14. Oligonucleotide Synthesis on Polystyrene-Grafted Poly(tetrafluoroethylene) Support

by **Eckhard Birch-Hirschfeld**[a]), **Zeno Földes-Papp**[b]), **Karl-Heinz Gührs**[a]), and **Hartmut Seliger**[b])*

[a]) Institut für Molekulare Biotechnologie e.V., Beutenbergstrasse 10, D–07745 Jena
[b]) Universität Ulm, Sektion Polymere, Albert-Einstein-Allee 11, D–89069 Ulm

(25.X.95)

Supports consisting of a thin layer (2–10%) of polystyrene (PS) grafted onto a poly(tetrafluoroethylene) (PTFE) core are an interesting alternative to controlled pore glass (CPG) carriers in oligonucleotide synthesis. The beads are mechanically stable, do not show significant swelling, and allow effective removal of substrates by short washing steps with organic solvents. PTFE-PS as an organic polymer has generally more hydrophobic properties than inorganic polymer supports and, therefore, is well compatible with organic solvents such as anhydrous MeCN. We found PTFE with a content of 2–3% PS graft to be a very good support for the synthesis of oligonucleotides of extended length. In comparison, PTFE with 5–10% grafted PS is especially useful for large-scale syntheses. Functionalization procedures minimized in the extent of side reactions are described as well as examples for the use of the supports in syntheses of oligonucleotides both on large scale and of extended chain length.

1. Introduction. – Most oligonucleotide syntheses during the last years were done on porous, non-swellable inorganic carriers, especially silica gel or controlled-pore glass. While these support materials yielded excellent results in routine automated preparations of smaller oligonucleotides, some difficulties occurred in the assembly of longer chains. In particular, steric and diffusion effects were observed during the preparation of long oligonucleotides, when supports with average pore diameters of 75 nm or lower were used [1]. More recently, we explored the potential of nonporous surface-coated silica-gel microbeads as carriers in oligonucleotide synthesis. We demonstrated that such a support system where oligonucleotide reactions only proceed on the outside of spheres internally inaccessible are useful for the preparation of even very long oligonucleotide chains [2]. Although conceptionally attractive, such support systems, in their practical application, suffer from the fact that the relatively small area available on the surface of microbeads has the consequence of extremely low loading of nucleoside groups.

A system similarly composed of an impenetrable inner core covered with a reactive coating was described in several publications from the laboratory in Jena [3] [4]. This system is based on polystyrene grafted onto *Teflon* granules in the gas phase.

Previous studies of large-scale syntheses of shorter oligonucleotide chains by modified phosphotriester approaches in the 5′→3′ as well as in 3′→5′ direction on polystyrene-coated *Teflon* supports of high nucleotide loadings yielded promising results [5] [6]. Therefore, we were interested to explore the potential of the above mentioned hydrophobic supports which provide good compatibility between the hydrophobic nature of the fully protected growing oligonucleotide chain and aprotic solvent systems in automated oligonucleotide synthesis, using the phosphoramidite approach.

2. Results. – 2.1. *Support Functionalization.* The results of the grafting reactions of styrene onto poly(tetrafluoroethylene) (PTFE) powder are shown in *Fig. 1*. The knots of the PTFE network are totally surrounded by polystyrene (PS) which thereby fills the cavities of the PTFE lattice. The copolymer particles exhibit a strongly structured PS surface with cavities and pores of different size.

