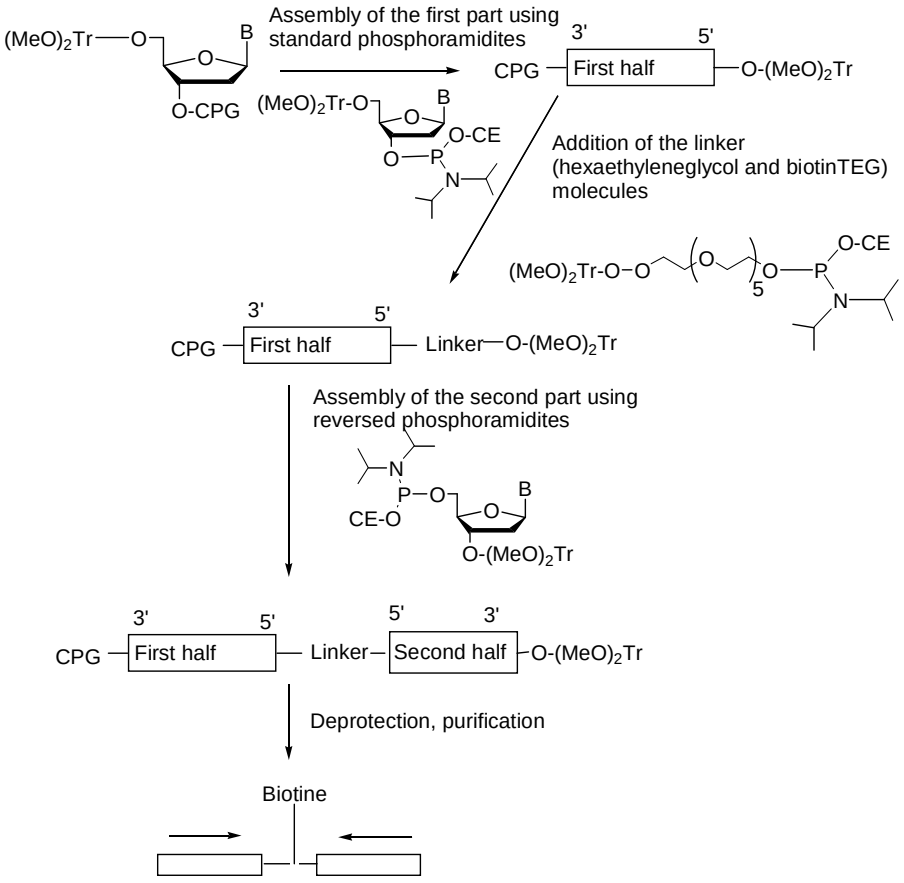
Oligonucleotide **12.** Lane 5: oligonucleotide **7**. The double band of oligonucleotide **7** is due to the diastereoisomers of biotin-tetraethyleneglycol molecule.

Figure 3: Representative TEM images of trimeric DNA-gold nanoparticle assemblies.

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

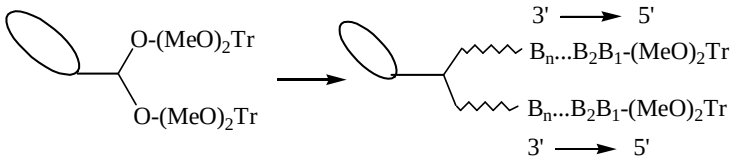

Biotin
oligonucleotide
oligonucleotide
oligonucleotide
Biotin
oligonucleotide
oligonucleotide
oligonucleotide
oligonucleotide
oligonucleotide
oligonucleotide

Scheme 1


Assembly of the first part using standard phosphoramidites
Addition of the linker (hexaethyleneglycol and biotinTEG) molecules
Assembly of the second part using reversed phosphoramidites
Deprotection, purification
Biotine
First half
Second half
Linker
CPG

