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Amide group assisted 3′-dephosphorylation of oligonucleotides synthesized on universal A-supports

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Abstract—(±)-3-Amino-1-(4,4′-dimethoxytriphenylmethyl)-2-propanediol was attached to succinylated alkylamino-controlled pore glass via the second amide bond. The resulting solid phase was acylated to give seven new universal solid supports, compatible with the preparation of all common types of oligodeoxyribonucleotides. These resins allow for fast elimination of the 3′-terminal phosphodiester or phosphorothioate function by ammonia in methanol at room temperature.

1. Introduction

A universal solid support, permitting the direct coupling of any nucleoside residue and the straightforward elimination of the terminal phosphodiester linkage at the same time as the deprotection step, would help eliminate the possibility of errors in parallel synthesis applications where up to 192 wells may contain different supports. Such an approach would eliminate the need of several supports for oligonucleotide synthesis and simplify the preparation of oligonucleotides having modified nucleosides at the 3′-terminus, or oligonucleotide mixtures with varying nucleosides at the 3′-termini.

McLean and coworkers[1] have described the solid support **U-1** (Scheme 1) for universal applications. This solid support allows for the detritylation, the addition of the first nucleoside monomer and the remainder of the oligomer preparation to proceed without changes to standard protocols. The elimination of the terminal phosphodiester group utilizes the same reagents, which are needed for the routine deprotection of oligonucleotides, but requires very aggressive and lengthy conditions (e.g. concentrated ammonium hydroxide/80°C/17 h, compared to the standard oligomer deprotection conditions of ammonium hydroxide/55°C/5–6 h). More recently Lyttle and coworkers[2] proposed a uridine-based linker immobilized onto polystyrene particles via a phosphotriester group (**U-2**) as an alternative to **U-1**. The support **U-2** gives oligonucleotide cleavage with aqueous ammonia in about 8 h at 60°C. Although all conditions mentioned above are suitable for the preparation of unmodified oligonucleotides, they are not compatible with base-labile nucleoside analogues. Furthermore, prolonged treatment with basic volatile reagents, or the need to employ a desalting step with sodium hydroxide, makes this solid support unattractive in industrial high output multi-well synthesizers.

Azhayev[3,4] reported on the new universal solid supports **U-3**–**U-5** (Scheme 1), which are not only compatible with the preparation of all common types of oligonucleotides but also function well for oligomers with unusual base labile units. These supports allow for a faster elimination of the 3′-terminal phosphodiester function (concentrated ammonium hydroxide/80°C/2–8 h) when compared with the earlier reported universal support **U-1**. In addition, these phases permit the use of neutral conditions for the terminal dephosphorylation with aqueous zinc chloride or even water. Apparently supports **U-3**–**U-5** appear to be more attractive than supports **U-1** and **U-2**. Nevertheless, they also require prolonged treatment with basic or neutral reagents at elevated temperatures. In addition, multistep preparations with supports **U-2**–**U-5** result in relatively high production costs. These characteristics also prevent these solid phases from being ideal for general industrial application.

Thus, the new universal supports, which lack the limitations of **U-1**–**U-5** remain important. We now report on new universal solid phases, which allow faster elimination of the 3′-terminal phosphodiester group in methanolic ammonia solutions at room temperature.

2. Results and discussion

Azhayev[3,4] demonstrated that aminomethyl- and diaminoethyl- substituents are capable of facilitating cleavage of a phosphodiester group linked to a cis-diol-containing bridge on a universal support. Thus, the idea of neighboring group

Keywords: oligonucleotides; amide group; solid supports.