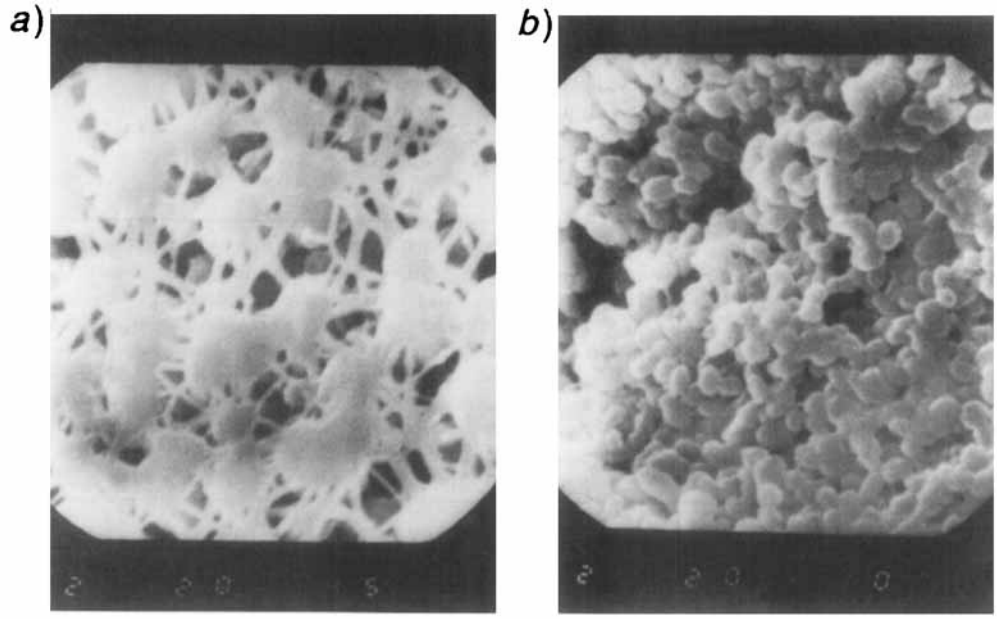


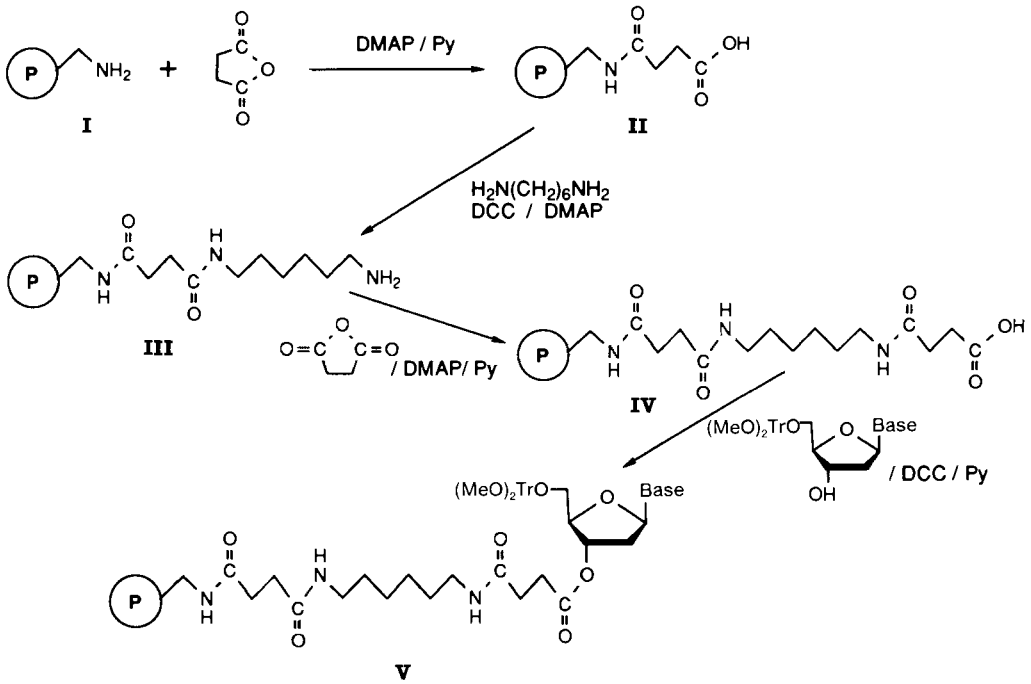
Fig. 1. *Microstructure of PTFE and of PTFE-PS granules demonstrated by electron microscopy. a*) PTFE particle in 20000-fold enlargement; *b*) Grafting product P_{20} (12–15% styrene) in 20000-fold enlargement.

The reactions leading to support functionalization are shown in the *Scheme*. Loadings could be varied within a wide range, from *ca.* 15 to 140 μmol nucleoside/g support. As expected, the nucleoside loading increases with increasing content of amino groups, the latter being itself related to the degree of grafting.

2.2. *Oligonucleotide Syntheses.* 2.2.1. *Application of PTFE-PS Copolymers for Routine Small-Scale Preparations of Average-Size Oligonucleotides As Well As of Long Sequences.* PTFE-PS Copolymers were used routinely for small-scale oligonucleotide syntheses applying the same 0.2-μmol standard cycles also used for syntheses with CPG supports. Sequences thereby obtained ranged from 34 to 146 bases; a representative list is given in *Table 1*. As seen from *Table 1*, the average yields per elongation, as determined from detritylation, were excellent in most cases (> 99% average) and comparable to the best average yields per elongation obtained with conventional CPG carriers. Most noteworthy, there was no decrease in these yields even for very long oligonucleotide sequences.

Table 1 also shows cases where the average yields per elongation were less than expected (*ca.* 98%). Investigating on these syntheses, we found an influence of the support matrix, in particular of the spacer length, on the average overall yields. As can be seen from *Fig. 2*, the interaction of the fully protected incoming phosphoramidites with the

Scheme. *Anchoring of Nucleotides to PTFE-PS Supports* via *a Long-Chain Spacer*[a])



Base = Thy, ib^2Gua, bz^6Ade, an^4Cyt

[a]) P = polymer support consisting of a thin layer of polystyrene onto a poly(tetrafluoroethylene) core; DMAP = 4-(dimethylamino)pyridine; DCC = dicyclohexylcarbodiimide; $(MeO)_2Tr$ = dimethoxytrityl.

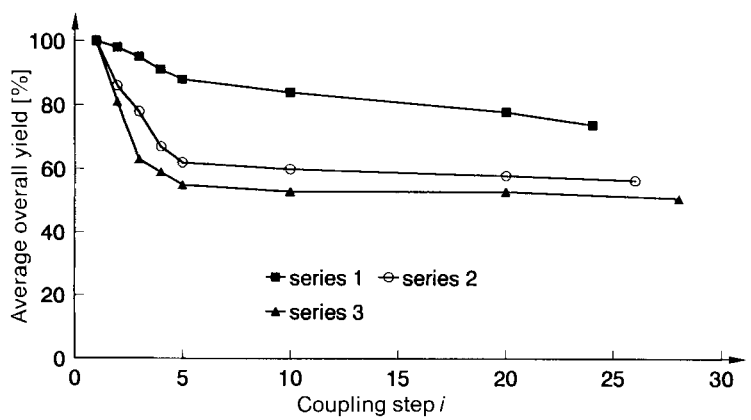


Fig. 2. *Influence of spacer on the average overall yield* [%] *of coupling steps* (*i*) *in oligonucleotide synthesis.* The yields were measured from detritylation by VIS absorption at 495 nm. *Series 1:* Synthesis on P_{20} with long spacer; *series 2* and *3:* Syntheses using P_{20} without long spacer.

Table 1. *Results of Oligonucleotide Syntheses on Different PTFE-PS Supports with Long Spacer* (type **IV**; see *Scheme*) *in Comparison with 500-Å CPG or 1000-Å CPG with Lcaa Spacer*[a]). Conditions: 0.2-μmol standard cycles of *Applied-Biosystems-380B* DNA synthesizer.

