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A New Universal Solid Support For Oligonucleotide Synthesis

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Abstract: Starting from 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose, benzyl 5,6-ditrifluoroacetamido-5,6-dideoxy-2-*O*-(4,4'-dimethoxytrityl)-α-D-mannofuranose was prepared and attached to a controlled pore glass to generate a new universal solid support for oligonucleotide synthesis. This support is applicable for the preparation of all types of oligonucleotides as well as oligomers with unusual base-labile nucleosides.

INTRODUCTION

Extensive research has been focused on standard oligonucleotide synthesis procedures which utilize a nucleoside-specific solid support, one containing the first nucleoside covalently bound by a succinate[1] or hydroquinone-*O,O'*-diacetate[2] linker. This bound nucleoside becomes the 3'-terminal residue of the oligomer upon cleavage and deprotection steps. It is obvious that this approach requires the use of at least four solid supports for general DNA synthesis. An additional four supports are required for RNA synthesis along with various solid supports containing unusual nucleosides for specific applications. Therefore a universal solid support, permitting direct coupling of any residue and the straightforward elimination of the terminal phosphodiester linkage at the same time as the deprotection step, will:

(i) eliminate the possibility of errors in parallel synthesis applications where up to 200 wells may contain different supports;
(ii) eliminate the need of four supports for DNA synthesis and four for RNA synthesis;
(iii) simplify the preparation of oligonucleotides with modified nucleosides at the 3'-terminus;
(iv) simplify the preparation of oligonucleotide mixtures with varying nucleosides at the 3'-termini.

McLean and coworkers[3] have recently described the solid support **1** (*Scheme 1*) for universal applications. This solid support possesses the following features: the detritylation, the addition of the first nucleoside monomer and the remainder of the oligomer preparation proceeds without any changes from standard procedures. The elimination of the terminal phosphodiester group utilizes the same reagents (ammonium hydroxide, aqueous methylamine, aqueous sodium hydroxide, etc.), needed for routine deprotection of oligonucleotides but requires more aggressive and lengthy conditions (e.g. concentrated ammonium hydroxide/80 °C/17 h as compared to the standard oligomer deprotection conditions of ammonium hydroxide/55 °C/5-6 h). Although these conditions are suitable for the preparation of unmodified oligonucleotides in scientific laboratories, they are certainly not compatible with base-labile nucleoside analogues. Furthermore, the prolonged treatment with basic volatile reagents or the need to employ the desalting step in the case of sodium hydroxide, makes this solid support unattractive for the use in industrial highly efficient parallel synthesizers. Thus, the design and synthesis of new universal solid supports, which lack the above mentioned shortcomings, remains a very important and

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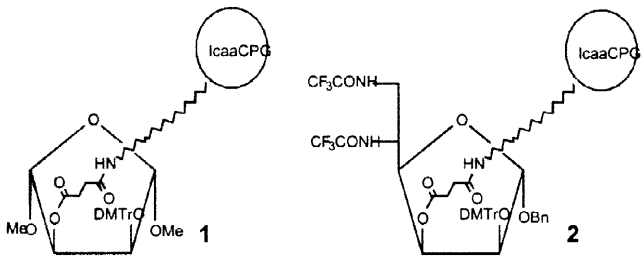
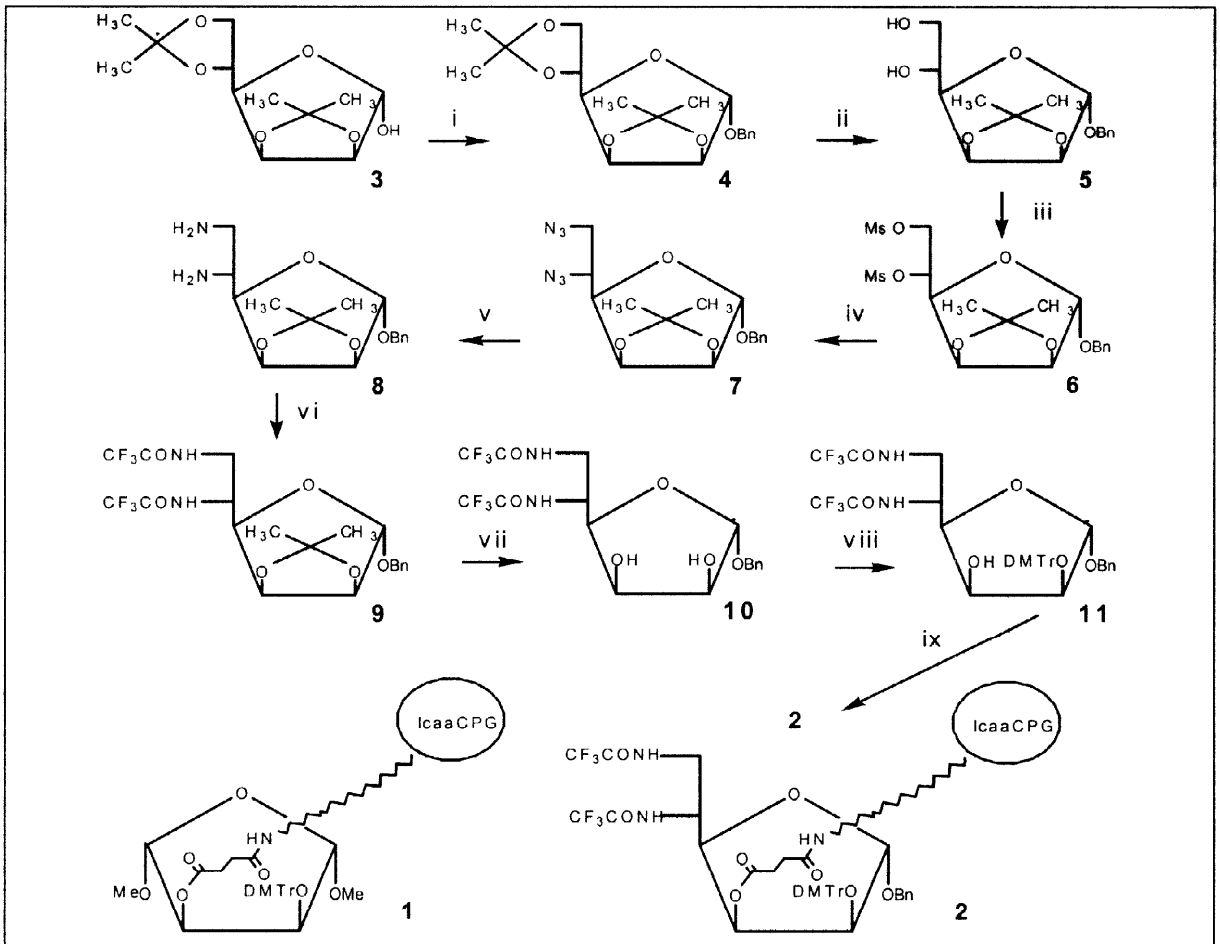


Scheme 1.

challenging goal. I now report on the new universal solid support **2** (*Scheme 1*), which is compatible with labile unusual nucleoside units and allows faster elimination of the 3'-terminal phosphodiester or phosphorothioate functions, when compared with the previously described commercial universal support **1**.