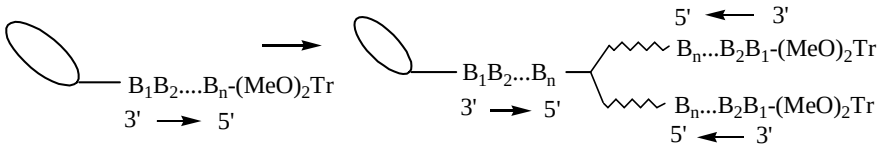
Scheme 2

Scheme 3



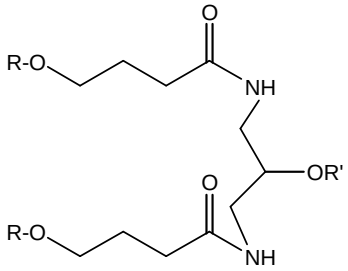
4

Oligonucleotides **8, 9**

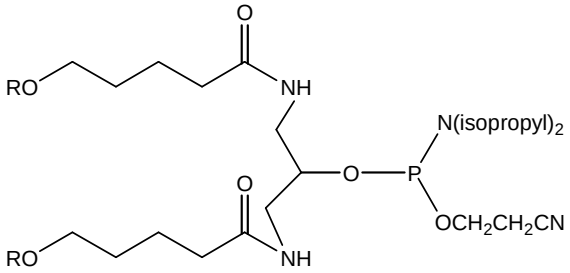


Oligonucleotides **11**, **12**

Scheme 4



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 5

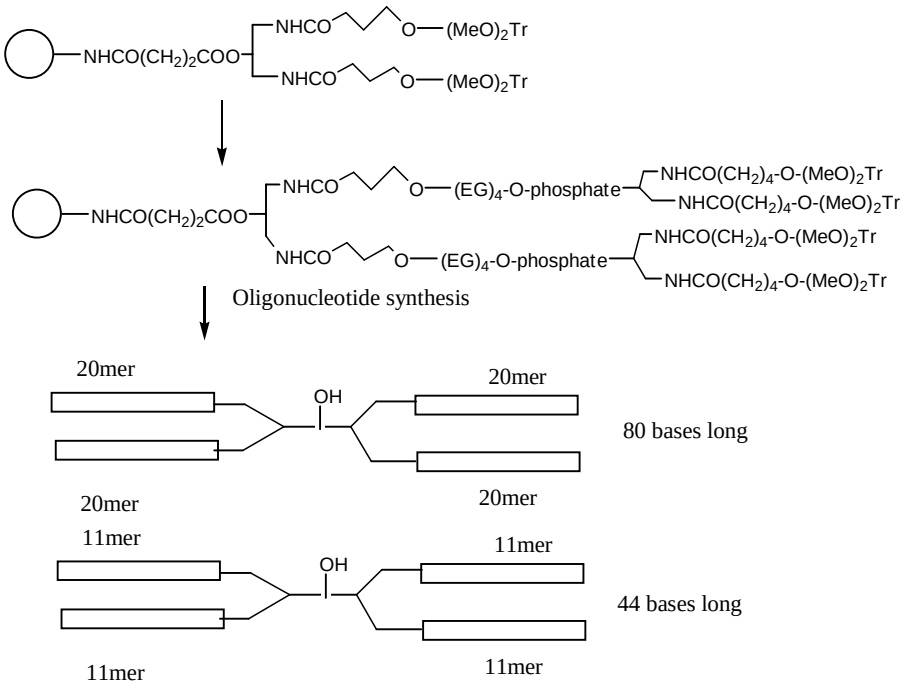

NHCO
O—(MeO)2Tr
NHCO(CH2)2COO
NHCO
O—(MeO)2Tr
NHCO
O—(EG)4-O-phosphate
NHCO(CH2)4-O-(MeO)2Tr
NHCO(CH2)4-O-(MeO)2Tr
NHCO(CH2)2COO
NHCO
O—(EG)4-O-phosphate
NHCO(CH2)4-O-(MeO)2Tr
NHCO(CH2)4-O-(MeO)2Tr
Oligonucleotide synthesis
20mer
20mer
OH
80 bases long
20mer
20mer
11mer
11mer
OH
44 bases long
11mer
11mer

Figure 1.

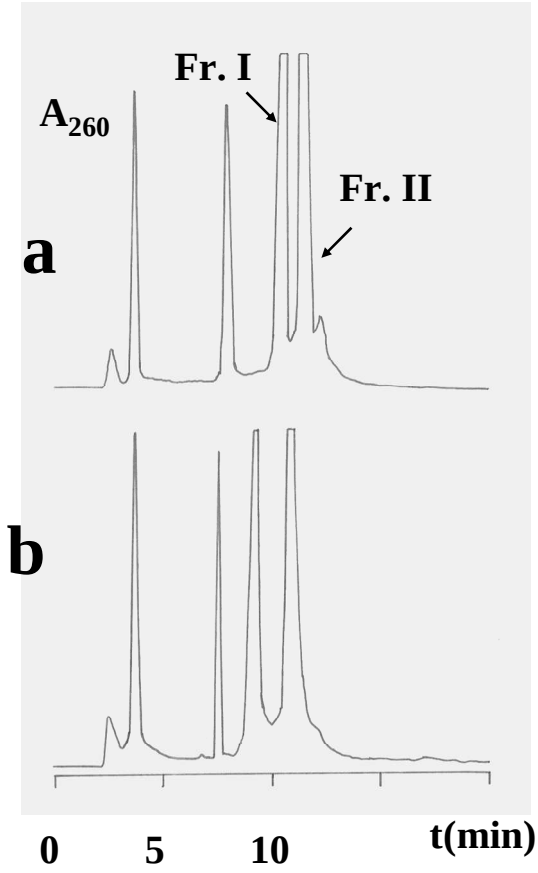


Figure 2.