Support	Nucleoside loading [μmol/g]	Target length *N* of oligonucleotide[a])	Purified amount [OD_{260}]	Total yield after *N*-1 cycles [% $(MeO)_2Tr$]	Average yield per elongation [% $(MeO)_2Tr$]
P_{29}	16.5	47mer **4**	29	70	99.2
P_{29}	12.5	45mer **5**	32	80	99.5
P_{29}	16.5	70mer **6**	23.8	85.6	99.8
P_{29}	16.5	146mer **7**	20.1	31.9	99.2
P_{19}	48	69mer **8**	16	63.4	99.3
P_{20}	87	44mer **9**	10.3	40	97.9
P_{20}	80	46mer **10**	9	35	97.7
CPG 500	29.4	34mer **1**	21	74.8	99.1
CPG 500	41	33mer **2**	19	83.4	99.4
CPG 100	41	67mer **3**	14	28.4	98.1

[a]) The following sequences were synthesized (for convenience, the hyphens representing the phosphodiester links are omitted):

1 (34mer) d(TCTAGAGGATCCGAATTCGTTGTAAAAATTGGGG)

2 (33mer) d(ACAGGCCCGTATGAGCTCATAAATGTGACTGGA)

3 (67mer) d(CCTGTTGGTTCAAAATAACTCGCGTCATCGCCTTGTTCATCTTCTCCTTTGTCGAATGAACTTGACA)

4 (47mer) d(ATGGGCAACCTTGACCTTTGAAGAGAACCTGGGAGTAGATCTCTGCA)

5 (45mer) d(ACAGCGATACGACTGATCGTGTGTGAGTTAGTAGAACATGAGTAG)

6 (70mer) d(TCTAGAGAATTCACGTATCATAGATATAAATGTAATGGCATAGGGCGGGTTTTATTTTCAGCCTGGATCG)

7 (146mer) d(AATTCAGGCCTCATATGGGCAAACCCCAGGTGGAACCGAAAAAATGCCCGGGTCGTGTGGTTGGCGCTTGTGTTGCCCATC-CGCATAGCTGGCCATGGCAGGTTTCTCTGCGTACCCGATTCGGTGTTCAGTTTTAAGGATCCTTC)

8 (69mer) d(TATGTCAAGTTCATTCGACAAAGGAGAATATAAAAAAGGCGATGACGCGAGTTATTTTGAACCAACAGG)

9 (44mer) d(AACAGCGATACGACTGATCATGTGAGTTAGTAGAACATGAGTAG)

10 (46mer) d(TGGGCAACCTTGACCTTTGAAGAGAACCTGGGAGTAGATCTCTGCA)

hydrophobic surface of the support particles leads to relatively low average overall yields of the initial coupling steps (*ca.* 70%), when supports containing a short spacer were used (*Scheme*, structure **II**). The introduction of a longer spacer, consisting of succinic residues and a bifunctional amino component [7] (structure **IV**) significantly reduced the number of spacer-ended amino groups as compared to amino functionality originally provided by the support. This is an indication of limited accessibility of a considerable fraction of the amino groups directly bound to the styrene coating of the support.

A slight decrease in average overall yields during the first cycles was even found for supports with long spacers (*Figs. 2* and *3*). This, however, could be overcome by prolonging the reaction time in the initial five coupling steps to 60 s. With this minor modification, syntheses on a 0.2-μmol scale could be run on routine protocols for automated synthesizers. Based on a support loading of typically around 12–17 μmol nucleoside/g, but also up to 48 μmol/g, average yields of the chain elongation were well above 99%, as shown in *Table 1*. These yields were of the same values, if not better, as those obtained in similar syntheses using CPG 500 with long-chain alkylamine spacers (also shown in *Table 1*).

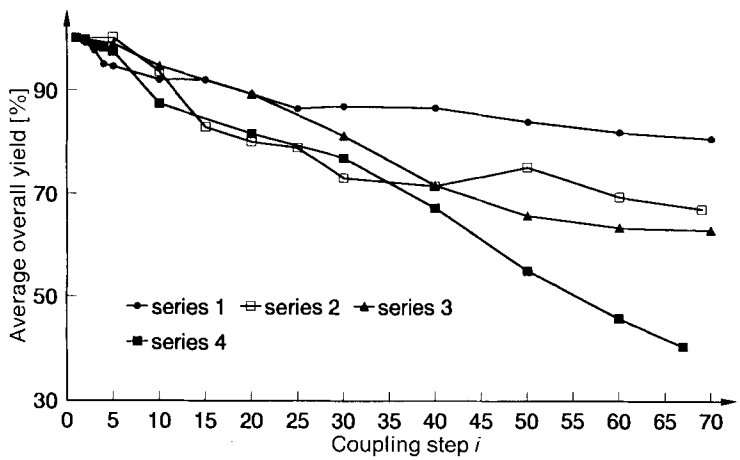


Fig. 3. *Comparison of the average overall yield* [%] *of coupling steps* i *during the course of oligonucleotide syntheses of extended chain length using PTFE-PS and CPG supports, respectively.* The yields were measured by VIS-absorption at 495 nm. *Series 1:* 70mer prepared on P_{29} with spacer of type **IV**; *series 2:* 70mer prepared on 1000-Å CPG (Lcaa); *series 3:* 69mer prepared on P_{19} with spacer of type **IV**; *series 4:* 67mer prepared on 500-Å CPG (Lcaa).

Fig. 4 shows an autoradiograph of a polyacrylamide-gel electrophoretic separation of the products of several syntheses listed in *Table 1*. The appearance of the gel separation, again, confirms that grafted polystyrene-PTFE supports give similar yields as controlled-pore glass. The low content of *N*-1 error sequences in the crude product of a 30mer synthesis on P_{19} support is demonstrated by high-performance capillary electrophoresis in *Fig. 5*.