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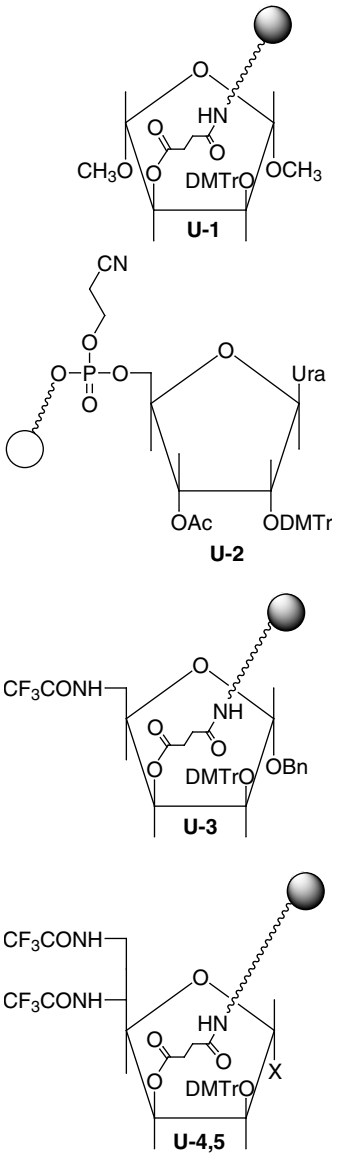
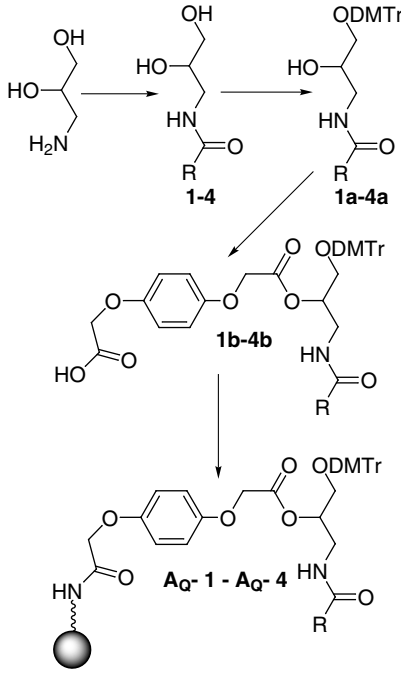


Scheme 1. U-4: X=–OBn; **U-5**: X=H; DMTr=4,4′-dimethoxytriphenylmethyl; ●〰=lcaaCPG; ○〰=polystyrene beads.

assistance for elimination of the terminal phosphodiester to generate termini with 3′-hydroxyls seems to be worthwhile.

Amides of carboxylic acids may be regarded as weak N–H acids.[5] Amide groups will give rise to amide anions in, for example methanolic ammonia or ammonium hydroxide. These amide anions, in turn, display basic properties. Therefore, it seemed interesting to see if a vicinal amide group of 3-acylamido-1,2-propanediol tether would enhance the elimination of a terminal phosphodiester in a basic solution, along with a linker originally on the solid support.

We report the preparation of four solid supports, $\mathbf{A_Q}$**-1**–$\mathbf{A_Q}$**-4** (Scheme 2) in which a (±)-3-acylamido-1-(4,4′-dimethoxytriphenylmethyl)-2-propanediol is attached to a long chain alkylamino controlled pore glass (lcaaCPG) via a labile hydroquinone-*O*,*O*′-diacetyl linker.[6] The starting (±)-3-amino-1,2-propanediol was first acylated to give the 3-acylamido derivatives **1**–**4**, and then converted to their dimethoxytrityl derivatives **1a**–**4a** in good yield. The

