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A Combinatorial Protecting Group Strategy for Oligonucleotide Synthesis

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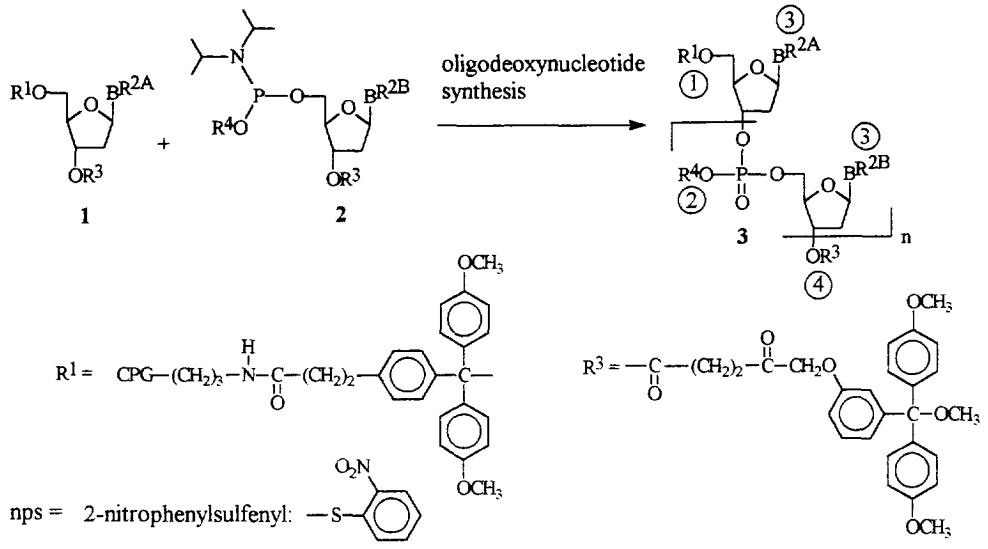
Abstract: A novel 5'-3' directed DNA synthesis will be described. Together with additional investigations on model compounds a synthetic strategy is established which allows multiselective deprotections. This offers the potential to either generate oligonucleotides in different sequence specific protection/ functionalization states or to create a combinatorial set of molecules available for specific molecular interaction or recognition experiments. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The approach to use the antisense or triplex DNA concept for the therapy of molecular diseases[1] requires the synthesis of modified oligonucleotides. Assembling nucleoside building blocks employing the β-cyanoethyl phosphoamidite functionality[2-4] and automated DNA synthesizers is a convenient and efficient process. By changing synthesis from the standard 3'-5' to the 5'-3' direction using a new colorimetric protecting group cleavable under neutral conditions[5] and the possibility to anchor the growing oligonucleotide chain via acid labile functionality a new synthetic strategy for chemical oligonucleotide synthesis can be envisioned which bears the potential for multiselective deprotection. This allows by sequence dependent preprogrammed selection of appropriate nucleotide building blocks to create oligonucleotides with a predetermined set of modifications or/ and functionalities. Various combinations of these sequence specifically modified/ functionalized oligonucleotides of the same base sequence can generate a combinatorial set of molecules available for specific molecular interaction or recognition experiments.

RESULTS AND DISCUSSION

Synthetic strategy: An 2'-deoxyoligonucleotide **3** is synthesized by the phosphoamidite method[2-4] inverse to the usual process in 5'-3' direction, using the building blocks **1** and **2** (scheme 1). During an elongation cycle the temporary protecting group R^3 is removed with neutral hydrazine reagent IV (table 1) before the condensation step and the acidified filtrate of the hydrazinolysis solution is spectrophotometrically measured to determine the preceding condensation yield. By this way a trityl assay as in the usual process with the 4, 4'-dimethoxytrityl group is possible , but without risking depurinations, due to the absence of acidic conditions during the synthesis cycles.



R^4 = a) β-cyanoethyl as protecting group for selective and orthogonal deprotection according to table 1 *or* b) a protecting group stable with reagent II (table1) - B^{R2A}, B^{R2B} = thymine *or* a) protected bases N^4-nps-cytosine (C^{nps}), N^6-nps-adenine (A^{nps}), N^2-nps-guanine (G^{nps}),[6] for selective and orthogonal deprotection according to table 1 *or* b) protected bases stable under the deprotection conditions of table 1, e.g.: N^4-npeoc-cytosine (C^{npeoc}), N^6-npeoc-adenine (A^{npeoc}), N^2-npeoc-O^6-npe-guanine ($G^{npeoc/npe}$),[7] npeoc = 2-(4-nitrophenyl)-ethoxycarbonyl, npe = 2-(4-nitrophenyl)-ethyl - n: number of condensation reactions; ①, ②, ③, ④: deprotection positions; CPG = Controlled-Pore-Glass.

Scheme 1.

Selective and orthogonal deprotections are possible if at the linkages ①- ④ of oligomer **3** deprotections are selectively done as shown in table 1.

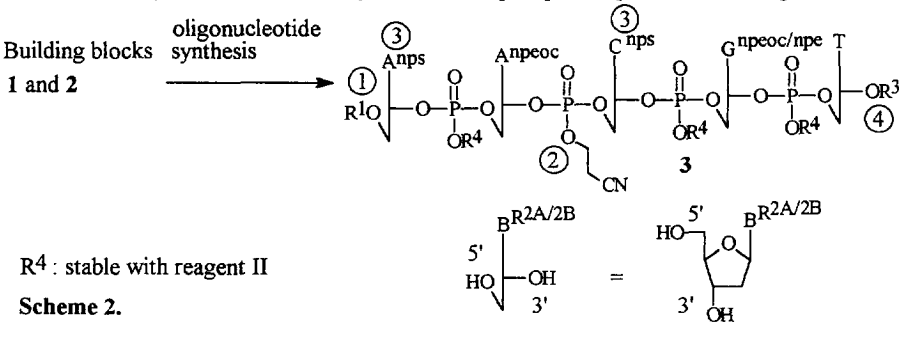
Table 1. Selective and orthogonal deprotection at oligomer **3**.

Deprotection at linkage in **3**	reaction	deprotection reagent	
①	detritylation	I:	80% acetic acid
② a)	decyanoethylation	II:	*tert*-butyl amine/ pyridine 1/9 (v/v)
③ b)	base deprotection	III:	p-thiocresole in pyridine/DMF 3/7 (v/v): 3 mmol/ ml
④	hydrazinolysis	IVa:	1M hydrazinium hydrate in pyridine/ glacial acetic acid/ water (4:3:0.35, v/v), pH 5.4
		IVb:	0.5M hydrazinium hydrate in pyridine/ glacial acetic acid/ water (4:1:0.25, v/v), pH 6.5

a) R^4 = β-cyanoethyl b) R^{2A}/R^{2B} = nps

Selective deprotections allow the following 16 deprotection combinations: fully protected oligomer, fully deprotected oligomer (①, ②, ③, ④) or partially deprotected oligomers after the following combinations of deprotection reactions, deprotected at positions: ①, ①+②, ①+②+③, ①+③, ①+④, ①+③+④, ①+②+④, ②, ③, ④, ②+③, ②+④, ③+④, ②+③+④. Within the combinations of deprotection reactions the order of deprotection can be chosen. In addition the use of differently base or/ and phosphorus protected building blocks **1** and **2** (scheme 1) during the oligonucleotide synthesis furnishes sequences with base or / and phosphate specific open functionalities. After each deprotection the nucleophilic group of the oligomer can be reacted with a new substituent or remains unprotected. Only a little quantity of support is needed to carry out such a new derivatized oligomer, so that a large variety of derivatizations is possible. New substituents are introduced at the immobilized oligonucleotide by the reaction of an excess of the appropriate reagents in their reactive forms (e.g. acyl chlorides, anhydrides), in case of the phosphate group in presence of condensing agents (e.g. aromatic sulfonyl chlorides or derivatives thereof).[6,8] The excess of the reagent can be removed by filtration. The simplicity of these substitutions can be compared with the "capping"-step in DNA synthesis and in principle can be performed on an automated DNA synthesizer.

Scheme 2 shows an example of an immobilized fully protected oligomer **3** for sequence specific derivatizations by the use of differently base or/ and phosphorus protected building blocks **1** and **2** of scheme 1.



R^4 : stable with reagent II

Scheme 2.

The selective and orthogonal deprotections and the derivatizations by introducing new substituents could be carried out at positions ①-④, at ② and ③ in a *sequence specific* way. During the derivatizations at ①-④ only the npeoc/npe base protection remains intact. In contrast, the phosphate protecting group R^4 needs to remain intact if derivatizations at ② are to be performed. These two protecting groups only serve to carry out *sequence specific* derivatizations at ② and/ or ③. *After* the derivatizations at least the bases, protected with npeoc/npe groups have to be deprotected without removing new substituents at ①-④ at the same time. The removal of the npeoc/npe groups is necessary to guarantee sufficient hybridization properities of the derivatizated oligomers with complementary nucleic acid sequences.

1) Base protection:

We found that npeoc/npe protection is stable during deprotection conditions of compound **3** at position ①-④ (scheme 1 and 2) with the reagents I -IV (table 1).