Also included in *Table 1* and *Fig. 4* is the preparation of a 146mer sequence done on a long-chain alkylamine spacered polystyrene-PTFE support. The average yield per elongation (cycle) in this case was found to be 99.2%. The gel separation shown in *Fig. 4*

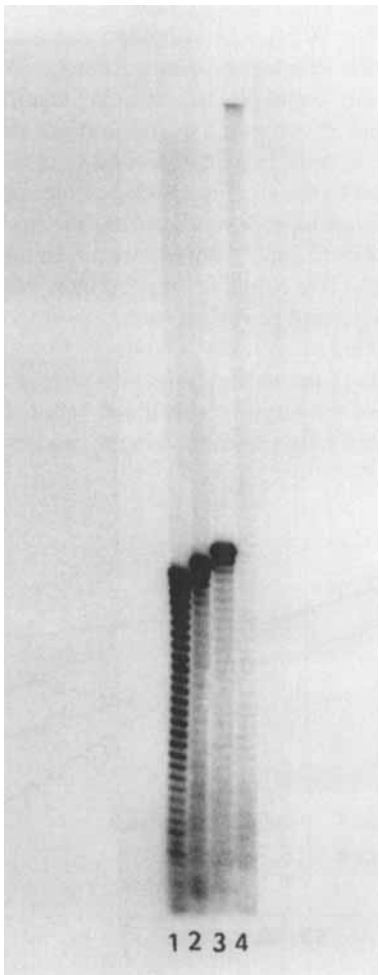


Fig. 4. *Autoradiograph of gel-electrophoretic separation of crude synthesis products using 500-Å CPG* (Lcaa) *and PTFE-PS supports.* Crude products were separated on a 6% polyacrylamide gel containing 7M urea. *Lane 1:* 67mer prepared on CPG; *lane 2:* 69mer prepared on P_{19} with type-**IV** spacer; *lane 3:* 70mer prepared on P_{29} with type-**IV** spacer; *lane 4:* 146mer prepared on P_{29} with type-**IV** spacer.

clearly demonstrates the applicability of this support material to the preparation of unusually long oligonucleotide chains.

2.2.2. *Application of PTFE-PS Copolymers to Large-Scale Synthesis of Oligonucleotides.* Recently, in the context of physico-chemical studies, but especially for future therapeutic applications, there is a demand for supports that allow large-scale preparations of oligonucleotides. To test this possibility, we prepared a series of supports with medium-to-high loading ranging from 42 to 130 μmol nucleoside/g (*Table 2*). Some of these carriers were used to prepare oligonucleotides on a 10-μmol scale. In these syntheses, up to 1400 OD_{260} units were obtained in yields averaging more than 99% per

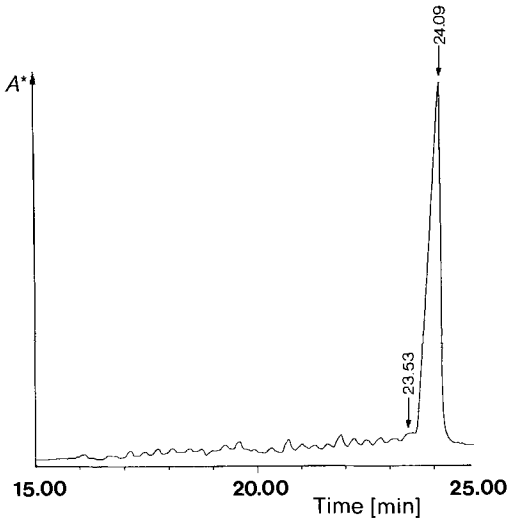


Fig. 5. *Profile of separation by high-performance capillary electrophoresis.* The 30mer crude product was synthesized on a 0.2-μmol scale with support P_{19}. The main peak at the retention time 24.09 min is the desired target (5′–3′) d(G-A-A-C-T-G-A-C-T-G-G-T-C-A-A-C-G-T-C-T-G-C-G-T-G-A-A-G-G-T). At retention time 23.53 min, the *N*-1 error sequences are recorded. The electrophoretogram was measured on a *Beckman* capillary electrophoresis system, model *P/ACE 5510* (*Beckman Instruments*, Palo Alto, CA, USA) at 30° equipped with a diode array detector and software *Beckman* System Gold Version 8.1. Running buffer: 250 mM *Tris*-borate with 7M urea, pH 8.4; detection at 254 nm. The capillaries (type ssDNA 100, 100 μm i.d.) filled with 12% polyacrylamide gel were purchased from *Beckman*.

Table 2. *Results of Large-Scale Oligonucleotide Syntheses on PTFE-PS Supports in Comparison with 500-Å Lcaa-CPG*[a])

Support	Loading [μmol/g]	Synthesis scale [μmol]	Target length *N* of oligonucleotide[a])	Purified amount [OD_{260}]	Total yield after *N*-1 cycles [% $(MeO)_2$Tr]	Average yield per elongation [% $(MeO)_2$Tr]
P_{19}[b])	42.2	10	12mer **11**	744	81	98.1
P_{19}[b])	42.8	10	25mer **12**	1165	65	98.3
P_{19}[b])	46.2	10	25mer **13**	1395	84	99.3
P_{19}[b])	42.8	10	25mer **14**	1265	83	99.3
P_{20} (manual)[c])	84	5	9mer **15**	280	60	93.8
P_{20} (manual)[c])	83.7	5	9mer **16**	291	67	95.1
P_{22}[c])	120	1	30mer **17**	38	33.8	96.3
P_{22}[b])	127	1	12mer **18**	24.8	69.6	96.8
CPG	41	1	25mer **19**	81.5	82.4	99.2