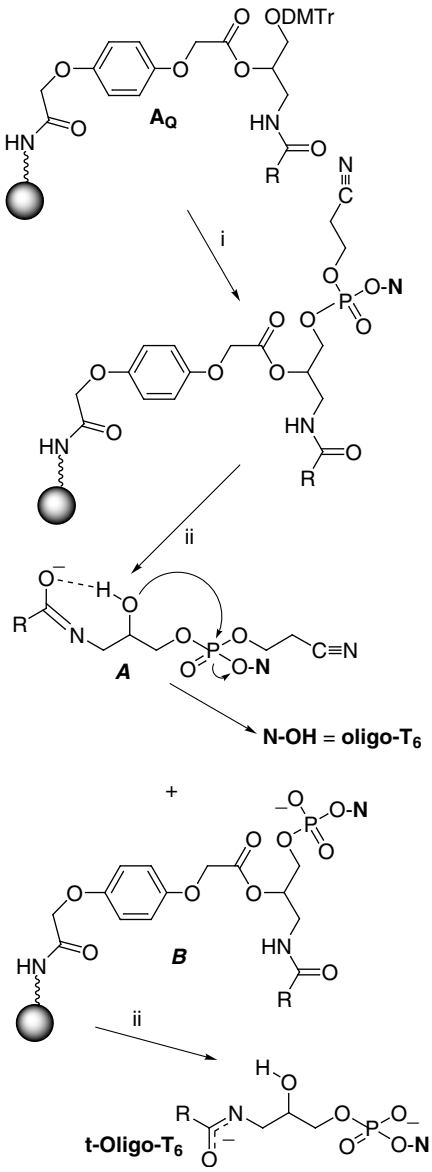


Scheme 2. 1, **1a**, **1b**, $\mathbf{A_Q}$**-1**: R=CF_3– **2**, **2a**, **2b**, $\mathbf{A_Q}$**-2**: R = CH_3– **3**, **3a**, **3b**, $\mathbf{A_Q}$**-3**: R=C_6H_5– **4**, **4a**, **4b**, $\mathbf{A_Q}$**-4**: R=$C(CH_3)_3$–.

2-hydroxy groups of **1a**–**4a** were acylated with hydroquinone-*O*,*O*′-diacetic acid and *N*,*N*′-diisopropylcarbodiimide to give compounds **1b**–**4b**. Finally, derivatives **1b**–**4b** were linked to a lcaaCPG, employing a *N*,*N*′-diisopropylcarbodiimide/*N*-hydroxybenzotriazole condensation. The resulting solid supports $\mathbf{A_Q}$**-1**–$\mathbf{A_Q}$**-4**, contained 40–100 μmol of DMTr-groups per gram of CPG.[7]

In order to investigate the usefulness of $\mathbf{A_Q}$ solid supports in the preparation of oligodeoxynucleotides, the short sequence oligo-T_6 was assembled on a synthesizer, using standard phosphoramidite chemistry and recommended protocols. No differences in coupling efficiency (>98% as determined by trityl assay) were detected between $\mathbf{A_Q}$-supports and the commercial **T**-derivatized support, which is consistent with the compatibility of $\mathbf{A_Q}$-supports with standard assembly steps. After completion of the synthesis, the addition of ammonia in methanol rapidly cleaved the hydroquinone-*O*,*O*′-diacetate linker and released a hexamer having a cyanoethyl-protected phosphotriester function linked to the 1-hydroxyl of (±)-3-acylamido-1,2-propanediol (Scheme 3, Structure ***A***). During subsequent treatment with ammonia, the acylamido fragment (as the acylamide anion) assists the vicinal 2-hydroxyl group in attacking the phosphorus and finally gives the desired oligo-T_6. At the same time, a fraction of phosphodiester (Scheme 3, Structure ***B***) was formed due to β-elimination of the cyanoethylene prior to formation of the deacylated phosphotriester (Scheme 3, Structure ***A***). This side reaction finally leads to a stable 3′-tethered oligomer *t*-oligo-T_6 (Scheme 3). Investigation of the properties of $\mathbf{A_Q}$-supports under various reaction conditions is reported hereafter.

The assembled sequences were first cleaved from $\mathbf{A_Q}$ supports with ammonia in organic solvents and the extent of dephosphorylation and cleavage of the terminal tether



Scheme 3. (i) oligonucleotide synthesis; (ii) NH_3/MeOH.