During the npeoc/npe deprotection with DBU reagent no removal of the new substituents at ①-④ is desired. These new substituents are linked for example at positions ① and ④ *via* (trityl) ether or carbonic acid ester bonds e.g., at ② *via* phosphate ester bonds e.g., at ③ *via* amide bonds to the oligomer. The stability of phosphate ester, carbonic acid ester and the nucleoside base amide bond during npeoc/npe

deprotection were described.[7,9] We found that these deprotection conditions do not affect the trityl ether bond and the nps base protection.

In addition to the npeoc/npe protection, the suitability of other groups should be expected.

2) Phosphate protection:

The stability of R^4 during the deprotections at ①, ③ and ④ is not absolutely necessary. In case R^4 is being removed during deprotection at position ④ using reagent IV, reaction of the OH group at position ④ with an acyl chloride would result in a mixed anhydride at the phosphate moiety which subsequently could be either transformed to a newly protected function or hydrolyzed to the phosphodiester. Of course, the substitution at position ② has to be carried out before; R^4 must be stable with reagent II, to guarantee a sequence specific derivatization at position ②.

The phosphate protection with the p-chlorophenyl group e.g. is stable with reagent II in contrast to the ß-cyanoethyl group.[10] The phosphate protection with the o-chlorophenyl group e.g. is stable with 0.5M hydrazine reagent.[11] The phosphate protection with the 2.5-dichlorophenyl group e.g. is stable with strong acids as p-toluenesulfonic acid in methylene chloride/ methanol.[9]

During the deprotection of R^4 no removal of the new substituents at ①-④ is desired. The o-, p-chlorophenyl and the 2.5-dichlorophenyl group e.g. can be removed without cleavage of the phosphate ester nucleotide bonds. The o-chlorophenyl group e.g. allows deprotection with 4-nitrobenzaldoximate without affecting benzoic acid ester and nps amide bonds.[6] Further the o-chlorophenyl group e.g. is easily removable with $(n\text{-butyl})_4NF$.[12] Under these conditions acetic acid ester, trityl ether bonds and the nucleoside base protection with the acetyl or benzoyl groups remain intact.[13]

Compared to oligodeoxynucleotide syntheses developed so far for the antisense and triplex DNA concept,[1,14,15] the new strategy shows a remarkable advantage. All possible derivatizations can be performed with only *one* oligonucleotide synthesis run.

Combinations of the described strategy with other schemes of oligonucleotide synthesis could be conceived. If in **3** (scheme 1) e.g. the oligomer would be connected at its 3'-OH or 5'-OH group to the CPG via a levulinic acid ester bridge (cleavable with neutral hydrazine reagent IV) instead of the trityl ether bridge in **3,** more simple 3'-5' as well as 5'-3' directed DNA syntheses would be available, keeping the advantage of multiselective deprotections, with the trityl moiety for easy detection and as "purification handle".[2-4] For these syntheses, amidites will be used, whose 5'-OH or 3'-OH groups respectively are protected with the 4,4'-dimethoxytrityl (DMTr) group. The following findings demonstrate the feasibility of this extension of the synthetic strategy. The base protection of nucleosides protected with the 2-nitrophenylsulfenyl (nps) group is rather stable with strongly acidic solutions.[6] We found that stability against depurination in 80% acetic acid decreases as follows: 2'-deoxy-N^6-nps-adenosine (A_d^{nps}) >> 2'-deoxy-N^2-nps-guanosine (G_d^{nps}) > 2'-deoxy-N^2-isobutyryl-guanosine (G_d^{ib}) >> 2'-deoxy-N^6-benzoyl-adenosine (A_d^{bz}) ; G_d^{ib} and A_d^{bz} are exposed to strong acids in every elongation cycle in the standard DNA synthesis process.[2-4] In accordance with ref.[6] A_d^{nps} does not depurinate with 80% acetic acid, although the main depurination problem in standard DNA synthesis is caused by the A_d^{bz} units. 2'-Deoxy-N^4-nps-cytidine (C_d^{nps}) is stable with 80% acetic acid. No problem caused by depurination is observed with npeoc/ npe base protection.[7]

To test the new strategy d(TAGCT) and d(TTTT) were synthesized by a 5'-3' directed DNA synthesis with support **1** (scheme 1, B^{R2A} = thymine) and amidites **2** (scheme 1, B^{R2B} = thymine, npeoc/ npe protected bases, R^4 = β-cyanoethyl, corresponding to **2a** - **d** of scheme 3). The 3'-OH protection was removed with hydrazine reagent IV (table 1) at near neutral pH, forming a heterocyclic compound which is detected in the visible spectral region with high sensitivity after being acidified.[5] To obtain the desired high condensation

yields, addition of the amidite solution had to be preceded by the activation with tetrazole. The oligomer could be removed from the support by a short treatment with 80% acetic acid without affecting the 3'-OH protection. Suitability of the 3'-OH protection group as "purification handle"[2-4] is comparable with the DMTr group. Removal of the ß-cyanoethyl group was carried out after synthesis of the protected d(TTTT) with *tert*-butyl amine reagent (table 1), after synthesis of the protected d(TAGCT) with 0.5M DBU in acetonitrile[7] (together with the removal of the base and the 3'-OH protection). Because of the lability of the 3'-OH protection with DBU reagent, the levulinyl group should be substituted by an acyl group stable with DBU reagent before removing the oligomer from the support, if maintaining of the purification handle effect is desired. The 3-{4-[bis-(4-methoxyphenyl)-methyl]-phenyl}-propionyl group of compound **12** (scheme 4), the triphenylmethoxy- acetyl[16] or the diphenyl-*tert*-butyl silyl group[17] could be such an acyl group. Further experimental steps were similar to the 3'-5' directed DNA synthesis.[2-4]

To demonstrate identity of the synthesized oligomers following the different synthetic routes (3'-5' versus 5'-3' direction) the oligomers were fully deprotected and analyzed by HPLC. Simultaneous analyses of either the two d(TAGCT) or d(TTTT) oligomers resulted in one single peak, proving identity (figure 1 and 2).

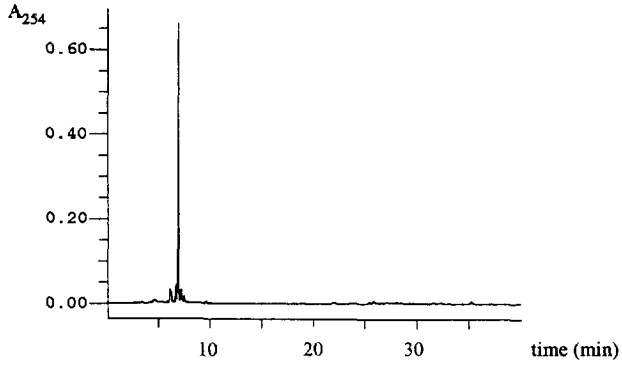


Figure1. HPLC analysis of a mixture of d(TAGCT) obtained by 5'-3' and 3'-5' directed syntheses.

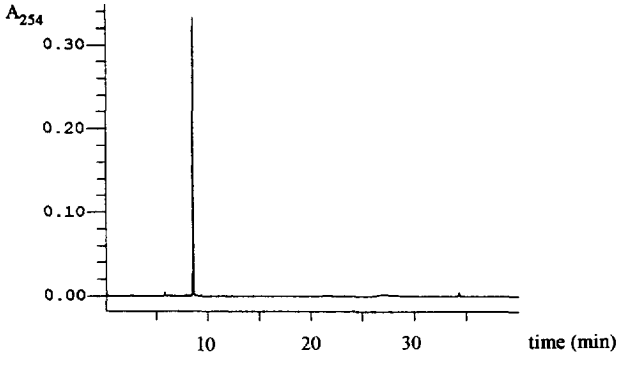


Figure 2. HPLC analysis of a mixture of d(TTTT) obtained by 5'-3' and 3'-5' directed syntheses.

The fully deprotected oligomers d(TAGCT) and d(TTTT) were further characterized by MALDI-TOF mass spectra (figures 3 and 4). In figure 5 a spectrum of d(TAGCT) obtained by the 3'-5' directed synthesis is shown.

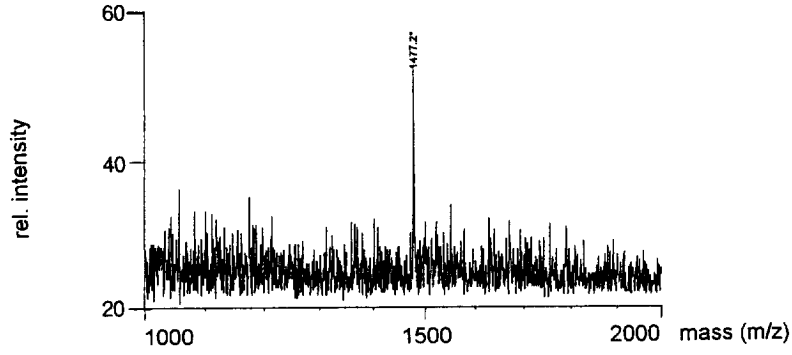


Figure 3. MALDI-TOF mass spectrum of d(TAGCT) obtained by 5'-3' directed synthesis. $M+H^+$: theoretical mass: 1478, found: 1477.