[a]) The following sequences were assembled (for convenience, the hyphens representing the phosphodiester links are omitted):

11 (12mer) d(CTTGGAGAATCC)
12 (25mer) d(AAAAAAAAAATAATTTTAAATATTT)
13 (25mer) d(TTTTTTTTTTATTAAAATTTATAAA)
14 (25mer) d(AAATATTTAAAATTATTTTTTTTTT)
15 (9mer) d(CGTTTTTGC)
16 (9mer) d(GCAAAAACG)
17 (30mer) d(CTATAATACCACTGGCGGTGATATCGAATT)
18 (12mer) d(CGAAAATTTTCG)
19 (25mer) d(TTTTTTTTTTATTAAAATTTATAAA)

[b]) Type-**IV** spacer; see *Scheme*.
[c]) Type-**II** spacer; see *Scheme*.

elongation (cycle) (*Fig. 6*). Very high loading (120–127 μmol nucleoside/g) makes the growing oligonucleotide chains somewhat less accessible resulting in yields decreasing to 96–97%. However, it has to be emphasized that these high-load supports could easily be handled in automated machines. Syntheses on larger scale with these systems are cur-

rently being explored. For purification of large amounts of crude oligonucleotide products, we developed a one-batch purification by liquid chromatography (*Fig. 7*). This method enables purification of up to 800 OD_{260} in one run.

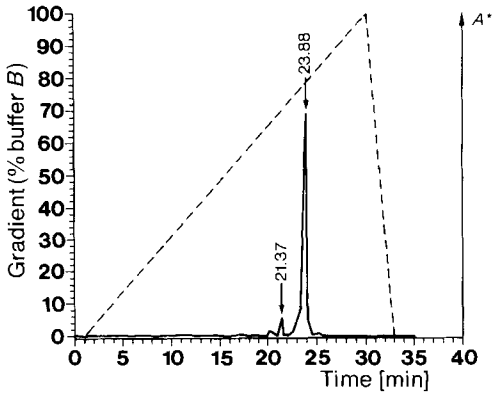


Fig. 6. *Profile of HPLC separation on a* MonoQ-HR-5/5 *column* (*Pharmacia*, Freiburg) *of the 25mer crude product of a large-scale synthesis with support* P_{19}. The main peak at retention time 23.88 min is the desired 25mer target (5′–3′) d(T-T-T-T-T-C-T-T-T-C-A-T-C-A-A-A-A-T-T-C-A-T-A-A-A). At retention time 21.37 min, the *N*-1 error sequences are measured. The chromatogram was obtained with a *BioRad-800* HRLC system at 60° using a gradient of 0 to 100% buffer *B* within 30 min at a flow of 1 ml/min. Buffer *A:* 10 mM NaOH (pH 11); buffer *B:* 1M NaCl in 10 mM NaOH (pH 11); detection at 260 nm.

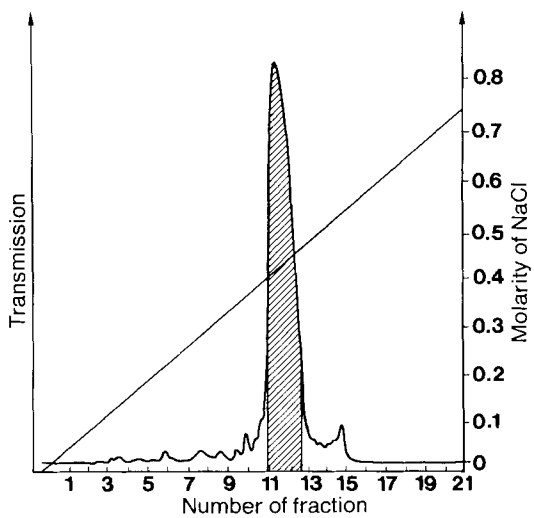


Fig. 7. *One-batch purification of large-scale synthesized oligonucleotides.* Crude product (462.8 OD_{260}) of a 17mer, (5′–3′) d(T-A-A-T-A-C-G-A-C-T-C-A-C-T-A-T-A), was purified by liquid chromatography using the column *Resource*™ *Q* (6 ml) from *Pharmacia*, Sweden. The chromatogram was recorded by *Pharmacia LKB Rec1* with a single-path monitor *UV-1*, 254 nm. A gradient of NaCl in 20 mM *Tris*, pH 9.2, was applied at a flow of 6.8 ml/min as indicated. Buffer *A:* 100 ml of 20 mM *Tris* (pH 9.2); buffer *B:* 100 ml of 1M NaCl in 20 mM *Tris* (pH 9.2); detection at 254 nm. From the fraction of the main peak indicated by hatching (purified target sequence), 417.7 OD_{260} were obtained.

3. Discussion. – In addition to easy handling and high efficiency in routine automated oligonucleotide synthesis, the preparation of chains of extended length as well as the production of shorter sequences in large quantities are declared goals of current oligonucleotide chemistry. Most supports predominantly in use, although well studied for routine small-scale preparations, seem to have limitations in one or the other of these aspects. Thus, in the case of CPG, the accessibility of the growing oligonucleotide chains is connected to pore size. In a study from our laboratory, CPG-type supports were shown to exhibit an almost equal density of surface nucleoside anchor groups (0.7–0.9 μmol/m^2), regardless of their average pore size. As a consequence of these findings, CPG supports of larger average pore size were recommended for the synthesis of oligonucleotides of higher chain length and were used also for combined chemical and enzymatic oligonucleotide chain lengthening [8]. Long chains can be made only with low-capacity supports. Small-pore CPG allows higher loading, but even this may not be a sufficient basis for the production of oligonucleotides in gram quantities and beyond. Nonporous surface-coated support systems such as silica-gel microbeads are conceptionally attractive for the synthesis of oligonucleotides of high chain length [2]. However, the loading of such surface-coated supports is generally so small that they offer no possibility for application to large-scale preparations [9].