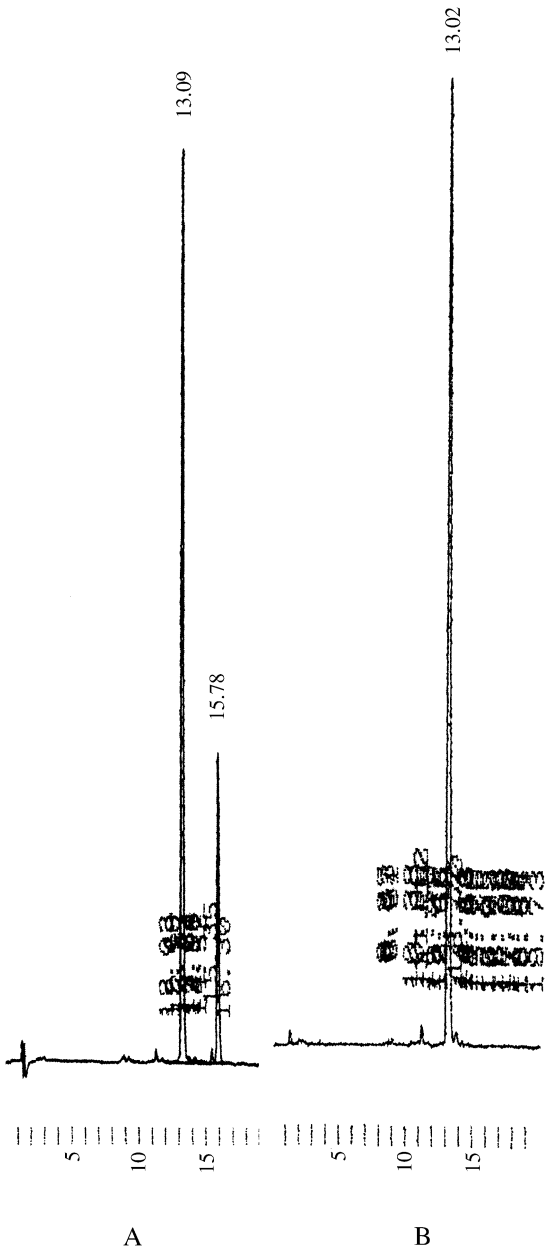


Figure 1. Ion exchange HPLC traces of oligo-T_6 synthesized on **T**-bound support and $\mathbf{A_Q}$-supports. A: oligo-T_6 synthesized on $\mathbf{A_Q}$**-3**-support and treated with 9 M NH_3/MeOH–dioxane (1:1), 1 h at 20°C; B: oligo-T_6 synthesized on **T**-bound support and treated with 32% ammonium hydroxide, 2 h at 20°C. Chromatograms of oligo-T_6 synthesized on $\mathbf{A_Q}$**-1**-, $\mathbf{A_Q}$**-2**- and $\mathbf{A_Q}$**-4**-supports were analogous to that shown for the $\mathbf{A_Q}$**-3**- support (data not shown). Time is in minutes.

was investigated, using ion exchange HPLC (Fig. 1, panel A). The effect of ammonium hydroxide at 20°C for 2 h on the cleavage from **T**-bound support is shown in Fig. 1, panel B for comparison. In each of the traces shown in Fig. 1, the product, with a retention time of 13 min, corresponds to oligo-T_6, as demonstrated by an authentic standard. The later eluting product in Fig. 1, panel A is the hexamer having a terminal phosphodiester linked to the 3-acylamido-1,2-propanediol tether—*t*-oligo-T_6. derived from supports $\mathbf{A_Q}$**-1**–$\mathbf{A_Q}$**-4** (Scheme 3; Fig. 1). All four *t*-oligo-T_6 oligomers derived from supports $\mathbf{A_Q}$**-1**–$\mathbf{A_Q}$**-4** were isolated by HPLC and their structure was confirmed by electrospray ionization mass spectrometry[8] (data not shown).