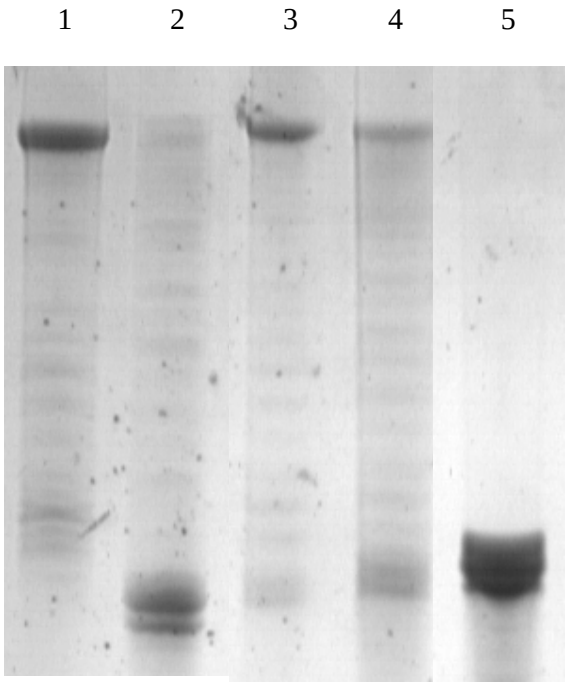


Figure 3.

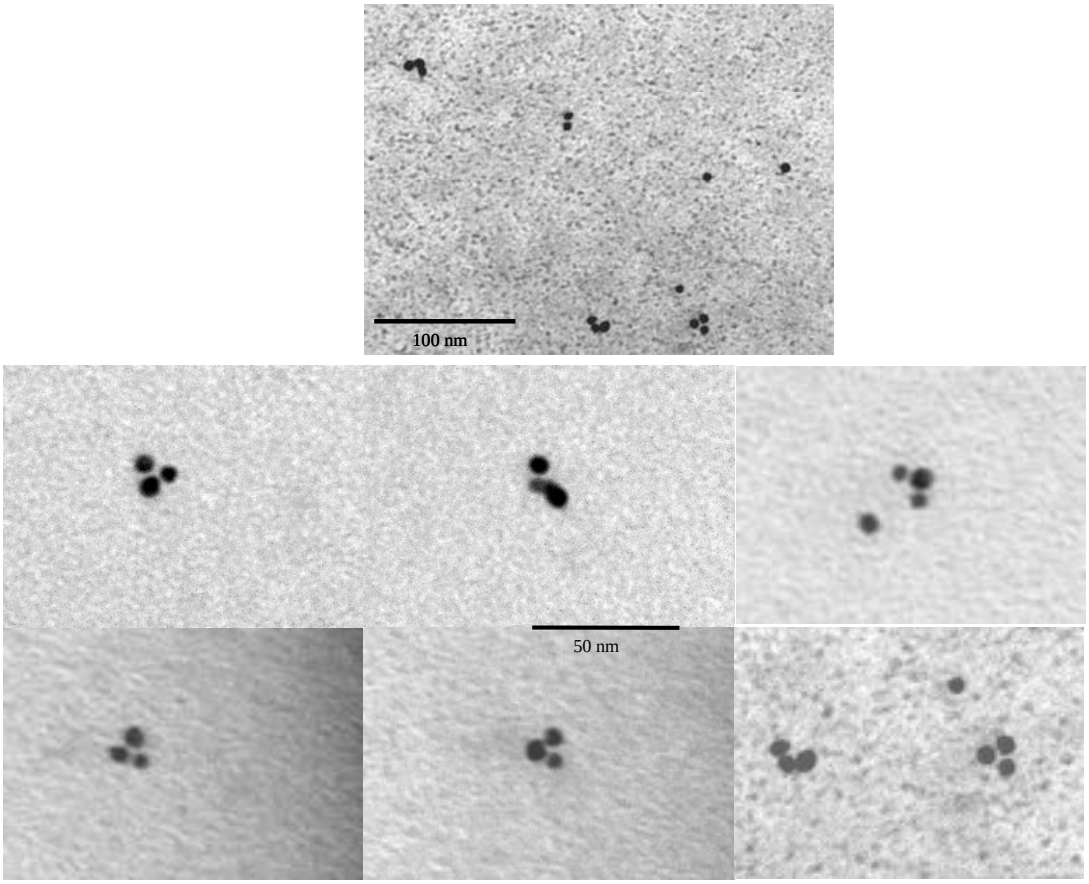


Figure 4.

1 2 3 4 5