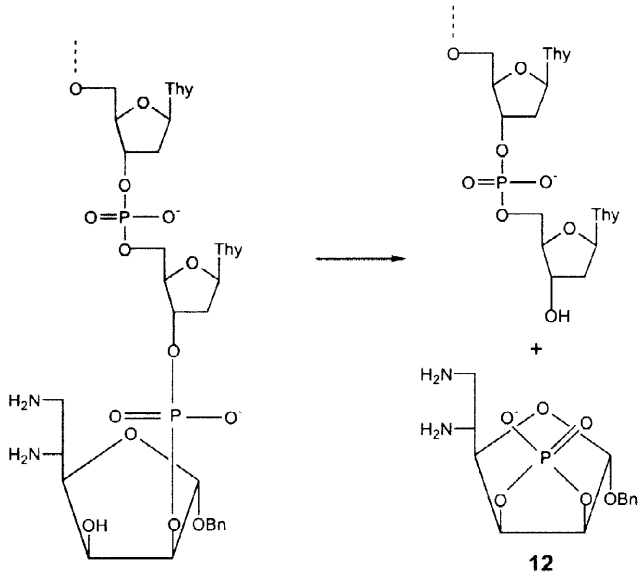
RESULTS AND DISCUSSION

Design and synthesis of the solid support **2**. - McLean *et al.*[3] have reported the effect of substituents in the ring of 1,4-anhydroerythritol on the efficiency of the terminal phosphodiester elimination step upon completion of oligonucleotide synthesis for their universal solid support. They concluded that reduction in electronegativity effects decreases the efficiency of the elimination reaction. This observation demonstrates that different substituents are able to dramatically influence the stability of a phosphodiester group linked to a ribose-like linker on a universal support. In this respect, substituents capable of facilitating phosphodiester bond cleavage are of special interest. Moreover, since the process of elimination resembles the base-catalyzed hydrolysis of RNA, some recent observations from the area of RNA cleavage provide useful information for the design of a more effective solid support. Recently, Komiyama *et al* [4] have reported that various diamines promote the hydrolysis of RNA. It was shown that among the diamines investigated, ethylenediamine appeared to be the most effective catalyst for phosphodiester bond cleavage. Therefore it seemed possible that the presence of an ethylenediamine residue in the ribose-like linker could enhance the elimination of terminal phosphodiester along with the sugar, originally linked to the solid support. Alternatively, the ethylenediamine fragment and the phosphodiester group could function as a tridentate ligand to complex ions like Zn^{2+},[5] which, in turn, would labilize the phosphodiester linkage through catalysis.[6] I have prepared a solid support **2** (*Scheme 1*) in which a protected 5,6-diamino-5,6-dideoxymannose is attached to the lcaa-CPG *via* a succinyl linker. The preparation of solid support **2** is outlined in *Scheme 2*. The starting 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose, **3**, is readily available, or it can easily be prepared from D-mannose in greater than 80% yield, following the literature procedure.[7] Compound **3** was benzylated[8] to produce crystalline **4** and converted into diol **5** in a good yield. The 5- and 6-hydroxy groups of **5** were activated by di-*O*-mesylation to give **6** as fine crystals and the two mesyloxy groups displaced by azide to give **7** as an oil in satisfactory yield. The azido groups of **7** were quantitatively reduced to amino functions resulting in **8**, which was successfully trifluoroacetylated to the sugar **9**. The 2,3-*O*-isopropylidene group of **9** was cleaved with aqueous formic acid at 50°C to give rise to diol **10**. The introduction of 4,4'-dimethoxytrityl group resulted in only one regioisomer - the 2'-*O*-dimethoxytrityl derivative **11** (as confirmed by the 2D COSY ^{1}H NMR spectrum, data not shown). It is noteworthy that most intermediate products were obtained as crystalline products and flash chromatography on silica gel was used only twice - to purify the oily diazide **7** and dimethoxytrityl derivative **11**, the latter as a pale yellow foam. The overall yield of **11** was about 24%. Finally, derivative **11** was linked to the lcaaCPG by a succinate linker between 3-hydroxyl of mannose sugar and amino group on the support.[1] The resulting solid support **2**, contained 24.5 μmols of DMTr-groups per gram of CPG.[9]



Scheme 2. i: NaH/BnBr; ii: 80% AcOH; iii: MsCl/Py; iv: LiN_3/DMF; v: a. Ph_3P/1.4-dioxane, b. ammonium hydroxide; vi: CF_3COOMe/DMF; vii: 50% HCOOH; viii: DMTrCl/Py; ix: a. SA/DMAP/Py, b. HOSu/DiPC/ lcaaCPG. Bn - benzyl; Ms - methanesulfonyl; Ph3P - triphenylphosphine; DMTr - 4,4'-dimethoxytrityl ; SA - succinic anhydride; DMAP - *N,N*-dimethylaminopyridine; HOSu - *N*-hydroxysuccinimide; DiPC - *N,N'*-diisopropylcarbodiimide; lcaaCPG - long chain alkylamino controlled pore glass

Synthesis of an oligo-T_6 on the solid support **2** *and investigation of dephosphorylation conditions.* - In order to investigate the usefulness of the solid support **2** in the preparation of oligodeoxynucleotides, the short sequence oligo-T_6 was assembled on a PE Applied Biosystems 392 DNA Synthesizer, using standard phosphoramidite chemistry and recommended protocols (40 nmol, 0.2 μmol and 1.0 μmol scales). No differences in coupling efficiency (>98% as determined from trityl assay) were detected between the solid support **2**, the commercial T-derivatized support or the commercial universal support **1**, consistent with the compatibility of **2** with standard assembly steps. After completion of the synthesis, addition of the base to the support cleaves the succinate bridge, removes the trifluoroacetyl groups from the diaminoethyl fragment of the sugar and releases the hexamer with a phosphodiester function linked to the benzylated diaminomannose residue. During the base treatment, the vicinal 3-hydroxyl group should attack the phosphorus, giving the desired oligo-T_6 and **12** (*Scheme 3*). If the diaminoethyl fragment of support **2** could increase the rate of phosphodiester elimination by assisting in the formation of the cyclic phosphate structure **12** relative to the unassisted reaction in the case of support **1**, then a significantly improved universal support would result. The investigation of the properties of support **2** reaction under various conditions is reported hereafter.



Scheme 3.

Dephosphorylation with concentrated ammonium hydroxide. - The assembled sequences were first cleaved from solid supports **1** and **2** with ammonium hydroxide (1.5 h at room temperature) and the extent of dephosphorylation and cleavage of the terminal carbohydrate was investigated, using ion exchange HPLC (Figure 1, panels A and E). Analogously, the effect of ammonium hydroxide at different temperatures and time on the cleavage is shown in Figure 1, panels B-D for support **2** and Figure 1, panel F for support **1**. In each of the traces shown in Figure 1, the product, with a retention time of about 7.5 minutes corresponds to oligo-T_6, as demonstrated by an authentic standard. The later eluting products are either the hexamer with terminal phosphodiester linked to the anhydroerythritol fragment, derived from support **1** (Figure 1. panels E,F) or the hexamer with phosphodiester attached to the diaminomannose ring, derived from support **2** (Figure 1, panels A-C)). While the oligomer with anhydroerythritol fragment elutes as a single peak with a retention time of about 8.8 minutes (Figure 1, panels E,F), the oligo-T_6 linked to the diaminomannose ring elutes as one very broad peak and an additional smaller peak with retention times of about 12 and 9 minutes correspondingly (Figure 1, panels A-C). We may assume that the presence of two peaks results from the formation of complex structures due to the self-association of diamino-containing hexamer. All of these compounds are transformed into the desired oligo-T_6 upon continued treatment with ammonium hydroxide (Figure 1, panel D).