The results of these studies under different conditions and at different temperatures are summarized in Table 1. The best yields of oligo-T_6 (75–88%) were achieved with $\mathbf{A_Q}$**-1** solid support, by incorporating trifluoroacetamido function and, more importantly, using 2–9 M ammonia in methanol–dioxane (or methanol–toluene, data not shown) for cleavage/dephosphorylation. It is noteworthy to mention that with ammonium hydroxide cleavage/dephosphorylation, only 43% of the oligo-T_6 was obtained with support $\mathbf{A_Q}$**-1**. Apparently, the presence of water facilitates the competing reaction (Scheme 3, Structure ***B***), making the use of aqueous ammonia for oligonucleotide cleavage/deprotection less efficient.

As follows from the experiments described above, the structure of the acylamido function incorporated onto the $\mathbf{A_Q}$ support did not significantly influence the yield of the desired oligo-T_6. Apparently, reactions with all of the

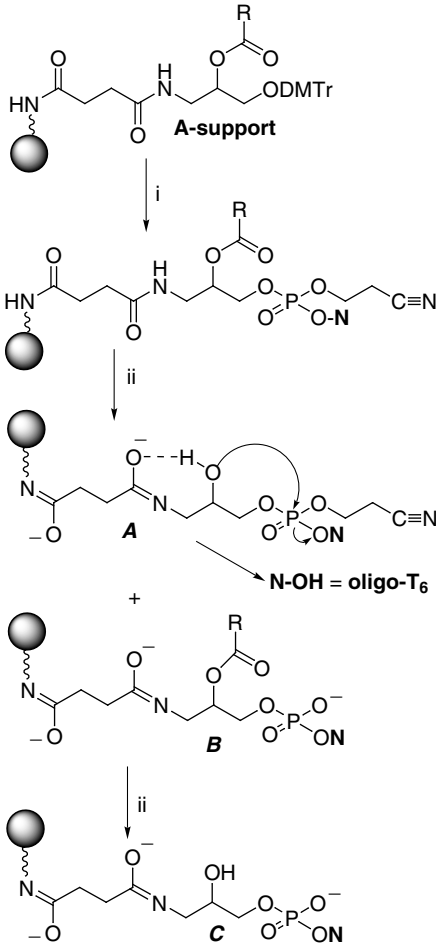
Table 1. Yield of oligo-T_6 derived from the universal solid supports $\mathbf{A_Q}$ under various cleavage/elimination conditions

Support	Dephosphorylation/cleavage procedure[a]	Purity (%)[b]
$\mathbf{A_Q}$-1	I	43
$\mathbf{A_Q}$-1	II	82
$\mathbf{A_Q}$-1	III	85
$\mathbf{A_Q}$-1	IV	87
$\mathbf{A_Q}$-1	V	88
$\mathbf{A_Q}$-2	III	75
$\mathbf{A_Q}$-2	IV	79
$\mathbf{A_Q}$-3	III	79
$\mathbf{A_Q}$-3	IV	83
$\mathbf{A_Q}$-4	IV	79

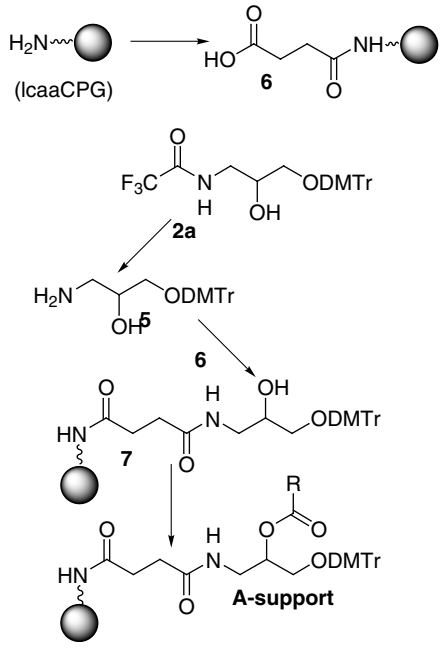
[a] Procedure I: 33% NH_3/H_2O, 20°C, 2h; procedure II: 9 M NH_3/MeOH–dioxane (1:1), 20°C, 12 h; procedure III: 9 M NH_3/MeOH–dioxane (1:1), 4°C, 1 h or 20°C, 1 h; procedure IV: 9 M NH_3/MeOH–dioxane (1:1), −20°C, 1 h or 20°C, 1 h; procedure V: 2 M NH_3/MeOH–dioxane (1:1), 4°C, 1 h or 20°C, 1 h.