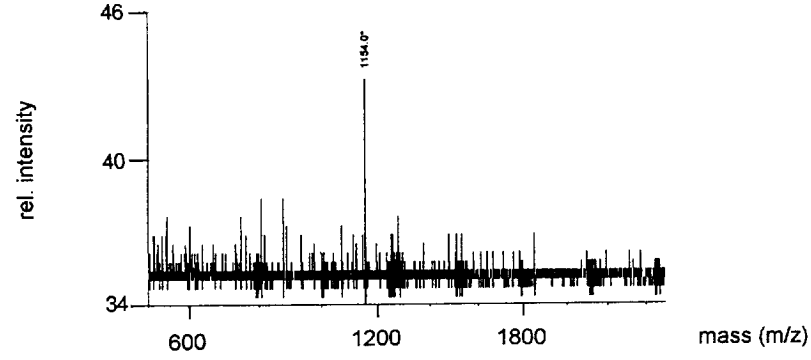


Figure 4. MALDI-TOF mass spectrum of d(TTTT) obtained by 5'-3' directed synthesis. $M+H^+$: theoretical mass: 1155, found 1154.

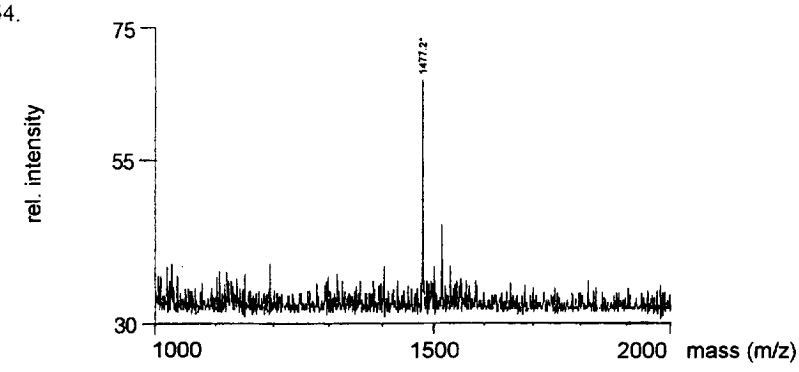
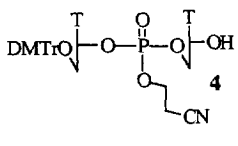


Figure 5. MALDI-TOF mass spectrum of d(TAGCT) obtained by 3'-5' directed synthesis. $M+H^+$: experimental mass: 1477.

The immobilized fully protected d(TTTT) (oligomer **3**, $B^{R^{2A}}$ and $B^{R^{2B}}$ = thymine) already shows eight different combinations of deprotection with the reagents I, II, IV of table 1. To demonstrate the orthogonal deprotection (16 deprotection combinations) for the fully protected mixed oligomer **3** of scheme 1 (for the bases A, G, C the protection group R^{2A}, R^{2B} = nps, R^4 = β-cyanoethyl) with the optimized deprotection reagents I -IV of table 1, additional deprotection experiments were carried out with the immobilized oligomers 5'-*O*-R^1-d(TTTT)-3'-*O*-R^3, 5'-*O*-R^1-d(TAGCT)-3'-*O*-R^3 (R^1, R^3 as in scheme 1) and in solution with the model compounds 5'-*O*-DMTr-2'-deoxythymidine [(DMTr)T_d], **4**, **5a**, **6a** (figure 6), G_d^{nps}, A_d^{nps}, C_d^{nps}. The deprotection reagents removed one protecting group quickly, while the other groups were stable under these conditions for at least 24h. This is demonstrated by the following results: 1) reagent I (80% acetic acid): the 3'-O-protected d(TTTT) and d(TAGCT) are removed from the support after 15 minutes by detritylation, in compound **4** only the DMTr group is cleaved, compound **6a** is only transformed to compound **5a** which is stable, the nps groups of the nps protected nucleosides are not removed (only at G_d^{nps} depurination is observed, but slower compared to G_d^{ib}); 2) reagent II (*tert*-butyl amine reagent): decyanoethylation is complete with compound **4** after 40 minutes, (DMTr)T_d, compounds **5a**, **6a** and the nps protected nucleosides are stable; 3) reagent III (thiocresolate reagent): nps groups are removed at G_d^{nps}, C_d^{nps} after 5 minutes, at A_d^{nps} after 45 - 60 minutes, compounds **4**, **5a**, **6a** are stable; 4) reagents IVa, IVb (hydrazine reagents): delevulination is complete with compounds **5a** and **6a** after 8 minutes, compound **4** and the nps protected nucleosides are stable.



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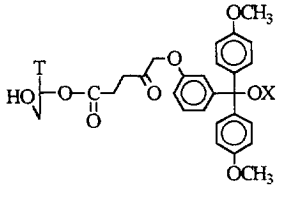


5a X = H

6a X = CH_3

T = thymine

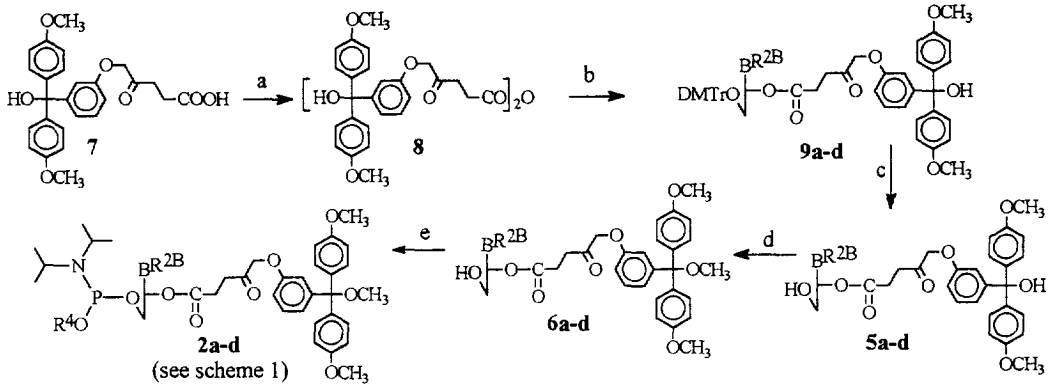
Figure 6.

G_d^{nps}, A_d^{nps} and C_d^{nps} are not affected by 0.02M iodine reagent which is used for oxidation reaction during oligodeoxynucleotide synthesis.

Experiments for base specific deprotections: G_d^{nps}, C_d^{nps} are stable with 0.5M DBU in acetonitrile for 24h, A_d^{nps} only shows a slight deprotection after this time.

Compounds **6b - d** were stable with reagents II and III for at least 24h, the stability of the npeoc/ npe base protection with reagents I and IV is demonstrated e.g. by the synthesis of the oligomer d(TAGCT).

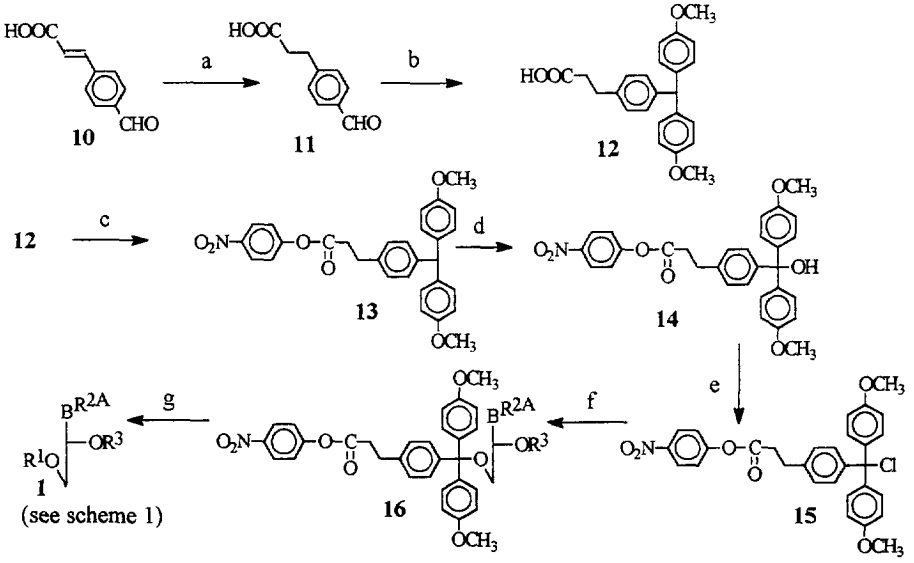
The syntheses of the building blocks **2** and **1** are shown in scheme 3 and 4.



a: condensation of 7[5] with DCC b: alcoholysis of **8** c: detritylation d: methylation e: alcoholysis of $[(CH_3)_2CH]_2NP(Cl)OCH_2CH_2CN$ with **6a-d** R^4 = ß-cyanoethyl

compounds **9, 5, 6** and **2**	B^{R2B}
a	thymine
b	N^4-npeoc-cytosine
c	N^6-npeoc-adenine
d	N^2-npeoc-O^6-npe-guanine

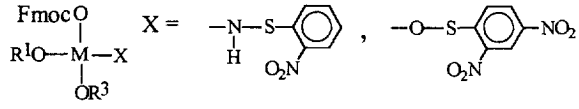
Scheme 3.



a: hydrogenation b: electrophilic aromatic substitution c: esterification d: oxidation e: chlorination of **14** f: alcoholysis with **6a** (scheme 3, B^{R2A}, B^{R2B} = thymine) g: aminolysis with aminopropyl CPG

Scheme 4.