The results of these studies are summarized in Table 1. Complete elimination of terminal phosphodiester linked to the diaminomannose function occurs within 27 h at 53 °C or within 7 h at 80 °C. While the usual deprotection of oligonucleotides proceeds within 2 h at 80 °C, additional 5 h at 80 °C are required to obtain the complete dephosphorylation and loss of the mannose residue using support **2**. It is noteworthy that under these conditions only 87% of oligo-T_6 was obtained using support **1**. Thus, the present support (**2**) has some advantages when compared with support **1**, under conditions of concentrated ammonium hydroxide. Apparently, the presence of diaminoethyl fragment in the case of support **2** increases the rate of elimination, by assisting in the formation of cyclic phosphate structure **12** and oligo-T_6 (*Scheme 3*).

Dephosphorylation with concentrated ammonium hydroxide/40% methylamine mixture. - Figure 2 shows traces of the oligo-T_6 samples, synthesized on supports **1** and **2** after treatment with concentrated ammonium hydroxide/40% methylamine (1:1) mixture. While treatment for 17 h at 53 °C leads to practically complete dephosphorylation in the case of support **2** (Figure 2, panel A), additional time is needed to cleave the hexamer with terminal phosphodiester linked to the anhydroerythritol fragment, derived from support **1** (Figure 2, panel B). Analogously, while oligo-T_6 may be recovered from the support **2** within 2 h at 80 °C, this time is not sufficient to recover all of the oligo-T_6 from the support **1**. Table 2 demonstrates the yields of hexamer obtained

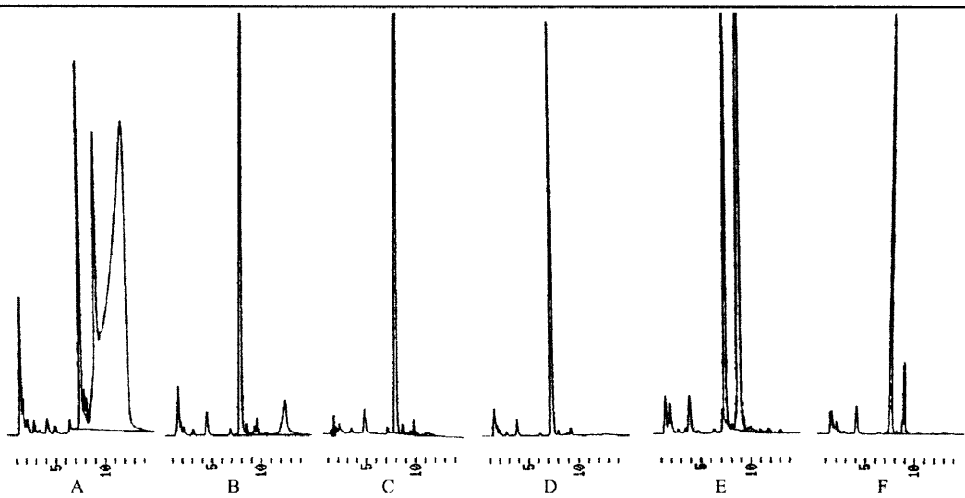


Figure 1. Ion exchange HPLC traces of oligo-T_6, synthesized on **1** and **2** and treated with concentrated ammonia. A, oligo-T_6, synthesized on **2** and treated at r.t. for 1.5 h; B, oligo-T_6, synthesized on **2** and treated at 53°C for 17 h; C, oligo-T_6, synthesized on **2** and treated at 53°C for 27 h; D, oligo-T_6, synthesized on **2** and treated at 80°C for 7 h; E, oligo-T_6, synthesized on **1** and treated at r.t. for 1.5 h; F, oligo-T_6, synthesized on **1** and treated at 80°C for 7 h.

from both supports using ammonium hydroxide/methylamine mixture. While support **2** leads to a nearly quantitative yield of product under both sets of conditions, only about 87% of oligo-T_6 was obtained from **1** under similar circumstances. The ammonium hydroxide/methylamine mixture leads to a faster elimination reaction than the ammonium hydroxide alone, and generates the desired oligo-T_6 (*Scheme 3*).

Table 1. Yield of oligo-T6 derived from the universal solid support **1** and the new universal solid support **2** under various elimination conditions.

Reagents	Conditions	Yield of oligo-T6 derived from 1, %[a]	Yield of oligo-T6 derived from 2, %[a]
Ammonium hydroxide	53°C / 27h	73	96
Ammonium hydroxide	80°C / 7h	87	97
Ammonium hydroxide/ 40% Methylamine	53°C / 17h	87	96
Ammonium hydroxide/ 40% Methylamine	80°C / 2h	86	96
0.5M $ZnCl_2$ in Ammonium hydroxide	80°C / 1h	97	98
Ammonium hydroxide, r.t./1.5h followed by $ZnCl_2$ in H_2O	80°C / 1h	77[b]	96[c]
Ammonium hydroxide, r.t./1.5h followed by H_2O	80°C / 24h	34	96

[a]As assessed from IE HPLC by integration of peaks at 260 nm. Only peak, corresponding to the oligo-T6 and peaks with higher retention times were integrated.
[b]treated with 0.5M $ZnCl_2$.
[c]treated with 50 mM $ZnCl_2$

Dephosphorylation with zinc chloride solutions. - It was shown previously that Zn^{2+} ions are capable of effective catalysis of RNA hydrolysis.[6] In addition, ethylenediamine is known to effectively complex Zn^{2+} ions, the equilibrium constant for the reaction Zn^{2+}+ ethylenediamine ↔ complex being about 10^6 M^{-1}.[5] The

diaminoethyl group of the terminal sugar of the oligonucleotide could provide a bidentate ligand to complex a Zn^{2+} and / or a $Zn^{2+}-OH^-$ species,[6] which, in turn, would further enhance the rate of phosphodiester bond

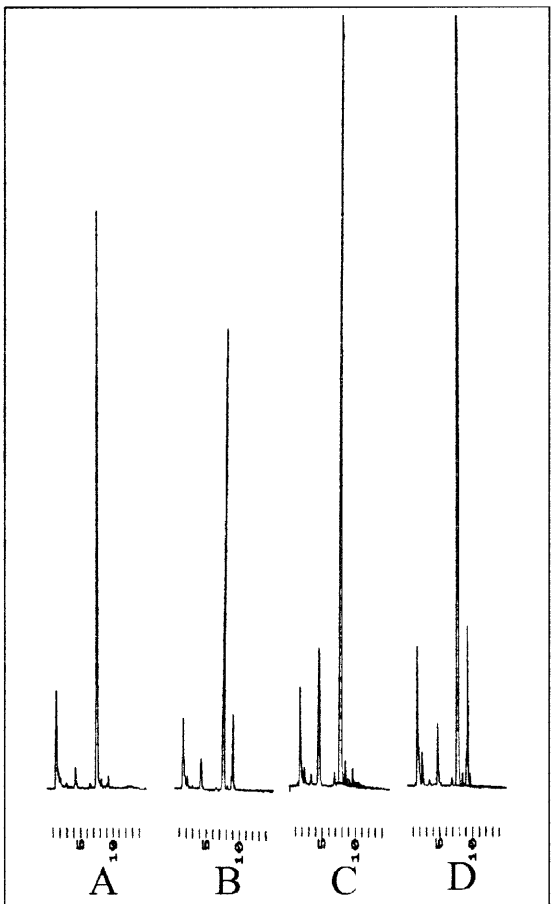


Figure 2. Ion exchange HPLC traces of oligo-T_6, synthesized on **1** and **2** and treated with concentrated ammonia/40% methylamine (1:1) mixture. A, oligo-T_6, synthesized on **2** and treated at 53°C for 17 h; B, oligo-T_6, synthesized on **1** and treated at 53°C for 17 h; C, oligo-T_6, synthesized on **2** and treated at 80°C for 2 h; D, oligo-T_6, synthesized on **1** and treated at 80 °C for 2 h.