[b] Purity was assessed using IE HPLC by integrating peaks at 260 nm.

acylamides reported here resulted in anions capable of assisting the vicinal 2-hydroxyl group in attacking the phosphorus (Scheme 3, Structure ***A***). On the other hand, the structure of the linker at the 2-hydroxy group appears to be critical. The cleavage of the hydroquinone-*O*, *O′*-diacetate group proceeds faster than β-elimination of



Scheme 4. (i) oligonucleotide synthesis; (ii): NH_3/MeOH.



Scheme 5. $\mathbf{A_f}$: R=H–; $\mathbf{A_p}$: R=$C_6H_5OCH_2$–; $\mathbf{A_{ac}}$: R=CH_3–; $\mathbf{A_{mac}}$: R=CH_3OCH_2–; $\mathbf{A_{mpac}}$: R=*p*-$CH_3OC_6H_4$–; $\mathbf{A_{dcac}}$: R=Cl_2CH–, $\mathbf{A_{tcac}}$: R=Cl_3C–.

the protecting group on the phosphorous. Therefore, attack on the phosphotriester function by the 2-hydroxyl group of the 3-acylamido-1,2-propanediol tether takes place, and the desired oligonucleotide is 3′-dephosphorylated.

In a basic solution, the diamide of dicarboxylic acid (e.g. succinic acid), linking lcaaCPG and the 3-amino-1,2-propanediol tether, should also be capable of assisting a similar attack of the 2-hydroxyl group on the phosphorous (Scheme 4, Structure ***A***), provided that this 2-hydroxy function of the tether is instantly deproptected with ammonia prior to phosphate deprotection. This attack should lead to the elimination of the desired oligonucleotide oligo-T_6. Interestingly, the competing reaction should lead to the formation of a lcaaCPG bounded oligonucleotide with the phosphodiester group at the 3′-terminus (Scheme 4, Structure ***B***). This intermediate should ultimately lead to a stable phosphodiester linked to the solid-phase (Scheme 4, Structure ***C***). Taking this kind of reasoning into consideration, we designed and synthesized several new universal **A**-supports (Scheme 5). In these structures the succinyl group bridged the lcaaCPG and 3-amino-1-(4,4′-dimethoxytriphenylmethyl)-2-propanediol via two amide bonds and the 2-hydroxyl function was acylated.

The preparation of **A** supports appeared to be straightforward (Scheme 5). Initially lcaaCPG was succinylated and capped to give succinylamido-CPG (**6**). (±)-3-Trifluoroacetamido-1-(4,4′-dimethoxytriphenylmethyl)-2-propanediol (**2a**) was converted to the corresponding amine **5**, which, in turn, was attached to **6** in the presence of *N*,*N′*-diisopropylcarbodiimide and *N*-hydroxybenzotriazole to give solid phase **7**.

Finally the free hydroxyl group on compound **7** was either acetylated to give $\mathbf{A_{ac}}$, phenoxyacetylated to give $\mathbf{A_{pac}}$,