Multifunctional molecules with core structure M (figure 7) different to the structure of oligomer **3** i.e. low molecular weight multifunctional molecules or biomolecules such as peptides, lipids and oligosaccharides could make it possible to create easily a high number of derivatives for combinatorial experiments.[18,19] The Fmoc group in 5'-*O*-Fmoc-2'-deoxythymidine[20] showed orthogonal deprotection properties with reagents I - IV (table 1). The 2,4-dinitrophenylsulfenyl (dnps) group in dnps ethyl ester[21] reveals comparable selective deprotection properties with reagents I - IVa to the 2-nitrophenylsulfenyl (nps) group in the nps amide moiety.



M: chosen core structure, R^1, R^3: see scheme 1, X: similar properties to the amide moiety of the nps group (scheme 1)

Figure 7. Orthogonal deprotection with additional core structures and protecting moieties.

Other variations can be conceived. Block condensations are possible using different derivatives. Because of the selectivity in deprotection the number of combinations to generate compounds with predetermined structures is large.

EXPERIMENTAL SECTION

^1H (400 and 250 MHz) and ^{13}C (101 and 63 MHz) NMR spectra were recorded on a Bruker AMX 400 and a AC 250-P instrument. Samples were dissolved in the presence of tetramethylsilane as internal standard, unless otherwise stated. ^{31}P NMR sprectra were recordered on a Varian Gemini 200 instrument. Internal standard: phosphoric acid in the solvent used for the sample (δ = 0.00 ppm). Chemical shifts are given in ppm. Mass spectra were obtained on a Finnigan MAT 311A mass spectrometer under EI conditions, a VG Analytical 70-250S mass spectrometer under FAB conditions (matrix: 3-nitro-benzyl alcohol, Xenon bombardment) and a Finnigan MAT Vision 2000 mass spectrometer under MALDI-TOF conditions (matrix solution: 0.7 mol/ l 3-hydroxy picolinic acid and 0.07 mol/ l ammonium citrate in acetonitrile/ water, 1/1, v/v). Elementary analyses were performed by the analytical department of the Institute of Organic Chemistry, University of Hamburg. Thin layer chromatography (tlc) was carried out on 60 PF_{254} silica gel coated aluminia sheets (Merck, Darmstadt, No 5562). Trityl and sugar containing compounds are visualized with sugar spray reagent (0.5 ml 4-metoxybenzaldehyde, 9 ml ethanol, 0.5 ml concentrated sulfuric acid and 0.1 ml glacial acetic acid) by heating with a fan or on a hot plate. p-Nitrophenyl ester containing compounds are visualized by ammonia vapour. Column chromatography was performed using silica gel from Merck. HPLC results were obtained on a Waters chromatography systems 625 LC with a photodiodearray detector 996 and using reversed phase columns (Waters Nova-Pak C18, 60 Å, 4 µm particles, 3.9 x 300mm, software: Millenium 2.0, eluants were: 0.1M triethylammonium acetate at pH 7.0 (A) and acetonitrile (B); the column was equilibrated at 30°C at 1ml per min, with 95% A/ 5% B, v/v, with elution using a linear gradient from 5% to 40% B in 40 min, monitored at 254 nm). Spectrophotometric measurements in the UV/ Vis region were performed on a Beckman UV35 and a LKB Ultrospec Plus UV/ Vis spectrophotometer. Solvents were dried and purified before use according to standard procedures. Extractions were monitored by tlc to optimize completion of extraction.

Synthesis of the building block 2

3'-O-levulinyl esters of the nucleosides 6a-d

Compound **8** is prepared *in situ* by reacting levulinic acid derivate 7[5] (3.78 g, 8.39 mmol) with N,N'-dicyclohexylcarbodiimide (1.80 g, 8.74 mmol) in dry dioxane (25 ml). N,N'-dicyclohexylurea is removed by filtration and washed with dioxane. The solution is divided in four equal parts and the solvents are evaporated *in vacuo*. To each of the four residues of anhydride **8** is added one of the four following protected nucleosides: 5'-*O*-DMTr-2'-deoxythymidine, 5'-*O*-DMTr-N^4-npeoc-2'-deoxycytidine, 5'-*O*-DMTr-N^6-npeoc-2'-deoxyadenosine, 5'-*O*-DMTr-N^2-npeoc-O^6-npe-2'-deoxyguanosine (1.00 mmol of each; base protected deoxynucleosides are from Chemogen, Konstanz)[7] and 4-dimethylaminopyridine (0.0100 g, 0.0819 mmol) in 1.64 ml pyridine. Completion of reaction is checked by thin layer chromatography. 30 min after the addition of a mixture of 0.130 ml of glacial acetic acid and 0.245 ml pyridine, 0.046 ml water are added, 60 min later an excess of ethyl acetate is added, the N,N'-dicyclohexylurea removed by filtration and washed with ethyl acetate. The mixture is extracted with water, 5% aqueous sodium hydrogen carbonate and water. After drying with sodium sulfate, the solvent is evaporated, then co-evaporated with toluene. The residues are directly detritylated with 80% acetic acid and the reaction is monitored by thin layer chromatography. The solutions are poured into an excess of water (about 10fold) and the aqueous mixtures are extracted with ethyl acetate. The organic phase is washed with 5% aqueous sodium hydrogen carbonate and water. After drying the solvent is evaporated, then co-evaporated with toluene (to remove remaining acetic acid). The residues are directly methylated by adding to each a solution of 200 ml methanol and 1 ml glacial acetic acid. If there is some insoluble material, it is dissolved in 5-10 ml dichloromethane and a mixture of 100 ml methanol and 0.5 ml glacial acetic acid is added. Monitoring by thin layer chromatography indicates completion of the reaction. The solvents are evaporated under reduced pressure, followed by co-evaporation with toluene (2-3 times). The residues of **6a-d** are purified by silica gel column chromatography (**6a**: silica gel 60H, No. 7736, **6b-d**: silica gel 60, No. 9385; Merck, Darmstadt). Silica gel used per gram raw product: **6a**: 25 g, **6b**: 51 g, **6c**: 65 g, **6d**: 51 g; using a step gradient from dichloromethane to dichloromethane/ methanol 98/2 (v/v), in the presence of 0.1% pyridine. Pure fractions are pooled, the solvents removed by evaporation, the residues dissolved in dichloromethane (15 ml per gram residue) and the solutions precipitated into hexane (315 ml per gram residue). Yields: **6a**: 68%, **6b**: 63%, **6c**: 62%, **6d**: 52%.

Compound 6a: *^1H NMR* (400 MHz, $CDCl_3$): δ = 1.88 (s, 3H, -C$\underline{H}_3$ of thymine), 2.5-2.34 (m, 2H, H2'^{a}/H2'^{b}), 2.64 (t, 2H, -C$\underline{H}_2$-CH_2-), 2.93 (t, 2H, -CH_2-C$\underline{H}_2$-), 3.04 (s, 3H, R_3C-OC$\underline{H}_3$), 3.8 (s, 6H, aryl-OC$\underline{H}_3$), 3.9 (m, 2H, H5'^{a}/H5'^{b}), 4.1 (m, 1H, H4'), 4.57 (s, 2H, -CO-C$\underline{H}_2$-O-), 5.38 (m,1H, H3'), 6.26 (t, 1H, H1'), 7.34-6.7 (m, 12H, aryl-$\underline{H}$), 7.55 (s, 1H, H6), 8.93 (s, 1H, N-$\underline{H}$ of thymine). - *^{13}C NMR* (101 MHz, $CDCl_3$): δ = 12.51 (q, -$\underline{C}H_3$ of thymine), 27.43 (t,-$\underline{C}H_2$-CH_2-), 33.79 (t, -CH_2-$\underline{C}H_2$-), 37.27 (t,C2'), 51.92 (q, R_3C-O$\underline{C}H_3$), 55.23 (q, aryl-O$\underline{C}H_3$), 62.39 (t, C5'), 72.77 (t, -CO-$\underline{C}H_2$-O-), 75.31 (d, C3'), 85.20 (d, C4'), 85.92 (d, C1'), 86.31 (s, $R_3\underline{C}$-OCH_3), 111.28 (s, C5 of thymine), 112.37, 113.11, 114.59, 121.83, 128.95, 130.20 (d, $\underline{C}$-H, aryl), 135.63, 147.58 (s, $R_2\underline{C}$-CR_2-OCH_3, aryl, quarternary), 136.34 (d, C6 of thymine), 150.52 (d, C2 of thymine), 157.32, 158.52 (s, $R_2\underline{C}$-OCH_3, and s, $R_2\underline{C}$-O-CH_2-CO-, aryl, position not defined), 163.69 (s, C4 of thymine), 172.26 (s, -$\underline{C}$OOR), 206.02 (s, -$\underline{C}$O-). - *^1H ^1H* and *^1H ^{13}C 2D NMR* spectra are determined (data not shown). - *MS* (FAB, pos. mode): m/z (rel. intensity): m/z calculated for $C_{37}H_{40}N_2O_{11}$ (M^+): 688; found: 688 (7), 657 (74, M - OCH_3 $^+$), 391 (78), 307 (100). - *Elementary Analysis* (%): Found: C, 64.99/ 64.73; H, 5.98/ 5.82; N, 4.02/ 3.99; $C_{37}H_{40}N_2O_{11}$ requires C, 64.53; H, 5.85; N, 4.07.