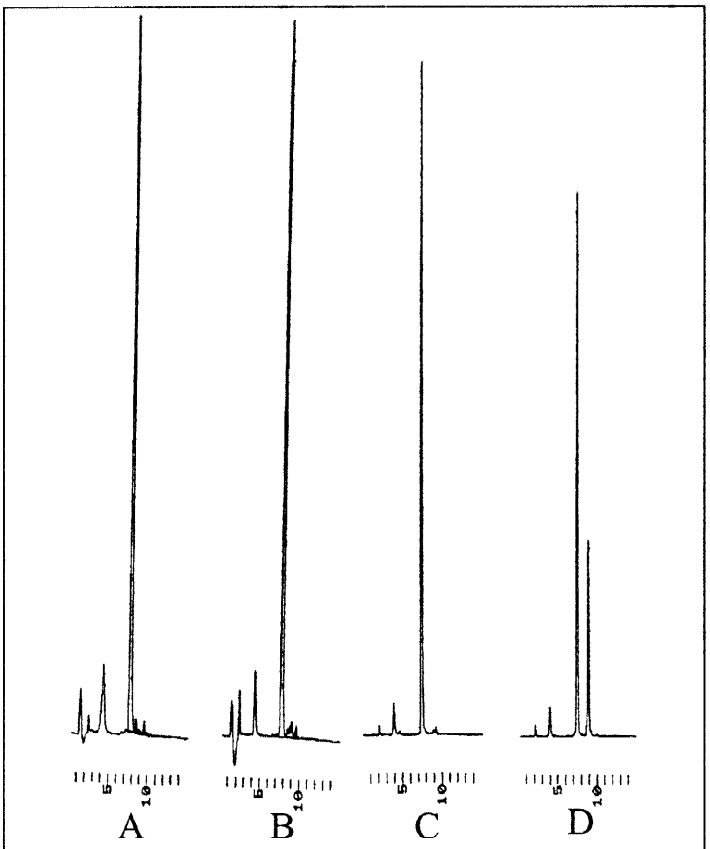


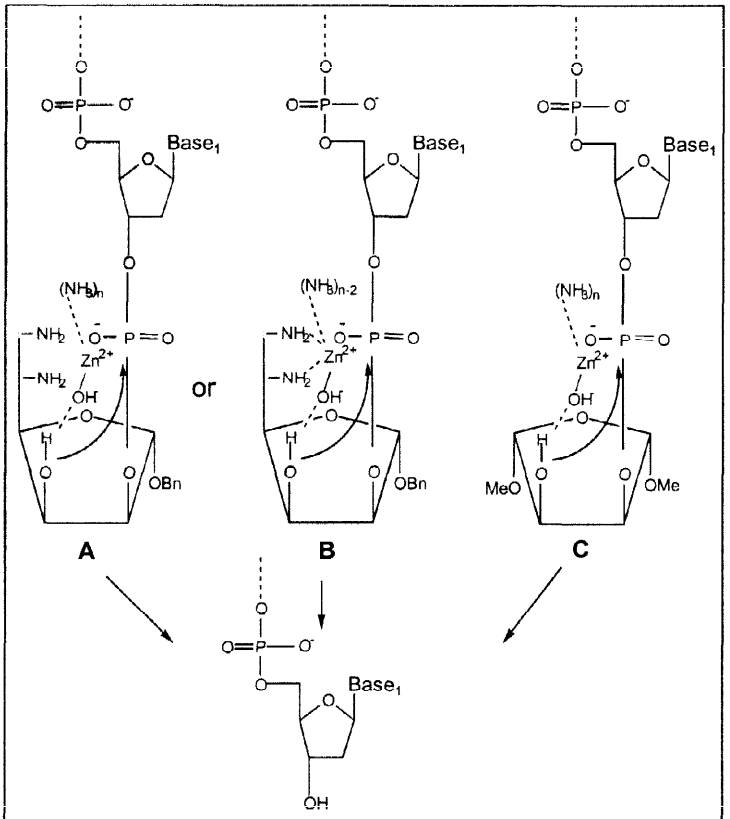
Figure 3. Ion exchange HPLC traces of oligo-T_6, synthesized on **1** and **2**, cleaved from the solid support (concentrated ammonia, at r.t. for 1.5 h), evaporated to dryness and treated with a zinc chloride solutions. A, oligo-T_6, synthesized on **2** and treated with 0.5M $ZnCl_2$ in concentrated ammonia at 80°C for 1 h; B, oligo-T_6, synthesized on **1** and treated with 0.5M $ZnCl_2$ in concentrated ammonia at 80°C for 1 h; C, oligo-T_6, synthesized on **2** and treated with 50mM $ZnCl_2$ in water at 80° C for 1 h; D, oligo-T_6, synthesized on **1** and treated with 0.5M $ZnCl_2$ in water at 80°C for 1 h

hydrolysis. Figure 3, panel A shows the analysis of oligo-T_6 synthesized on support **2** and treated with 0.5M $ZnCl_2$ in concentrated ammonia at 80°C for 1h. Figure 3, panel B shows that of the hexamer, prepared on support **1** and treated in the same manner. Very high yields were obtained in both cases (Table 1), indicating the efficiency of 0.5M $ZnCl_2$/concentrated ammonia conditions in elimination process. *Scheme 4, structure C* illustrates a possible mechanism for the elimination in the case of the structure derived from support **1**. According to this mechanism, a hydroxo ligand of the phosphate-coordinated ammonia-metal ion complex deprotonates the attacking 2'-hydroxy group, leading to the oligo-T_6, with a free 3'-terminus. Two alternative mechanisms can be proposed for the analogous process, taking place with support **2** (*Scheme 4, structures A*

and B). *Structure A* illustrates a mechanism similar to that outlined in *structure C*. This mechanism does not assume any engagement of the diaminoethyl function in the elimination process, and as in the case of support **1**, a hydroxo ligand of the phosphate-coordinated ammonia-metal ion complex deprotonates the attacking 2'-hydroxy group. The second mechanism shown as structure B is based on the assumption that the Zn^{2+}–OH^- hydroxo ligand is complexed in a bidentate manner to the diaminoethyl group and coordinated to ammonia. No data is available so far on the equilibrium constant for the diaminomannose + Zn^{2+} ↔ complex reaction. Assuming this equilibrium constant is similar to that for Zn^{2+} + ethylenediamine ↔ complex (~10^6 M^{-1},[9]), and that the equilibrium constants for NH_3 + nZn^{2+} ↔ complex were all found to be in the range of 10^2 - 10^3 M^{-1},[10] the reaction mechanism shown in structure B cannot be excluded. Of more interest was the action of $ZnCl_2$ in water for the elimination of the terminal diaminomannose residue and phosphodiester group. Figure 3C shows the analysis of oligo-T_6, synthesized on support **2**, cleaved from the support and finally treated with 50mM $ZnCl_2$ in water at 80°C for 1h. Figure 3D shows the analysis of oligo-T_6, synthesized on support **1** and treated with 0.5M $ZnCl_2$ in the same manner. A nearly quantitative yield in the case of support **2** relative to 77% yield in the case of support **1**, where 10 times higher concentration of $ZnCl_2$ was employed. These observations are consistent with the reaction mechanism depicted in *Scheme 5* as *structure A*. At 50mM $ZnCl_2$ it is likely that the diamino fragment of the sugar is entirely present as the zinc complex: one Zn^{2+}–OH^- is bound bidentally to two aminogroups and additionally coordinated to the phosphodiester function. This hydroxo ligand should act as an effective base and deprotonate the adjacent 3'-OH in the sugar analog. With the oligonucleotide derived from support **1**, Zn^{2+}–OH^- has only the phosphodiester residue available for monodentate coordination. This difference in zinc coordination can be expected to lower reaction rate in the case of the product derived from **1** relative to that derived from **2**.