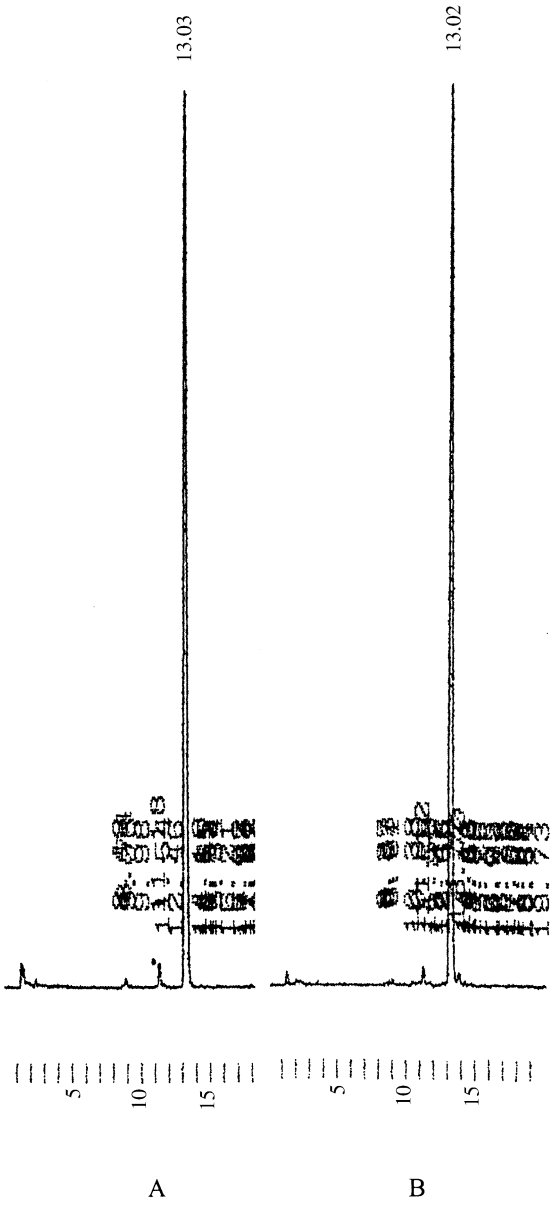


Figure 2. Ion exchange HPLC traces of oligo-T_6 synthesized on $\mathbf{A_f}$-support and **T**-bound support. A: oligo-T_6 synthesized on $\mathbf{A_f}$-support and treated with 2 M NH_3/MeOH, 1 h at 20°C followed by 32% ammonium hydroxide, 1 h at 20°C; B: oligo-T_6 synthesized on **T**-bound support and treated with 32% ammonium hydroxide, 2 h at 20°C. Time is in minutes.

methoxyacetylated to give $\mathbf{A_{mac}}$, 4-methoxyphenoxyacetylated to give $\mathbf{A_{mpac}}$, dichloroacetylated to give $\mathbf{A_{dcac}}$, trichloroacetylated to give $\mathbf{A_{tcac}}$ or formylated to give solid support $\mathbf{A_f}$. The resulting solid supports contained 70–100 μmol of DMTr-groups per gram of CPG.[7]

As in the case of $\mathbf{A_Q}$-supports, the short sequence oligo-T_6 was assembled on all five **A**-supports using standard phosphoramidite chemistry and recommended protocols. No differences in coupling efficiencies (>98% as determined from trityl assay) were detected between **A**-supports and the commercial **T**-bound solid support (**T**-CPG), which is consistent with the compatibility of **A**-supports and standard assembly steps.

We also investigated the properties of **A**-supports under various reaction conditions. The assembled oligo-T_6 sequence was cleaved from **A**-support with ammonia in methanol, and the extent of dephosphorylation/cleavage from the controlled pore glass was investigated by ion exchange HPLC (Fig. 2, panel A). The effect of ammonium hydroxide at 20°C for 2 h on cleavage from **T**-CPG is shown in Fig. 2, panel B, for comparison. In each of these chromatograms the product, with a retention time of about 13 min, corresponds to oligo-T_6, as demonstrated by an authentic standard.