Compound 6b: *^1H NMR* (400 MHz, $CDCl_3$): δ = 2.38 (m, 1H, H2'^{a}), 2.65 (m, 1H, H2'^{b}, t, 2H, -C$\underline{H}_2$-CH_2-, 3'-OH protecting group), 2.9 (t, 2H, -CH_2-C$\underline{H}_2$-, 3'-OH protecting group), 3.04 (s, 3H, R_3C-OC$\underline{H}_3$), 3.1 (t, 2H, -C$\underline{H}_2$-CH_2-, base protection), 3.8 (s, 6H, aryl-OC$\underline{H}_3$), 3.99-3.88 (m, 2H, H5'^{a}/H5'^{b}), 4.19 (m, 1H, H4'), 4.44 (t, 2H, -CH_2-C$\underline{H}_2$-, base protection), 4.55 (s, 2H, -CO-C$\underline{H}_2$-O-), 5.38 (m, 1H, H3'), 6.26 (m, 1H, H1'), 7.34-6.7 (m, 13H, aryl-$\underline{H}$ and H5), 7.38 (d, 2H, O_2N-aryl-$\underline{H}$, meta), 8.17 (d, 2H, O_2N-aryl-$\underline{H}$, ortho), 8.3-8.2 (s, 1H, N-$\underline{H}$ and d, 1H, H6 of cytosine). - *^{13}C NMR* (101 MHz, $CDCl_3$): δ = 27.43 (t,-$\underline{C}H_2$-CH_2-,3'-OH protecting group), 33.78 (t, -CH_2-$\underline{C}H_2$-, 3'-OH protecting group),34.97 (t, -C$\underline{H}_2$-CH_2-, base protection), 38.58 (t,C2'), 51.91 (q, R_3C-O$\underline{C}H_3$), 55.23 (q, aryl-O$\underline{C}H_3$), 62.12 (t, C5'), 65.49 (t, -CH_2-C$\underline{H}_2$-, base protection), 72.76 (t, -CO-$\underline{C}H_2$-O-), 74.98 (d, C3'), 85.98 (d, C4' and d, C1'), 123.86 (d, $\underline{C}$-H, O_2N-aryl, ortho),129.77 (d, $\underline{C}$-H, O_2N-aryl, meta), 112.35, 113.1, 114.58, 121.83, 128.95, 130.2 (d, $\underline{C}$-H, aryl, d, C5 and d, C6 of cytosine, position not defined),86.31 ($R_3\underline{C}$-OCH_3), 123.22, 135.64, 147.58, 149.14, 149.40, 149.67 (s, aryl, quarternary, C2, C4 of cytosine and -NH-$\underline{C}$O- of the base protection, position not defined), 157.32, 158.52 (s, $R_2\underline{C}$-OCH_3, and s, $R_2\underline{C}$-O-CH_2-CO-, aryl, position not defined), 172.32 (s, -$\underline{C}$OOR), 205.97 (s, -$\underline{C}$O-). - *^1H ^1H* and *^1H ^{13}C 2D NMR* spectra are determined (data not shown). - *MS* (FAB, pos. mode): m/z (rel. intensity): m/z calculated for $C_{45}H_{46}N_4O_{14}$ (M^+): 866; found: 866 (9), 835 (100, M - OCH_3 $^+$), 307 (87).

Compound 6c: *^1H NMR* (400 MHz, $CDCl_3$): δ = 2.48 (m, 1H, H2'^{a}), 2.67 (t, 2H, -C$\underline{H}_2$-CH_2-, 3'-OH protecting group), 2.96 (t, 2H, -CH_2-C$\underline{H}_2$-, 3'-OH protecting group), 3.05 (s, 3H, R_3C-OC$\underline{H}_3$), 3.15 (t, 2H, -C$\underline{H}_2$-CH_2-, base protection and m, 1H, H2'^{b}), 3.78 (s, 6H, aryl-OC$\underline{H}_3$), 4.0-3.84 (m, 2H, H5'^{a}/H5'^{b}), 4.29 (m, 1H, H4'), 4.55 (t, 2H, -CH_2-C$\underline{H}_2$-, base protection and s, 2H, -CO-C$\underline{H}_2$-O-), 5.58 (m,1H, H3'), 6.35 (m, 1H, H1'), 7.34-6.7 (m, 12H, aryl-$\underline{H}$ of DMTr),7.42 (d, 2H, O_2N-aryl-$\underline{H}$, meta), 8.08 (s, 1H, H2 or H8 of adenine), 8.14 (d, 2H, O_2N-aryl-$\underline{H}$, ortho), 8.73 (s, 1H, H2 or H8 of adenine), 9.04 (-N-$\underline{H}$ of adenine). - *^{13}C NMR* (101 MHz, $CDCl_3$): δ = 27.49 (t, -$\underline{C}H_2$-CH_2-,3'-OH protecting group), 33.86 (t, -CH_2-$\underline{C}H_2$-, 3'-OH protecting group), 35.01 (t, -C$\underline{H}_2$-CH_2-, base protection), 37.85 (t,C2'), 51.91 (q, R_3C-O$\underline{C}H_3$), 55.22 (q, aryl-O$\underline{C}H_3$), 63.16 (t, C5'), 65.52 (t, -CH_2-C$\underline{H}_2$-, base protection), 72.77 (t, -CO-$\underline{C}H_2$-O-), 76.57 (d, C3'), 87.25 (d, C4'), 87.49 (d, C1'), 123.84 (d, $\underline{C}$-H, O_2N-aryl, ortho), 112.32, 113.11, 114.55, 121.87, 128.97, 130.21 (d, $\underline{C}$-H, aryl), 129.81 (d, $\underline{C}$-H, O_2N-aryl, meta), 86.30 ($R_3\underline{C}$-OCH_3)135.57, 145.24, 147.02, 147.72, 149.18, 149.72, 150.04, 150.69 (s, aryl, quarternary, C4-C6 of adenine and -NH-$\underline{C}$O- of the base protection, position not defined), 142.31 (d, C2 or C8 of adenine), 152.3 (d, C2 or C8 of adenine), 157.3, 158.54 (s, $R_2\underline{C}$-OCH_3, and s, $R_2\underline{C}$-O-CH_2-CO-, aryl, position not defined), 171.98 (s, -$\underline{C}$OOR), 206.14 (s, -$\underline{C}$O-). - *^1H ^1H* and *^1H ^{13}C 2D NMR* spectra are determined (data not shown). - *MS* (FAB, pos. mode): m/z (rel. intensity): m/z calculated for $C_{46}H_{46}N_6O_{13}$ (M^+): 890; found: 859 (5, M - OCH_3 $^+$), 307 (100). - *Elementary Analysis* (%): Found: C, 62.14/ 62.00; H, 5.26/ 5.17; N, 9.06/ 9.01; $C_{46}H_{46}N_6O_{13}$ requires C, 62.02; H, 5.20; N, 9.43.

Compound 6d: *^1H NMR* (400 MHz, $CDCl_3$): δ = 2.44-2.40 (m, 1H, H2'^{a}), 2.67 (t, 2H, -C$\underline{H}_2$-CH_2-, 3'-OH protecting group), 2.96 (t, 2H, -CH_2-C$\underline{H}_2$-,3'-OH protecting group), 3.04 (s, 3H, R_3C-OC$\underline{H}_3$), 3.12 (t, 2H, -C$\underline{H}_2$-CH_2-, npeoc base protection and m, 1H, H2'^{b}), 3.30 (t, 2H, -C$\underline{H}_2$-CH_2-, npe base protection), 3.8 (s, 6H, aryl-OC$\underline{H}_3$), 3.99-3.82 (m, 2H, H5'^{a}/H5'^{b}), 4.23 (m, 1H, H4'),4.49 (t, 2H, -CH_2-C$\underline{H}_2$-, npeoc base protection), 4.56 (s, 2H, -CO-C$\underline{H}_2$-O-), 4.82 (t, 2H, -CH_2-C$\underline{H}_2$-, npe base protection) 5.57 (m,1H, H3'), 6.24 (m, 1H, H1'), 7.7-6.7 (m, 16H, aryl-$\underline{H}$ of DMTr, O_2N-aryl-$\underline{H}$, meta and s, 1H, -N-$\underline{H}$ of guanine), 7.89 (s, 1H, H8 of guanine), 8.18-8.13 (m, O_2N-aryl-$\underline{H}$, ortho, npe and npeoc group). - *^{13}C NMR* (101 MHz, $CDCl_3$): δ = 27.46 (t,-$\underline{C}H_2$-CH_2-,3'-OH protecting group), 33.86 (t, -CH_2-$\underline{C}H_2$-, 3'-OH protecting group), 35.04 (t, -C$\underline{H}_2$-CH_2-, npe and npeoc base protection), 37.37 (t, C2'), 51.92 (q, R_3C-O$\underline{C}H_3$), 55.23 (q, aryl-O$\underline{C}H_3$),