Here, we describe a 'compromise' support system, that gives excellent yields in routine small-scale synthesis, but has the unique feature that it can also be used with equally high efficiency for the preparation of oligonucleotides of extended length and in large quantity. This support is based on impenetrable, chemically inert, and mechanically stable PTFE microbeads as core material [10]. On the outer surface of the PTFE core, the support contains a shell of polystyrene covalently bound to the PTFE by graft copolymerization. Depending on the conditions of grafting [4], copolymers of different composition, *i.e.*, different content of polystyrene graft, can be obtained. This enables the introduction of variable nucleoside loadings and simultaneously a good accessibility of the surface-bound growing oligonucleotide chains.

As shown in *Fig. 1*, the PTFE core is completely coated by polystyrene, especially in copolymers with higher graft content. Since the polystyrene layer is highly structured, a large surface area is available for further functionalization. The polystyrene lattice is well compatible with solvents and reagents applied during the elongation cycles, as was recognized in work originating from other groups [11–13]. Whereas other supports which contain an impenetrable core and only a shell of growing oligonucleotide chains are usually confined to a very low loading and have particle sizes that may present difficulties for handling in automated apparatus [2], the support system described here is based on particles of a diameter of 50–1000 μm which present no problem to filtration and washing. Nucleoside loadings can be varied within a wide range, from *ca.* 15 up to *ca.* 160 μmol/g. The introduction of a longer spacer, consisting of succinic residues joined by a bifunctional amino component, significantly reduces the number of spacer-ended amino groups, as compared to amino functionality originally provided by the support, but increases the accessibility of the functional growing chain ends.

PTFE-PS Copolymers of different styrene content and different loading were tested for their performance in oligonucleotide synthesis in comparison with CPG of different pore size as a reference. In *Figs. 2* and *3*, the average overall yield is plotted against the number of cycles *i*. Monitoring of trityl yields provides some obstacles (see [14]). Com-

puter simulations of average yields per elongation [15] [16] clearly demonstrated that trityl yields never reflect rigorously yields of truncated or failure sequences as well as the target sequence. To obtain more accurate synthesis parameters, we will determine the propagation probability function (d(i)) and the termination probability function (p(i)) from measured elution profiles by mathematical (iterative) methods [16]. Nevertheless, the data of *Figs. 2* and *3* give some indications for a non-constant chain growth during synthesis of long target sequences above $N \approx 30$ which strongly depends on the support and spacer type used. Further, they indicate an exponential or polynomial propagation probability function (d(i)) for the synthesis of target sequences above $N \approx 30$. It is emphasized that this result is not obtained from a local picture of the nonlinear growth system, *e.g.* from cloning of N-1 error sequences [17].

The average overall yields using PTFE-PS supports were shown here to be compatible to those of CPG. However, different from controlled-pore glass, matrix effects of the support surface seem to play a role during initial cycles, as demonstrated in *Figs. 2* and *3*. Especially, the cleavage of dimethoxytrityl groups preceding the coupling reactions was slowed down during the first cycles. This adverse effect could be partly compensated by the introduction of the longer spacer (see above) and by doubling the time for detritylation and coupling during the first five cycles. Prolonged detritylation steps are used for synthesis of long oligonucleotides [18–20] [2]. With these adjustments, the excellent results given in *Table 1* and *Fig. 4* could be obtained with low-grafted PTFE-PS supports in the synthesis even of unusually long oligonucleotides. As shown in *Fig. 4* with remarkably small fractions of truncated oligonucleotides in gel-electrophoretic separations, the results are the better the lower the content of polystyrene graft. Steric hindrance due to the filling of pores, as observed during the preparation of very long oligonucleotide chains on CPG supports [1], does not seem to be a problem with surface-loaded supports. As demonstrated in *Fig. 4*, the supports with a low styrene content (P_{29}) are best suited for the preparation of unusually long oligonucleotides. Thus, using supports with longer spacer, low styrene content (2–3%), and low nucleoside loading (*ca.* 15 μmol/g), oligonucleotides of length up to 100 bases were synthesized in yields per elongation averaging > 99% (see *e.g.* P_{29}, *Table 1* and *Fig. 5*). These yields were of the same values, if not better, as those obtained in similar syntheses using CPG 500 or CPG 1000 with long-chain alkylamine spacers. The synthesis of a 146mer target sequence yielded 99.2% per elongation (cycle). This clearly demonstrates the applicability of the support material with an extended amino-terminated succinamide spacer to the preparation of unusually long oligonucleotide chains.

The synthesis of gram amounts up to the kilogram range [21] is a challenge to oligonucleotide chemistry [22] as well as to separation techniques for analysis of crude products [16]. CPG Supports employed for small- and medium-scale synthesis have limited nucleoside loading as reported by *Pon et al.* [23] (typically 30–40 μmol nucleoside/g support). Copolymers of polyethylene glycol and polystyrene were functionalized up to 190 μmol nucleoside/g support and were applied to the phosphoramidite method [24] and H-phosphonate method [25], but their properties are mainly influenced by the hydrophilic polyethylene glycol component [26]. Rigid polystyrene supports loaded from 80 to 340 μmol nucleoside/g support were used for synthesis of 8mer and 10mer oligonucleotides [27]. The larger content of nucleoside anchored to PTFE-PS supports with a higher fraction of polystyrene graft enabled the preparation of large quantities of syn-