Dephosphorylation with water. - In principle the presence of diamino function in the fragment by itself (*Scheme 3*) could enhance the reaction rate by general acid/base catalysis. Figure 4 shows the analysis of oligo-T_6, synthesized on supports **1** and **2**, cleaved from the latter and treated with water at 80°C. Panel A of Figure 4 was taken after short reaction time, while panel D shows that within 22h practically all of the intermediate, bearing diamino-sugar has been converted into the desired hexamer with free 3'-terminal hydroxyl (See also Table 1). In contrast, panels E and F of Figure 4 indicate that in the case of oligo-T_6, derived from **1**, only a very small fraction of the starting terminal phosphodiester linkage was hydrolyzed during reaction with water. The 34% yield obtained can be largely attributed to base-catalyzed elimination during the initial ammonia treatment. *Structure B* in *Scheme 5* describes the proposed mechanism for the elimination reaction. At a pH value of about 8, at least one of the amino groups - one at primary carbon atom would be largely protonated. Under these circumstances one might expect an interaction between the positively charged protonated amino group and the negatively charged phosphodiester bond. Simalteneously, the non protonated NH_2 function at position 5 may act as a general base for deprotonation of the 3'-OH and facilitating its attack on the phosphorus atom.

Synthesis of oligo-T_6 phosphorothioate on the solid support 2. - Oligo-T_6 phosphorothioate was synthesized on commercial T-derivatized support and support **2**, following the recommended protocol. No differences in



Scheme 4.

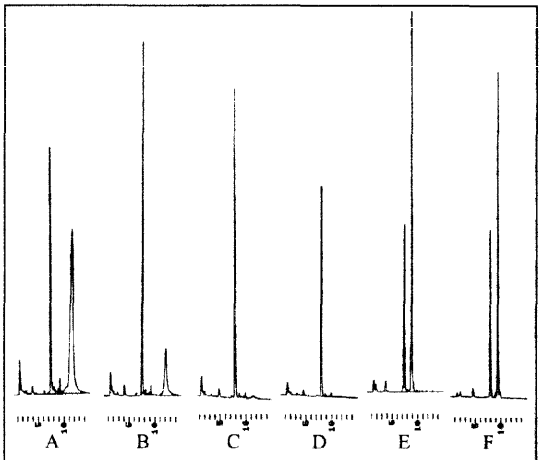


Figure 4. Ion exchange HPLC traces of oligo-T_6, synthesized on **1** and **2**, cleaved from the solid support (concentrated ammonia, at r.t. for 1.5 h), evaporated to dryness and treated with water. A, oligo-T_6, synthesized on **2** and treated at 80°C for 1 h; B, oligo-T_6, synthesized on **2** and treated at 80°C for 6 h; C, oligo-T_6, synthesized on **2** and treated at 80°C for 19 h; D, oligo-T_6, synthesized on **2** and treated at 80°C for 22 h; E, oligo-T_6, synthesized on **1** and treated at 80°C for 1 h; F, oligo-T_6, synthesized on **1** and treated at 80°C for 22 h.

coupling efficiency (>98% as determined from trityl assay) were detected between support **2** and the T- support. In both cases the support bound oligonucleotide phosphorothioate was kept in ammonium hydroxide at 80 °C for 2 h and then analyzed by ion exchange HPLC. Figure 5 shows analyses of oligo-T_6 phosphorothioate prepared on commercial T-support (panel A) and that prepared on support **2** (panel B) after treatment. A 2 h reaction at 80 °C was not sufficient to obtain quantitatively the desired phosphorothioate with a free hydroxyl at 3'-terminus (yield ~ 80%, Table 2). An additional 1 h heating in concentrated ammonia increased the yield to 90% (Figure 5, panel C; Table 2) and a 4 h treatment gave the product with the yield of ~ 95% (data not shown). In a separate experiment, the support **2** bound oligonucleotide phosphorothioate was kept in concentrated ammonium hydroxide at 80 °C for 2 h , evaporated to dryness, redissolved in water and kept additionally at 80 °C for 1 h. Panel D shows the analysis of oligo-T_6 phosphorothioate, resulting from this experiment. The yield of the desired product was 98% (Table 2). From the comparison of data for cleavage of phosphodiester and phosphorothioate diester, given in Figures 4 and 5, as well as in Tables 1 and 2, it appears obvious that the elimination of the diaminosugar linked *via* phosphorothioate diester proceeds much faster than that of the diaminosugar linked to a phosphodiester. *Scheme 5* suggests a possible mechanism for elimination of the diaminosugar linked *via* phosphorothioate diester (*structure C*) and analogous mechanism for phosphodiester cleavage (*structure B*). I cannot explain the considerable differences in elimination rates for these two reactions. One can only speculate that either the more negative charge on sulfur atom or its larger atomic radius or both are making the transition state more favorable than that for phosphodiester. Nevertheless

it is clear that the solid support **2** can be successfully employed for the preparation of phosphorothioates. In fact, by incorporating a phosphorothioate diester during the first coupling, unmodified oligonucleotides can be removed from the support and cleaved during the "normal" ammonia deprotection step followed by heating in water.

Table 2. Yield of oligo-T_6 phosphorothioate derived from the new universal solid support **2** under various elimination conditions.

Reagents	**Conditions**	**Yield of oligo-T_6 phosphorothioate, %[a]**
Ammonium hydroxide	80°C / 2h	79
Ammonium hydroxide	80°C / 3h	89
Ammonium hydroxide followed by water	80°C / 2h; 80°C / 1h	98

[a]As assessed from IE HPLC by integration of peaks at 260 nm. Only peak, corresponding to the oligo-T6 and peaks with higher retention times were integrated.

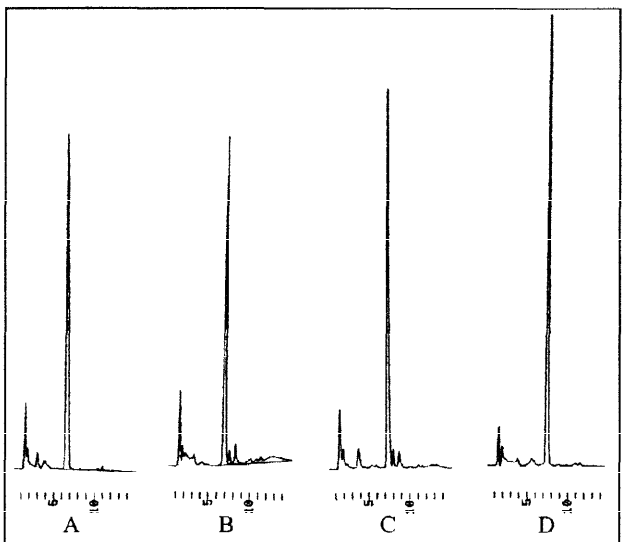


Figure 5. Ion exchange HPLC traces of oligo-T_6 phosphorothioate. A, oligo-T_6 phosphorothioate, synthesized on **T-support** and treated with concentrated ammonia at 80°C for 2 h; B, oligo-T_6 phosphorothioate, synthesized on **2** and treated with concentrated ammonia at 80°C for 2 h; C, oligo-T_6 phosphorothioate, synthesized on **2** and treated with concentrated ammonia at 80°C for 3 h; D, oligo-T_6 phosphorothioate, synthesized on **2**, treated with concentrated ammonia at 80°C for 2 h, evaporated to dryness and additionally treated with water at 80°C for 1 h.