63.01 (t, C5'), 64.97 (t, -CH_2-$\underline{C}H_2$-, npeoc base protection), 67.07 (t, -CH_2-$\underline{C}H_2$-, npe base protection) 72.77 (t, -CO-$\underline{C}H_2$-O-), 76.24 (d, C3'), 86.55 (d, C4'), 86.79, (d, C1'), 123.75, 123.82 (d, $\underline{C}$-H, O_2N-aryl, ortho, npe and npeoc base protection, position not defined), 141.33 (d, C8 of guanine), 112.32, 113.11, 114.55, 121.87, 128.96, 129.77, 130.04, 130.21 (d, $\underline{C}$-H, aryl), 86.30 ($R_3\underline{C}$-OCH_3), 135.58, 145.58, 146.94, 147.7, 149.15, 149.42, 149.69, 151.13, 151.47, 152.06, 161.01 (s, aryl, quarternary, C2, C4-C6 of guanine and -NH-$\underline{C}$O- of the base protection, position not defined), 157.31, 158.54 (s, $R_2\underline{C}$-OCH_3, and s, $R_2\underline{C}$-O-CH_2-CO-, aryl, position not defined), 172.04 (s, -$\underline{C}$OOR), 206.10 (s, -$\underline{C}$O-). - *^1H ^1H and ^1H ^{13}C 2D NMR* spectra are given. - *MS* (FAB, pos. mode): m/z (rel. intensity): m/z calculated for $C_{54}H_{53}N_7O_{16}$ (M^+): 1055; found: 1024 (6, M - OCH_3 $^+$), 307 (100).

Phosphoamidites 2a-d

All steps are carried out under inert athmosphere (argon). Organic solvents are free from water and other impurities. Compounds **6a-d** (0.5 mmol of each) are azeotropically dried with small amounts of pyridine and toluene and dissolved in 2.43 ml ethyl acetate. After the addition of N,N-diisopropyl ethylamine (1.75 mmol, 0.226 g, corresponding to 0.30 ml at room temperature) the reaction flask is capped with a septum and cooled with an ice bath. Chloro-ß-cyanoethoxy-N,N-diisopropylaminophosphane (0.610 mmol, 0.144 g, corresponding to 0.117 ml at room temperature, Biosyntech, Hamburg) is added dropwise by a syringe. 15 min later the reaction is allowed to raise to room temperature. Monitoring by thin layer chromatography (about 60 min after starting the reaction) indicates complete conversions to the amidites **2a-d**. The precipitated amine hydrochloride is filtered off using a column type reactor fitted with a sintered glass fritt and washed with 1.5 ml ethyl acetate. The solution is extracted in a separation funnel with cold 5% sodium hydrogen carbonate (2 x 2:8 ml). The organic solution is filtered using the described reactor which contains sodium sulfate, followed by washing of the sodium sulfate layer with ethyl acetate (2 x 1.8 ml). After evaporation of the solvents of the filtrate, a foam is obtained. The amidite is dissolved in 5 ml ethyl acetate (containing 0.1% pyridine) and precipitated into 120 ml of hexane (at -20°C). After filtration using the described reactor the amidite is washed with 12 ml of hexane, dried and stored at -20°C. Yields: **2a**: 86%, **2b**: 72%, **2c**: 78%, **2d**: 80%. - *^{31}P NMR* (81 MHz, CD_3CN/ CH_3CN, 1/1,v/v and a trace of N,N-diisopropyl ethylamine): **2a**: δ = 149.18, 149.35 (diastereomers), **2b**: δ = 149.25, **2c**: δ = 149.07, **2d**: δ = 148.89, 149.16 (diastereomers).

Synthesis of the building block 1

3-(4-Formylphenyl)-propionic acid (11)

Hydrogenation of compound **10**[22-24] is carried out in the presence of 5% Pd on activated carbon. *^1H NMR* (250 MHz, [D_6]DMSO): δ = 2.6 (t, 2H, -$\underline{C}H_2$-CH_2-), 2.95 (t, 2H, -CH_2-$\underline{C}H_2$-), 7.45 (d, 2H, $\underline{H}$-aryl-CHO, meta), 7.85 (d, 2H, $\underline{H}$-aryl-CHO, ortho), 9.96 (s, 1H, -C$\underline{H}$O), 12.16 (s, 1H, -COO$\underline{H}$).

3-{4-[Bis-(4-methoxyphenyl)-methyl]-phenyl}-propionic acid (12)

Compound **11** (25.7 g, 144 mmol) and methoxybenzene (36.8 g, 340 mmol) are stirred in 450 ml glacial acetic acid to dissolve most of the material. The mixture is cooled in an ice bath and immediately concentrated sulfuric acid (225 g, 2290 mmol) added dropwise. The reaction mixture is then stirred at room temperature until thin layer chromatography (dichloromethane/ methanol: 8/2, v/v) demonstrates quantitative conversion. The reaction mixture is poured into 3 l ice/ water. Subsequently the reaction flask is washed with ether and the ether solution is poured into the ice/water. The orange-white raw product between the aqueous and organic

layer is filtrated by suction (if there is still a considerable amount of the raw product under the aqueous and/or dissolved in the ether layer, it is also worked up). The raw product is triturated with 200 ml water, filtrated by suction , again triturated with petroleum ether (bp 60-70°C) and filtrated. It is recrystallized from ether. Yield: 22.3 g (41%). Note: More product **12** can be purified from the crystalline residue of the the mother liquor by silica gel column chromatography or by Soxhlet extraction with petroleum ether (bp 30-50°C). - *^1H NMR* (250 MHz, $CDCl_3$): δ = 2.65 (t, 2H, -$\underline{C}H_2$-CH_2-), 2.92 (t, 2H, -CH_2-$\underline{C}H_2$-), 3.78 (s, 6H, -O$\underline{C}H_3$), 5.40 (s, 1H, R_3C-$\underline{H}$), 7.13-6.77 (m, 12H, aryl-$\underline{H}$). - *^{13}C NMR* (63 MHz, $CDCl_3$, internal standard $CDCl_3$ at 77.00 ppm): δ = 30.1 (t, -$\underline{C}H_2$-CH_2-), 35.48 (t, -CH_2-$\underline{C}H_2$-), 54.8, 55.19 (q, aryl-O$\underline{C}H_3$ and d, $R_3\underline{C}$-H, position not defined), 113.63, 128.1, 129.41, 130.21 (d, $\underline{C}$-H, aryl), 136.46, 137.91, 142.7 (s, aryl, quarternary), 157.93 (s, $R_2\underline{C}$-OCH_3, aryl), 178.85 (s, -$\underline{C}$OOH).- *MS* (EI): m/z (rel. intensity): m/z calculated for $C_{24}H_{24}O_4$ (M^+): 376; found: 376 (100), 345 (9, M-OCH_3 $^+$), 227 (35, M - HOOC-CH_2-CH_2-C_6H_4 $^+$). - *MS* (FAB, pos. mode): m/z (rel. intensity): m/z calculated for $C_{24}H_{24}O_4$ (M^+): 376; found: 376 (48), 345 (8, M-OCH_3 $^+$), 269 (53, M - C_6H_4-OCH_3 $^+$), 227 (38, M - HOOC-CH_2-CH_2-C_6H_4 $^+$). - *Elementary Analysis* (%): Found: C, 76.55/ 76.35; H, 6.71/ 6.53;$C_{24}H_{24}O_4$ requires C, 76.57; H, 6.43.

p-Nitrophenyl-3-{4-[bis-(4-methoxyphenyl)-methyl]-phenyl}-propionate (13)