thetic oligonucleotides using a relatively small input of polymer support. Thus, PTFE carriers with 12–17% grafted polystyrene allowed loadings up to 160 μmol nucleoside/g support. Very high loading (120–127 μmol nucleoside/g support) made the growing oligonucleotide chains somewhat less accessible resulting in average yields per elongation that decreased to 96–97%. However, it has to be emphasized that these high-load supports could easily be handled in automated machines. Besides the reduction of expense for support materials, this results in a lower consumption of phosphoramidites and other reagents due to the smaller volume and better solvation of supports within large-scale reaction columns [28]. As can be seen from *Figs. 5* and *6*, even in the case of large-scale synthesis with highly grafted supports, the buildup of truncated and failure sequences is rather small. A good compromise are supports with 5–10% styrene content and loadings of up to 50 μmol/g. They afford routine synthesis of shorter oligonucleotides, *e.g.* 25mers, in optimum yield, producing quantities of up to 1400 OD_{260} units per batch in yields averaging more than 99% per elongation (cycle). Applications of the PTFE-PS supports to preparations of structurally modified oligonucleotides are under investigation. An alternative option for large-scale preparations are solution-phase syntheses (for a review, see [22]).

Considerable interest has concentrated recently on polystyrene support materials cross-linked by different amounts of divinylbenzene. Although these are now widely in use, they seem to have limitations in one or the other aspects. *McCollum* and *Andrus* [11] reported on polystyrene material which is especially suitable for small-scale syntheses (40 nmol) up to *ca.* 50mers. Furthermore, they claim to have synthesized a 209mer. Theoretical analyses of syntheses of extremely long oligonucleotides (above *ca.* 200 nucleotides) show that this is possible, but a very low amount of target sequence may result [15] [29] [30]. However, the literature so far gives no direct evidence for the composition of the crude product before applying cloning procedures as well as site-directed mutagenesis. *Bardella et al.* [31] described syntheses with similar average coupling efficiency in large scale, but this was exemplified for 8mers only (the overall yield for the purified product was 21%). More recently, *Wright et al.* [24] reported on syntheses from a 25-μmol up to 1-mmol scale. In the case of polystyrene materials especially designed for high loading [32], swelling/deswelling complicates their use in automated synthesizers.

E. Birch-Hirschfeld and *K.-H. Gührs* want to thank Mrs. *Christel Hartmann* for excellent technical assistance. *Z. Földes-Papp* and *H. Seliger* gratefully acknowledge financial support from the *Deutsche Forschungsgemeinschaft*.

Experimental Part

General. The 5′-*O*-(dimethoxytrityl)deoxynucleoside 3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl)phosphoramidites were purchased from *MWG Biotech, Roth,* and *Applied Biosystems.* The standard solns. for activation, oxidation, and detritylation were also from these companies.

Characteristics of the PTFE-PS Supports. The following types of our PTFE-PS supports were used throughout this study: P_{29}, P_{19}, P_{20}, and P_{22}. The supports were characterized by the degree of grafting (DG), NH_2 groups bound to the support and to the spacer, as well as by the nucleoside loading. A comprehensive list of these data is reported in [6] and [9]. In brief, P_{29} has a DG of 2–3% styrene which enables a nucleoside loading of 11–18 μmol/g support. For P_{19}, P_{20}, and P_{22}, the values are 5–7% styrene and 42–48 μmol nucleoside/g support, 12–15% styrene

and 50–80 μmol nucleoside/g support, and 15–17% styrene and 120–140 μmol nucleoside/g support, resp. The experimental conditions for grafting of styrene onto PTFE and functionalization of polymer supports are given in detail below.

Grafting of Styrene onto PTFE. A coarse-grained PTFE powder (*Polychrom I*, gift from Dr. *Potapov*) with an average particle diameter of 500–1000 μm was chosen as starting material. The grafting of styrene onto this material was accomplished by ^{60}Co radiation in an eddy flow reactor in which the PTFE gravel was flown through by a styrene-saturated N_2 stream [4]. This procedure yielded products highly homogeneous in degree of grafting (DG) and avoided the formation of large amounts of styrene homopolymers. The DG was determined either as weight increase of the support material during the grafting reaction or from the ratio of intensities of the IR bands at 1560 and 1610 cm^{-1}, respectively, compared to a calibration curve. Moreover, DG was estimated from corrected values of the C-content of polymers as determined in elementary analysis [4]. Unfortunately, all the methods mentioned failed to provide precise data at DG lower than 5%, where rough estimates could only be taken from the amino-group content of the support in the course of further functionalization.

Functionalization of Polymer Supports (see *Scheme*). The resin was treated with *N*-(chloromethyl)phthalimide/trifluoromethanesulfonic acid 1:2 (mol/mol) and the mixture was subsequently hydrolyzed [33]: *resin-methanamide* **I**. The amounts of incorporated amino groups could be regulated, to some extent, by shortening or prolonging the time of reaction. A valuation of the content of amino groups linked to the resin was obtained by colorimetric determination of picrate released by picrate complexes of support aliquots [34].

Spacer groups were optionally linked to amino groups of the support *via* succinylation followed by subsequent reaction with hexane-1,6-diamine and further succinic anhydride. In a typical succinylation, 5 g of **I** (P_{20}, DG = 10–12%, 185 μmol NH_2/g) were suspended in 25 ml of abs. pyridine and treated with 2.5 g (25 mmol) of succinic anhydride and 2.05 g (25 mmol) of 1-methyl-1*H*-imidazole (25 mmol). After incubation at r.t. for 12 h, the *4-oxo-4-[(resin-methyl)amino]butanoic acid* **II** was filtered and subsequently washed with pyridine, pyridine/H_2O 1:1, pyridine, MeOH, and Et_2O and dried *in vacuo*.