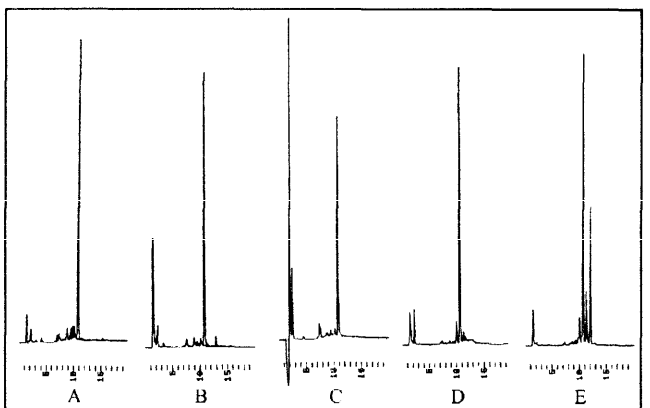


Figure 6. Ion exchange HPLC traces of decamer **13**. A, **13**, synthesized on **T-support** and treated with concentrated ammonia at 80°C for 2 h; B, **13**, synthesized on **2** and treated with concentrated ammonia at 80°C for 7 h; C, **13**, synthesized on **2**, treated with concentrated ammonia at 80°C for 2 h, evaporated to dryness and treated additionally with 50mM $ZnCl_2$ in water at 80°C for 1 h, D, **13**, synthesized on **2** by introducing the first phosphorothioate linkage, followed by nine phosphodiester linkages, treated with concentrated ammonia at 80°C for 2 h, evaporated to dryness and additionally treated with water at 80°C for 1 h; E, **13**, synthesized on **1** by introducing the first phosphorothioate linkage, followed by nine phosphodiester linkages, treated with concentrated ammonia at 80°C for 2 h, evaporated to dryness and additionally treated with water at 80°C for 1 h.

Synthesis of oligonucleotides of mixed sequences on the solid support 2. - In order to investigate the applicability of support **2** for the preparation of oligodeoxyribonucleotides of mixed sequences, I have synthesized the decamer 5'-TCAGTACTCT-3' (**13**) on **2** and studied the conditions for terminal phosphodiester

elimination. Figure 6, panel A shows the analysis of decamer **13** prepared on commercial T-support and deblocked at 80 °C for 2 h. Panels B and C of Figure 6 demonstrate analyses of the same oligomer **13** prepared on support **2** and treated either with concentrated ammonium hydroxide at 80 °C for 7 h or concentrated ammonium hydroxide at 80 °C for 2 h (deprotection of hyterocyclic bases), followed by 50mM $ZnCl_2$ in water at 80 °C for 1h correspondingly. The yield in both cases was greater than 95%, indicating that support **2** is suitable for the preparation of oligomers of mixed sequences. Additionally I used **2** to synthesize the same decamer **13** by introducing a 3'-terminal phosphorothioate linkage, followed by nine phosphodiester bonds. Upon the completion of the assembly, the decamer bearing solid support was kept in concentrated ammonium hydroxide at 80 °C for 2 h, followed by evaporation to dryness and additional heating in aqueous solution at 80 °C for 1 h. The results of this experiments are shown in panel D of Figure 6. The yield of the desired decamer **13** was more than 95%. I have prepared a number of different 20 - 30mer PCR primers, employing solid support **2** and thus generating 3'-termini bearing any of four 2'-deoxynucleosides. All primers performed well in PCR experiments. In order to investigate if support **2** is equally suitable for the preparation of RNA fragments, I have synthesized the relatively long ribooligonucleotide sequence 5'-UACUGUUGGUAAAAUGGAAGACGCCAAAAACAUA-3' (**14**) on **2** and A-bound support, using phosphoramidites in which 2'-OH groups were protected with TBDMS and N^4-acetylcytidine monomer. The cleavage from supports and deprotection of heterocyclic bases was achieved by employing the standard subroutine - concentrated ammonium hydroxide/40% methylamine mixture at 65 °C for 10 minutes, followed by evaporation. In the case of the oligomer derived from **2**, an additional treatment with 50 mM $ZnCl_2$ in water at 80 °C for 1 h was required to ensure the elimination of terminal phosphodiester. The oligonucleotide was then desalted using Dowex-50 (Py+) and dried. TBDMS protecting groups were finally cleaved in usual manner.[11] Ion exchange HPLC analysis of the oligonucleotide synthesized on **2** is shown in Figure 7. The integration of peaks with retention times higher than 5 minutes have revealed the 61 % content of **14** in the oligonucleotide mixture. The ribooligomer prepared on **2** was identical to the 34mer prepared on the A-bound support (data not shown). These results demonstrate the applicability of the new solid support for the RNA synthesis as well as DNA synthesis.

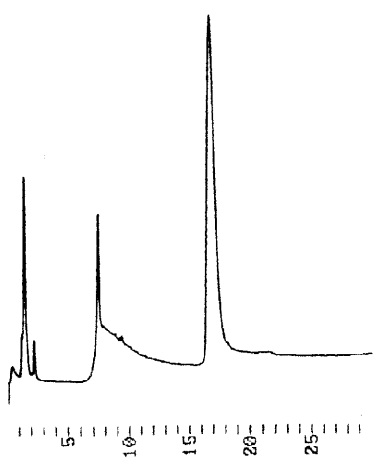
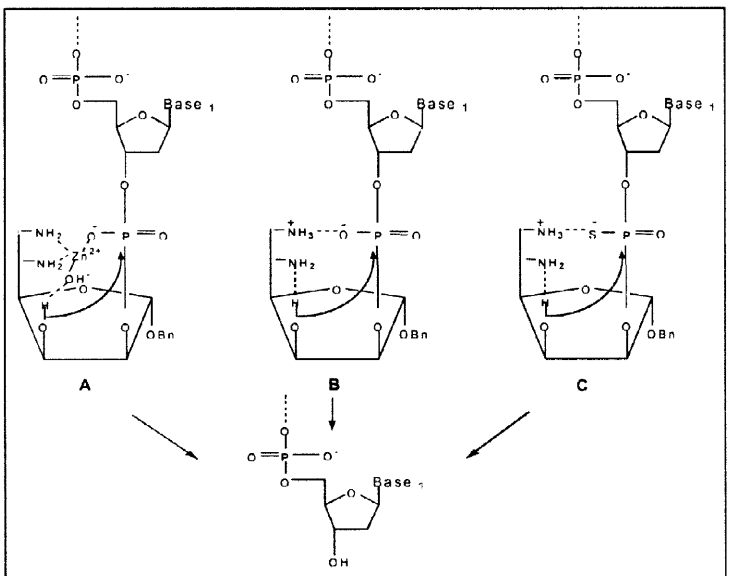


Figure 7. Ion exchange HPLC traces of ribooligonucleotide **14**



Scheme 5.

Conclusion.- I have designed, synthesized and tested a new universal solid support, which is compatible with the preparation of all common types of oligonucleotides and should function well for oligomers with unusual base labile nucleoside units. This support allows for faster elimination of the 3'-terminal phosphodiester or phosphorothioate function when compared with the earlier reported universal support[3] and, additionally, permits the use of neutral conditions for the terminal dephosphorylation, employing aqueous zinc chloride solution or even water. I believe that the idea of neighboring group assistance by various functions for the elimination of the terminal phosphodiester to generate termini with 3'-hydroxyls may lead to the creation of truly universal polymers, working as fast as conventional modified controlled pore glasses or resins.