Compound **12** (22.2 g, 59.0 mmol) and p-nitrophenol (8.23 g, 59.2 mmol) are dissolved in dry dioxane (272 ml) and dry pyridine (14.7 ml). After addition of a solution of N,N'-dicyclohexylcarbodiimide (13.9 g, 67.4 mmol) in dry dioxane (66 ml) the mixture is stirred at room temperature until thin layer chromatography (dichloromethane/ methanol: 9/1, v/v) revealed quantitative conversion (4-18 h). N,N'-Dicyclohexylurea is removed by filtration, the precipitate washed with dioxane until no UV absorbing material can be detected. The solvent is evaporated, the residue azeotropically dried with toluene, dissolved in dichloromethane (70 ml) and remaining dicyclohexylurea removed by filtration. After evaporating the solvent, the residue is directly converted to compound **14**. Remaining DCC can be removed with a small amount of hexane. Yield: 29.1 g (99%). - *^1H NMR* (250 MHz, $CDCl_3$): δ = 2.90 (t, 2H, -$\underline{C}H_2$-CH_2-), 3.14 (t, 2H, -CH_2-$\underline{C}H_2$-), 3.78 (s, 6H, -O$\underline{C}H_3$), 5.43 (s, 1H, R_3C-$\underline{H}$), 7.18-6.76 (m, 14H, aryl-$\underline{H}$), 8.22 (d, 2H, O_2N-aryl-$\underline{H}$, ortho). - *^{13}C NMR* (63 MHz, $CDCl_3$): δ = 30.42 (t, -$\underline{C}H_2$-CH_2-), 35.9 (t, -CH_2-$\underline{C}H_2$-), 54.88 (d, $R_3\underline{C}$-H), 55.24 (q, aryl-O$\underline{C}H_3$), 113.69, 122.43, 125.17, 128.31, 129.57, 130.24 (d, $\underline{C}$-H, aryl), 136.39, 137.38, 143.14, 145.32, 155.35 (s, aryl, quarternary), 158.02 (s, $R_2\underline{C}$-OCH_3, aryl), 170.5 (s, -$\underline{C}$OOR).- *MS* (EI): m/z (rel. intensity): m/z calculated for $C_{30}H_{27}NO_6$ (M^+): 497; found: 497 (16), 480 (3), 51 (100). - *MS* (FAB, pos. mode): m/z (rel. intensity): m/z calculated for $C_{30}H_{27}NO_6$ (M^+): 497; found: 497 (26), 466 (4, M-OCH_3 $^+$), 390 (45, M - C_6H_4-OCH_3 $^+$), 375 (24, M - O_2N-C_6H_4 $^+$), 227 (100, M - O_2N-C_6H_4-OOC-CH_2-CH_2-C_6H_4 $^+$).

p-Nitrophenyl-3-{4-[bis-(4-methoxyphenyl)-hydroxymethyl]-phenyl}-propionate (14)

Compound **13** (29.1 g, 58.5 mmol) is dissolved in 670 ml glacial acetic acid and freshly prepared lead dioxide[25] (9.95 g, 41.6 mmol) was added and the mixture placed in a preheated oil bath until a clear solution is obtained. Another 9.95 g (41.6 mmol) lead dioxide is added and dissolved. The reaction is monitored by thin layer chromatography (dichloromethane/ methanol 99/1, v/v). During the reaction a side product appears which travels between the educt **13** and product **14**. The reaction was terminated when UV intensity of the residual **13** spot equals the side product. The reaction mixture is poured on ice/ water (3 l) and extracted with dichloromethane. The organic layer is extracted with water and dried with Na_2SO_4. Solvents are evaporated and some remaining acetic acid removed by co-evaporation with toluene. The raw product (29.8 g) is dissolved in dichloromethane and purified by silica gel 60H (Merck, Darmstadt; No.7736, 1200 g) column

chromatography; elution is carried out in the presence of 0.03% pyridine using a step gradient from dichloromethane to dichloromethane / ethanol 99/1, v/v). Fractions containing compound **14** are combined and the solvents evaporated. The sirupous residue gradually crystallizes under a petroleum ether layer after rubbing with a glass rod. Yield: 15.1 g (50%). 2.15 g (7%) of the starting material **13** are recovered. - *^1H NMR* (250 MHz, $CDCl_3$): δ = 2.70 (s, 1H, R_3C-OH), 2.94 (t, 2H, -$\underline{C}H_2$-CH_2-), 3.1 (t, 2H, -CH_2-$\underline{C}H_2$-), 3.78 (s, 6H, -$OC\underline{H}_3$), 7.28-6.78 (m, 14H, aryl-$\underline{H}$), 8.21 (d, 2H, O_2N-aryl-$\underline{H}$, ortho). - *^{13}C NMR* (63 MHz, $CDCl_3$): δ = 30.37 (t, -$\underline{C}H_2$-CH_2-), 35.8 (t, -CH_2-$\underline{C}H_2$-), 55.26 (q, aryl-$O\underline{C}H_3$), 81.27 ($R_3\underline{C}$-OH), 113.22, 122.41, 125.17, 127.87, 128.08, 129.08, (d, $\underline{C}$-H, aryl), 138.35, 139.41, 145.32, 145.85, 155.32 (s, aryl, quarternary), 158.7 (s, $R_2\underline{C}$-OCH_3, aryl), 170.45 (s, -$\underline{C}$OOR). - *MS* (EI): m/z (rel. intensity): m/z calculated for $C_{30}H_{27}NO_7$ (M^+): 513; found: 513 (22), 496 (9, M - OH $^+$), 406 (12, M - C_6H_4-OCH_3 $^+$), 243 (93, M - O_2N-C_6H_4-OOC-CH_2-CH_2-C_6H_4 $^+$), 135 (100). - *MS* (FAB, pos. mode): m/z (rel. intensity): m/z calculated for $C_{30}H_{27}NO_7$ (M^+): 513; found: 513 (19), 496 (90, M - OH $^+$), 406 (16, M - C_6H_4-OCH_3 $^+$), 391 (11, M - O_2N-C_6H_4), 375 (3, M - O_2N-C_6H_4-O-), 243 (52, M - O_2N-C_6H_4-OOC-CH_2-CH_2-C_6H_4 $^+$), 135 (100). - *Elementary Analysis* (%): Found: C, 70.47/ 70.78; H, 5.35/ 5.38; N, 2.75/ 2.74; $C_{30}H_{27}NO_7$ requires C, 70.16; H, 5.3; N, 2.73.

p-Nitrophenyl-3-{4-[bis-(4-methoxyphenyl)-chlormethyl]-phenyl}-propionate (15)

Compound **14** (0.600 g, 1.17 mmol) is refluxed in acetyl chloride (6 ml) for 3 h. Solvents are evaporated and remaining traces of acetic acid or/ and acetyl chloride removed by co-evaporation with toluene. The residue is directly converted to compound **16**.

Alcoholysis of compound 15 with 6a to compound 16

The sirupous raw product of compound **15** (max. 1.17 mmol) is dissolved in dry pyridine (4.1 ml) and compound **6a** (0.450 g, 0.653 mmol) is added. After 19 h another 0.140 g (0.203 mmol) **6a** is added. 24 h later monitoring by thin layer chromatography indicates no further conversion. Pyridine (3 ml) and ethanol (0.3 ml) are added and the solution poured into an excess of water after 5 min. The water solution is extracted with ethyl acetate. The organic phase is extracted with water and dried with Na_2SO_4. Solvents are evaporated and the residue azeotropically dried with toluene. The raw product (1.38 g) is dissolved in dichloromethane and purified by silica gel 60 (Merck, Darmstadt; No. 9385, 80 g) column chromatography; elution is carried out in presence of 0.1% pyridine with dichloromethane/ ethanol 99/1, v/v). Fractions containing **16** are combined and the solvents evaporated. The residue is dissolved in dichloromethane (11 ml) and precipitated into hexane (220 ml). Yield: 0.468 g (46%). - *^1H NMR* (400 MHz, $CDCl_3$ and a trace of [D_5]pyridine): δ = 1.36 (s, 3H, -$C\underline{H}_3$ of thymine), 2.5-2.4 (m, 2H, H2'^{a}/H2'^{b}), 2.63 (t, 2H, -$\underline{C}H_2$-CH_2-, 3'-OH protecting group), 2.9 (t, 2H, -CH_2-$C\underline{H}_2$-, 3'-OH protecting group), 2.9 (t, 2H, -$\underline{C}H_2$-CH_2-, 5'-OH protecting group), 3.04 (t, 2H, -CH_2-$C\underline{H}_2$-, 5'-OH protecting group), 3.04 (s, 3H, R_3C-$OC\underline{H}_3$), 3.45 (m, 2H, H5'^{a}/H5'^{b}), 3.77 (s,12H, aryl-$OC\underline{H}_3$), 4.14 (m, 1H, H4'), 4.54 (s, 2H, -CO-$C\underline{H}_2$-O-), 5.47 (m,1H, H3'), 6.43 (t, 1H, H1'), 7.37-6.7 (m, 26H, aryl-$\underline{H}$), 7.6 (s, 1H, H6), 8.21 (d, 2H, O_2N-aryl-$\underline{H}$, ortho), 8.9 (s, 1H, N-$\underline{H}$ of thymine). - *^{13}C NMR* (101 MHz, $CDCl_3$ and a trace of [D_5]pyridine): δ = 11.66 (q, -$\underline{C}H_3$ of thymine), 27.4 (t, -$\underline{C}H_2$-CH_2-, 3'-OH protecting group), 30.26 (t, 2H, -$\underline{C}H_2$-CH_2-, 5'-OH protecting group), 33.77 (t, 2H, -CH_2-$\underline{C}H_2$-, 3'-OH protecting group), 35.68 (t, 2H, -CH_2-$\underline{C}H_2$-, 5'-OH protecting group), 37.88 (t, C2'), 51.90 (s, 3H, R_3C-$O\underline{C}H_3$), 55.21, 55.27 (q, aryl-$O\underline{C}H_3$, 3'-OH and 5'-OH protecting group, position not defined), 63.74 (t, C5'), 72.74 (t, -CO-$\underline{C}H_2$-O-), 75.69 (d, C3'), 83.96 (d, C4'), 84.38 (d, C1'), 86.29, 87.05 (s, $R_3\underline{C}$-OCH_2-, 5'-OH protecting group, $R_3\underline{C}$-OCH_3, 3'-OH protecting group, position not defined), 111.49 (s, C5 of