For the further elongation of the spacer, **II** was suspended in 15 ml of abs. pyridine and then 203 mg (1.75 mmol) of hexane-1,6-diamine, 516 mg (2.5 mmol) of dicyclohexylcarbodiimide and 61 mg (0.5 mmol) of 4-(dimethylamino)pyridine were added. After stirring at r.t. *N-(6-aminohexyl)-N-(resin-methyl)butanediamide* for 10 h, **III** was filtered and washed successively with pyridine, MeOH, and Et_2O. In case of P_{20} with a content of 185 μmol/g styrene bound NH_2 groups, the reaction yielded a content of 89 μmol/g of amino groups, as measured by the picrate assay.

A second succinic residue mediating the linkage to the first deoxynucleoside was introduced by stirring 5 g of dried **III** together with 165 mg of succinic anhydride and 0.977 g of 4-(dimethylamino)pyridine in 15 ml of abs. pyridine at r.t. for 10–12 h. The resulting *4-{6-{1,4-dioxo-4-[(resin-methyl)amino]butylamino}hexylamino}-4-oxobutanoic acid* **IV** was isolated by filtration and dried *in vacuo* after stepwise washing with pyridine, MeOH, and Et_2O.

For linking of deoxynucleosides to the support, typically 1 g of **IV** suspended in 3 ml of abs. pyridine was mixed with 0.3 mmol of a 2′-deoxy-5′-*O*-(dimethoxytrityl)nucleoside and 412 mg (2 mmol) of dicyclohexylcarbodiimid as well as with 24 mg (0.2 mmol) 4-(dimethylamino)pyridine and stirred at r.t. for 4 h. The reaction was stopped by addition of 0.5 ml of MeOH. After 2 more h of stirring, the mixture was filtered, and the formed *2′-deoxy-5′-O-(dimethoxytrityl)-3′-O-{4-{6-{1,4-dioxo-4-[(resin-methyl)amino]butylamino}hexylamino}-1,4-dioxobutyl}nucleoside* **V** was washed with pyridine, dimethylformamide, MeOH, and Et_2O. The product was dried *in vacuo*, and then unreacted amino groups were blocked by a 4 h reaction at r.t. with 0.5 ml of Ac_2O in 0.5 ml abs. pyridine catalyzed by 24 mg (0.02 mmol) of 4-(dimethylamino)pyridine. Finally, the resin was isolated by filtration and washed with pyridine, MeOH, and Et_2O. After careful drying of the support *in vacuo*, loadings of 60 to 80 μmol of 2′-deoxynucleoside per gram of resin were measured by the colorimetric determination of the acid-released trityl moiety.

Oligonucleotide Synthesis, Purification, and Analysis. Syntheses were carried out using an *Applied-Biosystems-380B* DNA synthesizer according to 0.2-, 1-, or 10-μmol standard cycles. Using polystyrene-grafted PTFE supports, coupling steps as well as washing steps during the initial five elongations were run for double time compared to the 0.2-μmol standard synthesis cycles.

Oligonucleotides were purified by HPLC using a *Pye-Unicam-PU-4000* apparatus in ion-exchange mode on *Partisil-10 SAX* (*Whatman*, 4,6 × 250 mm, 10 μm). Alternatively, oligonucleotides, especially those of extended chain length, were isolated from polyacrylamide gels (12–20%, 1–3-mm thickness) containing 7M urea and were

subsequently desalted using *DEAE* paper (*Whatman*). Large-scale purification was carried out by liquid chromatography: *Resource*™-*Q* column, 6 ml (*Pharmacia*); detection at 254 nm; flow rate 6.8 ml/min; linear gradients from 0 to 100% buffer *B* in *A* (buffer *A*, 20 mM *Tris*, pH 9.6; buffer *B*, 1M NaCl in 20 mM *Tris*).

Estimates of relative yields were obtained by relating the 495-nm absorptions of the solns. of the individual detritylation steps to that of the detritylation of the support-bound nucleoside. To get average overall yields, the solns. resulting from cleavage of the dimethoxytrityl protecting group were separately collected, and absorptions at 495 nm were manually determined after acidification by addition of 0.1M toluene-4-sulfonic acid in MeCN.

HPLC Measurements were performed on *Bio-Rad 2700* (*Bio-Rad*, CA, USA) equipped with a column oven, the *Bio-Rad* UV/VIS detector *UV-1806*, and with software Series *800 HRLC*®-System, Version 2.30.1a. The *MonoQ-HR-5/5* column (*Pharmacia*, Freiburg) was used. Detection at 260 nm; flow rate 1 ml/min; temp. 60°; linear gradients of 0 to 100% buffer *B* in *A* were used within 30 min (buffer *A*, 10 mM NaOH, pH 11; buffer *B*, 10 mM NaOH, 1M NaCl, pH 11); equilibration of the column was done by washing to 0% buffer *B* during 20 min.

High-performance capillary electrophoresis was carried out on the *Beckmann* capillary electrophoresis system, model *P/ACE 5510* (*Beckman Instruments*, Palo Alto, CA, USA) at 30° equipped with a diode array detector and software *Beckman* System Gold Version 8.1. Detection at 254 nm; injection, electrokinetic, 5 s, a voltage of 10 kV; (–) polarity; separation at 230–320 V/cm; running buffer, 250 mM *Tris*-borate with 7M urea, pH 8.4. Capillaries (type ssDNA 100, 100 μm i.d.) with linear 12% polyacrylamide gel were obtained from *Beckman*.

All synthetic oligonucleotides were characterized by denaturating polyacrylamide-gel electrophoresis with internal length standards. Sequence analysis was done by the method of *Maxam* and *Gilbert* [35].

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