EXPERIMENTAL

General.- The melting point values reported are uncorrected. Flash silica gel chromatography was performed with 230-400 mesh 60Å silica from Merck. All reagents used in sugar chemistry were from Aldrich. 4.4'-Dimethoxytrityl chloride was from ChemGenes and long chain alkylamino controlled pore glass (lcaaCPG) was from Sigma. Anion exchange HPLC was performed on DNAPac PA-100 (4 x 250 mm) column (Dionex) at 25 °C; buffer A: 0.1M LiOAc in 20% MeCN; buffer B: 0.4M $LiClO_4$ in A; flow rate 1 ml/min; a linear gradient from 3 to 18%B in 15 min was applied for oligo-T_6; a linear gradient from 3 to 23%B in 15 min, followed by a linear gradient from 23 to 30% B in 5 min was applied for decamer **13**; a linear gradient from 10 to 50% B in 15 min was applied for oligo-T_6 phosphorothioate; a linear gradient from A to 50% B in 30 min was applied for ribooligonucleotide **14**. ^{1}H NMR spectra (400 MHz, with tetramethylsilane as internal standard) were recorded on a Bruker AM 400WB spectrometer in $CDCl_3$. IR spectra were taken with a Perkin-Elmer 983 spectrometer.

Benzyl 2,3:5,6-di-O-isopropylidene-α-D-mannofuranose (**4**). - 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose (**3**, 16 g, 61.4 mmol) was converted into benzyl 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose (**4**) as described.[8] Crystallization from *n*-hexane gave 15.9 g (74 %) of **4** as large white crystals; m.p.54 - 56 °C; $[\alpha]_D$ = +72° (c = 2.307, $CHCl_3$); IR (KBr, cm^{-1}) ν 3092 -3031 (C-H arom.), 2989 - 2838 (C-H aliph.), 1608 and 1496 (C=C arom), 1453 (CH_2), 1381 (CH_3), 1265 and 1210 (C-O), 887 - 697 (=CH arom.); ^{1}H NMR ($CDCl_3$): δ = 1.33, 1.40, 1.47, 1.48 (each 3H, s, *i*-Pr); 3.97 - 4.01 (2H, m, H4, H6'); 4.12 (1H, dd, *J*= 6.3, 8.7 Hz, H6); 4.40 - 4.45 (1H, m, H5); 4.50, 4.66 (each 1H, d, *J* = 11.9 Hz, -$\underline{CH_2}$-C_6H_5); 4.67 (1H, d, *J* =5.9 Hz, H2); 4.81 (1H, dd, *J* =5.9, 3.6 Hz, H3) 5.09 (1H, s, H1); 7.28 - 7.39 (5H, m, -CH_2-$\underline{C_6H_5}$). Found : C, 65.01; H, 7.37. Calcd. For $C_{19}H_{26}O_6$: C, 65.13; H, 7.48 %.

Benzyl 2,3-O-isopropylidene-α-D-mannofuranose (**5**). - Compound **4** (15.3g, 43.7 mmol) was dissolved in 80% acetic acid (50 ml), kept for 15 h at room temperature and evaporated to dryness. The residue was coevaporated with n-butanol (2 x 25 ml), toluene (25 ml) and finally crystallized from toluene to give 13.3 g, 98%) of **5** as white crystals; m.p. 62 - 64 °C; $[\alpha]_D$ = +75° (c = 2.113, $CHCl_3$); IR (KBr, cm^{-1}) ν 3520 and 3353 (O-H), 3063 (C-H arom.), 2991 - 2835 (C-H aliph.), 1607 and 1496 (C=C arom), 1452 (CH_2), 1381 (CH_3), 1274 and 1206 (C-O ether),), 1085 and 1016 (C-O alcohol), 893 - 699 (=CH arom.); ^{1}H NMR ($CDCl_3$): δ = 1.34, 1.48 (each 3H, s, *i*-Pr); 2.33 (1H, m, OH6); 3.01 (1H, d, *J* = 5.0 Hz, OH5); 3.69 (1H, m, H6'); 3.85 (1H, m, H6); 3.97 (1H, dd, *J* = 3.7, 8.1 Hz, H4); 3.99 - 4.03 (1H, m, H5); 4.51, 4.64 (each 1H, d, *J* = 11.9 Hz, -$\underline{CH_2}$-C_6H_5); 4.67 (1H,

d, J = 6.0 Hz, H2); 4.87 (1H, dd, J =6.0, 3.7 Hz, H3); 5.12 (1H,s, H1); 7.28 - 7.38 (5H, m, -CH_2-C_6H_5). Found : C, 61.80; H, 7.01. Calcd. For $C_{16}H_{22}O_6$: C, 61.92; H, 7.15 %.

Benzyl 5,6-di-O-methanesulfonyl-2,3-O-isopropylidene-α-D-mannofuranose (**6**). - Compound **5** (13 g, 42 mmol) was dissolved in dry pyridine (50 ml) and the solution was cooled to 4 °C. Methanesulfonyl chloride (15 ml) was then added dropwise and the resulting mixture was left overnight at room temperature. Crushed ice (50 g) was added, the mixture was concentrated to about 20 ml and the residue was dissolved in methylene chloride. The organic solution was thoroughly washed with saturated solution of $NaHCO_3$, dried over Na_2SO_4 and evaporated to dryness. The residue was coevaporated with toluene (2 x 50 ml) and finally crystallized from methanol to give 18.9 g (97%) of **6** as white crystals; m.p. 102 - 104 °C; $[\alpha]_D$ = +46° (c = 1.987, $CHCl_3$); IR (KBr, cm^{-1}) ν 3029 (C-H arom.), 2986 - 2885 (C-H aliph.), 1500 (C=C arom), 1453 (CH_2), 1357 and 1348 and 1172 (S=O), 1088 - 705 (S-O and =CH arom.); ^{1}H NMR ($CDCl_3$): δ = 1.33, 1.49 (each 3H, s, *i*-Pr); 3.10, 3.15 (each 3H, s, CH_3-SO_2-); 4.29 (1H,dd, J =8.0, 3.6 Hz, H4);); 4.52 (1H, d, J = 11.6 Hz, H6'); 4.54 (1H, dd, J = 4.4, 11.6 Hz, H6'); 4.68 - 4.73 (3H, m, H2 + -CH_2-C_6H_5); 4.81 (1H, dd, J =5.8, 3.6 Hz, H3); 5.04 - 5.08 (1H, H5); 5.12 (1H, s, H1); 7.30 - 7.40 (5H, m, -CH_2-C_6H_5). Found : C, 46.57; H, 4.63. Calcd. For $C_{18}H_{26}O_{10}S_2$: C, 46.75; H, 4.79 %.

Benzyl 5,6-diazido-5,6-dideoxy-2,3-O-isopropylidene-α-D-mannofuranose (**7**). - Compound **6** (18.5 g, 39.7 mmol) was dissolved in N,N'-dimethylformamide (200 ml), lithium azide (29.25 g, 596 mmol) was added, the mixture was heated at 130 °C for 6 h with stirring and finally evaporated to dryness. The residue was dissolved in ether - *n*-hexane (1:1) mixture (100 ml), washed with water (100 ml), dried over Na_2SO_4 and evaporated to dryness. Compound **7** was isolated as a colorless oil by flash chromatography on silica gel, using ether in *n*-hexane (from 0 to 20%). The yield of diazide **7** was 8.4 g (59 %); $[\alpha]_D$ = +38° (c = 2.000, $CHCl_3$); IR (KBr, cm^{-1}) ν 3089 -3033 (C-H arom.), 2990 - 2876 (C-H aliph.), 2100 (N_3), 1607 and 1496 (C=C arom), 1452 (CH_2), 1374 (CH_3), 1212 (C-O), 880 - 699 (=CH arom.); NMR ($CDCl_3$): δ = 1.29, 1.45 (each 3H, s, *i*-Pr); 3.42 (1H, dd, J =5.9, 12.9 Hz, H6'); 3.56 (1H, dd, J =3.0, 12.9 Hz, H6); 3.89 (1H, dq, J =3.0, 5.9, 8.9 Hz, H5); 4.14 (1H, dd, J =2.6, 8.9 Hz, H4); 4.67 (1H, s, H2); 4.68 (1H, d, J =2.6 Hz, H3); 4.52, 4.72 (each 1H, d, J = 11.8 Hz, -CH_2-C_6H_5); 5,12 (1H, s, H1); 7.29 - 7.39 (5H, m, -CH_2-C_6H_5). Found : C, 52.96; H, 5.38; N, 23.64. Calcd. For $C_{16}H_{20}N_6O_4$: C, 53.33; H, 5.59; N, 23.32 %.