thymine), 112.29, 113.09, 113.37, 114.59, 121.81, 122.37, 128.07, 128.38, 128.93, 130.08, 130.18 (d, C-H, aryl), 125.17 (d, C-H, O_2N-aryl, ortho), 135.61 (d, C6 of thymine), 135.06, 135.4, 138.55, 142.89, 145.33, 147.57, 155.28 (s, aryl, quarternary), 150.37 (C2 of thymine), 157.3, 158.51 (s, R_2C-OCH_3, and s, R_2C-O-CH_2-CO-: 3'-OH protecting group, aryl, position not defined), 158.84 (s, R_2C-OCH_3, aryl, 5'-OH protecting group), 163.57 (s, C4 of thymine), 170.35 (s, -COOR, 5'-OH protecting group), 172.07 (s, -COOR, 3'-OH protecting group), 205.94 (s, -CO-). - *^1H ^1H and ^1H ^{13}C 2D NMR* spectra are determined (data not shown). - *MS* (FAB, pos. mode): m/z (rel. intensity): m/z calculated for $C_{67}H_{65}N_3O_{17}$ (M^+): 1183; found: 1183 (4), 1152 (35, M - OCH_3 $^+$), 1137 (2, M - NO_2 $^+$), 496 (100, fragment M - OH $^+$ of compound **14**). - *Elementary Analysis* (%): Found: C, 67.63/ 67.89; H, 5.63/ 5.69; N, 3.49/ 3.51; $C_{67}H_{65}N_3O_{17}$ requires C, 67.95; H, 5.53; N, 3.55.

Aminolysis of compound 16 with aminopropyl CPG to building block 1

Compound **16** (0.160 g, 0.135 mmol) is dissolved in dry dioxane (0.311 ml) and dry pyridine (0.032 ml). A suspension of aminopropyl CPG (0.405 g, CPG-10-500, Biosyntech, Hamburg) in 1.27 ml dry N,N-dimethylformamide and 0.160 ml (0.116 g, 1.15 mmol) dry triethylamine is added and the suspension shaken during 21.5 h. An intensive yellow colour indicates beginning reaction caused by released p-nitrophenolate ions. The suspension is shaken during 21.5 h. A ninhydrin test at this stage indicates the existence of free amino groups on the support. To acylate, "cap", these groups, dry triethylamine (0.030 ml) and acetic anhydride (0.090 ml) are added and the suspension is shaken for another 60 min. After this time a negative ninhydrin test is obtained. The support is washed successively with N,N-dimethylformamide, ethanol, dioxane, ether (100 ml each) and dried *in vacuo*. Analysis for the extent of 3'-OH protected nucleoside attached to the support is done spectrophotometrically. An accurately weighed sample is treated either with 5% dichloroacetic acid in dichloromethane (v/v) or with hydrazine reagent IV (table 1) followed by acidifing the solution with 40% trichloroacetic acid in dichloromethane (percentage by weight). The liquid phase is measured at 513 nm (extinction coefficient of an acid solution of the removed trityl derivate: ε = 78600). Amount of nucleoside bound to the support **1**: 45.6 µmol/ g.

Syntheses of the fully deprotected oligonucleotides d(TTTT) and d(TAGCT)

The apparatus for manual oligonucleotide synthesis consists in of column type reactor fitted with a sintered glass fritt, a stopcock and a connection to a vacuum pump to remove solvents by suction or to dry the support just before the condensation step (step 3, table 2). Only this step is carried out under inert gas athmosphere (argon). The inert gas is introduced to the apparatus *via* an injection needle through a septum at the top of the apparatus. Another needle through the septum guarantees equalizing of the gas pressure.

Notes and descriptions of the reagents and solvents:

1) For synthesis 0.0220 g of the support **1** with about 1 µmol loaded nucleoside are used.

2) For the synthesis of d(TTTT) 0.146 g amidite **2a** is dissolved in 1.4 ml acetonitrile (DNA grade). For the synthesis of d(TAGCT) 0.0800 g of **2a-d** each is dissolved in 0.8 ml each of acetonitrile (DNA grade).

3) Tetrazole reagent: 31.8 g 1-H-tetrazole in 1 l acetonitrile; GEN 905035.

4) Oxidation reagent: 4.3 g iodine in 1 l water/ pyridine/ tetrahydrofuran (THF), 9.05/ 0.41/ 90.54, v/v; GEN 905028.

5) Capping reagent 1: N-methylimidazole/ pyridine/ acetonitrile, 12/ 10/ 78, v/v, GEN 905027; capping reagent 2: acetic anhydride/ acetonitrile, 12/ 88, v/v, GEN 905026. The tetrazole, oxidation and the capping reagents are purchased from PerSeptive Biosystems GmbH, Hamburg.

6) Hydrazine reagent: 0.5M hydrazine reagent IVb (table 1).Reagent of high quality have to be used: bidestilled water, : acetic acid p.a. (Merck, Darmstadt No. 63), hydrazinium hydrate (Merck, Darmstadt No. 804608), pyridine p.a. (Merck, Darmstadt No. 7463).

7) TCA reagent: 40% trichloroacetic acid in dichloromethane (percentage by weight).

8) Amidite and tetrazole solutions and acetonitrile (DNA grade) to dissolve amidites and to carry out the last washing in step 2 (table 2) before the condensation are kept under molecular sieve 0.3 nm, freshly activated in a microwave oven, stored under argon and taken or added by syringes *via* septa.

9) Acetonitrile for washing steps only has to be "HPLC grade", except for the last washing before the condensation.

Table 2. Steps involved in one elongation cycle during synthesis.

Step	Operation	Reagent	Volume (ml)	Duration (min)
1	Delaevulination	Hydrazine reagent	0.7	60
2	Washing	N,N-Dimethylformamide	2 x 5	
		Acetonitrile	2 x 5[a)]	
		Acetonitrile (DNA grade)	1 x 5	
3	Drying	High vacuum		10
		(then flushing with argon)		
4	Condensation	a) Amidite solution	0.4	1[b)]
			0.8	3[c)]
		b) Tetrazole solution	0.8[d)]	9[b)]
			1.6[d)]	10[c)]
5	Washing	Acetonitrile	2 x 5	
6	Oxidation	Oxidation reagent	1.65	1
7	Washing	Acetonitrile	2 x 5	
8	Capping	Capping reagent 1	1.25	1
		Capping reagent 2	1.25	
9	Washing	Acetonitrile	2 x 5	
10	Drying	High vacuum		some min

a) before washing with acetonitrile (DNA grade) insert septum, b) in case of d(TTTT) synthesis, c) in case of d(TAGCT) synthesis d) add dropwise

Sufficent contact between support and solvent or reagent is guaranteed by occasionally gentle shaking, especially after addition of amidite solution and during the dropwise addition of the tetrazole reagent.

1/3 of the solution of step 1 (0.233 ml) is given in a 25 ml standard flask and filled up to the mark with the TCA reagent. The absorptions of the solutions of the elongation cycles are measured spectrophotometrically at 513 nm leading to the nucleoside loading of support **1** and to the yields of the condensation reactions.

Deprotection and purifications of the oligomers

A) d(TTTT) synthesis:

The support with the attached oligomer is washed with pyridine and the ß-cyanoethyl groups are removed with *tert*-butyl amine reagent II (table 1). After washing the support with pyridine and acetonitrile and drying *in vacuo*, the oligomer is removed from the support by treating it with 80% acetic acid for 15 min. After lyophilisation of the solution, the oligomer is purified by HPLC: the terminal 3'-OH protecting group (corresponding to the group of compound **5a** in scheme 3) serves here as purification handle. Treatment with 32% ammonia followed by lyophilisation leads to the fully deprotected oligomer d(TTTT). - *HPLC*: Ret. time (min): 8.57, *UV* detection: λ_{max} = 266.1 and 217.7 nm. - *MS* (MALDI-TOF): theoretical mass: M+H $^{+}$: 1155; found: 1154.

B) d(TAGCT) synthesis:

The oligomer is removed from the support by treatment with 80% acetic acid for 15 min. After lyophilisation, the base, the phosphate and the 3'OH protection are removed by treatment with 0.5M DBU in acetonitrile leading directly to the fully deprotected d(TAGCT). The reagent is evaporated *in vacuo*. - *HPLC*: Ret. time (min): 6.96, *UV* detection: λ_{max} = 259.0 and 216.5 nm. - *MS* (MALDI-TOF): M+H $^{+}$: theoretical mass: 1478; found: 1477.

Deprotection experiments with model compounds

The deprotection experiments with model compounds in solution were monitored by thin layer chromatography. The molar ratios of the deprotection reagents I-IV (table 1) to the model compounds were at least 100 : 1.

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