Benzyl 5,6-ditrifluoroacetamido-5,6-dideoxy-2,3-O-isopropylidene-α-D-mannofuranose (**9**). - Diazide **7** (8.1 g, 22.5 mmol) was dissolved in 1,4-dioxane (50 ml), triphenylphosphine (35.4 g, 135 mmol) was added and the mixture was stirred for 1 h at room temperature. Concentrated ammonium hydroxide (5 ml) was added to the solution, the resulting mixture was left for additional 3 h at room temperature and evaporated to dryness. The solids were extracted with water (3 x 50 ml), the combined extracts were evaporated to dryness and the extraction was repeated. The aqueous solution was evaporated to dryness, coevaporated with ethanol (3 x 50 ml), benzene (3 x 50 ml) and again evaporated to dryness. The white crystalline residue was dissolved in a mixture of N,N'-dimethylformamide - methyl trifluoroacetate (2:1, 90 ml), the clear solution was heated at reflux for 24 h and finally evaporated to dryness. Crystallization of the residue from methanol gave 9.45 g (84 %) of **9** as white crystals; m.p. 136 - 138 °C; $[\alpha]_D$ = +57° (c = 2.300, $CHCl_3$); IR (KBr, cm^{-1}) ν 3308 and 3098

(N-H), 3030 (C-H arom.), 2988 - 2891 (C-H aliph.), 1701 (C=O amide I), 1463 (NH amide II), 1463 (CH_2), 1383 (CH_3), 1271 - 1182 (C-F and C-O), 892 - 700 (=CH arom.); ^{1}H NMR ($CDCl_3$): δ = 1.27, 1.41 (each 3H, s, *i*-Pr); 3.62 (2H, m, H6 + H6'); 4.03 (1H, t, *J* =3.8 Hz, H4); 4.43 (1H, m, H5); 4.54, 4.62 (each 1H, d, *J* = 12.0 Hz, -$\underline{CH_2}$-C_6H_5); 4.65 (1H, d, , *J* =5.9 Hz, H2); 4.74 (1H, dd, *J* =5.9, 3.8 Hz, H3); 5.16 (1H, s, H1); 6.96 (1H, d, *J* =6.6 Hz, CF_3CONH-); 7.25 (1H, br.s, CF_3CONH-); 7.29 - 7.38 (5H, m, -CH_2-$\underline{C_6H_5}$).). Found : C, 47.90; H, 4.32; N, 5.48. Calcd. For $C_{20}H_{22}N_2O_6F_6$: C, 48.01; H, 4.43; N, 5.60 %.

Benzyl 5,6-ditrifluoroacetamido-5,6-dideoxy-α-D-mannofuranose (**10**). - The solution of compound **9** (9.0 g, 18 mmol) in 75 % formic acid (20 ml) was heated at 55 °C for 1.5 h and the solution was evaporated to dryness. Crystallization of the residue from *n*-hexane - ether gave 6 g (73 %) of **10** as white crystals; m.p. 149 - 151 °C; $[\alpha]_D$ = +72° (c = 1.310, $CHCl_3$); IR (KBr, cm^{-1}) ν 3500 - 3283 (O-H and N-H), 3113 (N-H), 3034 (C-H arom.), 2935(C-H aliph.), 1697 (C=O amide I), 1560 (NH amide II), 1497 (C=C arom.), 1452 (CH_2), 1345 (CH_3), 1270 - 1186 (C-F and C-O), 914 - 698 (=CH arom.); ^{1}H NMR ($CDCl_3$): δ = 3.57 - 3.68 (2H, m, H6 + H6'); 4.07 (1H, d, *J* = 5.1 Hz, H2); 4.18 (1H, dd, *J* = 6.8, 3.0 Hz, H4); 4.20 - 4.24 (1H, m, H5); 4.48 - 4.52 (1H, m, H3); 4.50, 4.67 (each 1H, d, *J* = 11.7 Hz, -$\underline{CH_2}$-C_6H_5); 5.05 (1H, s, H1); 7.29 - 7.33 (5H, m, -CH_2-$\underline{C_6H_5}$); 7,96, 8.29 (each 1H, br.s, $CF_3CO\underline{NH}$-). Found : C, 44.12; H, 3.82; N, 6.19. Calcd. For $C_{17}H_{18}N_2O_6F_6$: C, 44.36; H, 3.94; N, 6.09 %.

Benzyl 5,6-ditrifluoroacetamido-5,6-dideoxy-2-O-(4,4'-dimethoxytrityl)-α-D-mannofuranose (**11**). - Dimethoxytritylation of **10** (3 g, 6.5 mmol) was performed by adding 4,4'-dimethoxytrityl chloride (2.54 g, 7.5 mmol) to a solution of diol in dry pyridine (20 ml). Usual work up and flash chromatography on silica gel using ether in *n*-hexane (from 0 to 20 %) gave 4.6 g (93 %) of **11** as a pale yellow foam; $[\alpha]_D$ = +14° (c = 1.460, $CHCl_3$); IR (KBr, cm^{-1}) ν 3515 (O-H), 3307 and 3091 (N-H), 3034 and 3005 (C-H arom.), 2935 - 2838 (C-H aliph.), 1710 (C=O amide I), 1608 and 1508 (C=C arom.), 1559 (NH amide II), 1444 (CH_2), 1302 (CH_3), 1253 and 1033 (C-O methoxy), 1302 - 1179 (C-F and C-O), 913 - 700 (=CH arom.); ^{1}H NMR ($CDCl_3$): δ = 3.50 - 3.65 (2H, m, H6 + H6'); 3.72 - 3.80 (7H, m, H5 + 2xCH_3O-); 3.88 (1H, t, *J* =3.8 Hz, H4); 4.24 (1H, dd, *J* =2.5, 5.2 Hz, H2); 4.31 (1H, m, H3); 4.40, 4.61 (each 1H, d, *J* = 11.5 Hz, -$\underline{CH_2}$-C_6H_5); 5.08 (1H, d, *J* =2.5 Hz, H1); 7.16 - 7.44 (18H, m, arom.). Found : C, 60.94; H, 3.83; N, 3.57. Calcd. For $C_{38}H_{27}N_2O_8F_6$: C, 60.56; H, 3.61; N, 3.72 %.

Solid support 2. - Compound **11** was linked to the lcaaCPG (5 g) by a succinate bridge as described in literature.[1] The product **2** contained 24.5 μmol of DMTr-groups per gram of CPG.

Solid supports, monomers and oligonucleotides. - Oligomers reported here were assembled on a PE Applied Biosystems 392 DNA synthesizer on a 40 nmol, 0.2 μmol or 1.0 μmol scale, employing recommended protocols. Universal support **1**, nucleoside-bound solid supports, deoxyribonucleoside and ribonucleoside 3'-phosphoramidites were from Glen Research.

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