Table 2. Relative yield of oligo-T6 derived from the universal solid supports **A** under various cleavage/elimination conditions

Support	Dephosphorylation/cleavage procedure[a]	Relative yield (%)[b]	Purity (%)[c]
T-CPG	I	100	97
$\mathbf{A_f}$	I	42	82[d]
$\mathbf{A_{ac}}$	II	4	74
$\mathbf{A_{pac}}$	II	48	95
$\mathbf{A_{mac}}$	II	24	94
$\mathbf{A_{mpac}}$	II	30	95
$\mathbf{A_{dcac}}$	II	68	96
$\mathbf{A_{tcac}}$	II	72	96
$\mathbf{A_f}$	II	76	96

[a] Procedure I: 33% NH_3/H_2O, 20°C, 2 h; procedure II: 2 M NH_3/MeOH, 20°C, 1 h or 33% NH_3/H_2O, 20°C, 1 h.
[b] Yield of oligo-T_6 resulting from the commercial **T**-support was taken as 100%. Relative yield of oligomer, resulting from **A**-support, was calculated relative to the yield from the **T** support.
[c] Purity was assessed using IE HPLC by integrating peaks at 260 nm.
[d] Along with 7.8% of an unidentified product.

The optimal conditions for recovering oligo-T_6 from **A**-supports was assessed from IE HPLC by peak integration, corresponding to oligo-T_6 (260 nm) derived from 1 μmol of initial solid support. Typically, 1/50 of the total amount of obtained oligomer was injected and the oligonucleotide peak area was compared to the corresponding area of oligo-T_6, resulting from commercial **T**-CPG. The results of these are summarized in Table 2.

It can be seen that after completion of the synthesis, addition of ammonia in methanol to all **A**-supports released the oligo-T_6 in solution (Scheme 4; Fig. 2; Table 2). As expected, the competing reaction most probably led to the formation of some lcaaCPG bounded oligomer with the phosphodiester group at the 3′-terminus (Scheme 4, Structure ***C***). This assumption is supported by the relative yield data given in Table 2. Thus, the only oligonucleotide product in the solution appeared to be the desired oligomer, along with small amounts of truncated sequences resulting from the non-quantitative coupling steps in the process of oligonucleotide assembly.

When comparing different **A**-supports, the $\mathbf{A_{ac}}$-support leads to only traces of oligo-T_6. This fact makes $\mathbf{A_{ac}}$-support not so useful for oligonucleotide synthesis. Also, $\mathbf{A_f}$-, $\mathbf{A_{tcac}}$- and $\mathbf{A_{dcac}}$-supports appear to be superior to the $\mathbf{A_{pac}}$-, $\mathbf{A_{mac}}$- and $\mathbf{A_{mpac}}$-, leading to about two times higher overall yield of the desired oligomer (65–75%). As in the case of $\mathbf{A_Q}$-supports, the ammonium hydroxide cleavage/dephosphorylation led to a significantly lower yield of oligo-T_6, compared to the methanolic ammonia treatment (Table 2).

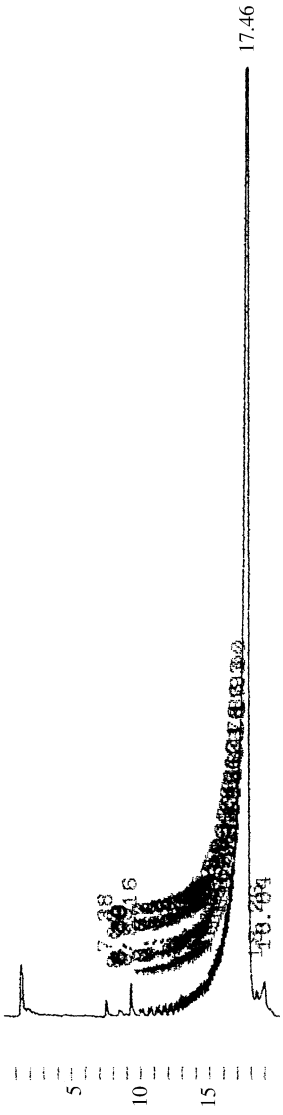


Figure 3. Ion exchange HPLC traces of oligo-T_{50} synthesized on $\mathbf{A_f}$-support and treated with 2 M NH_3/MeOH, 1 h at 20°C, followed by 32% ammonium hydroxide, 1 h at 20°C. Time is in minutes.

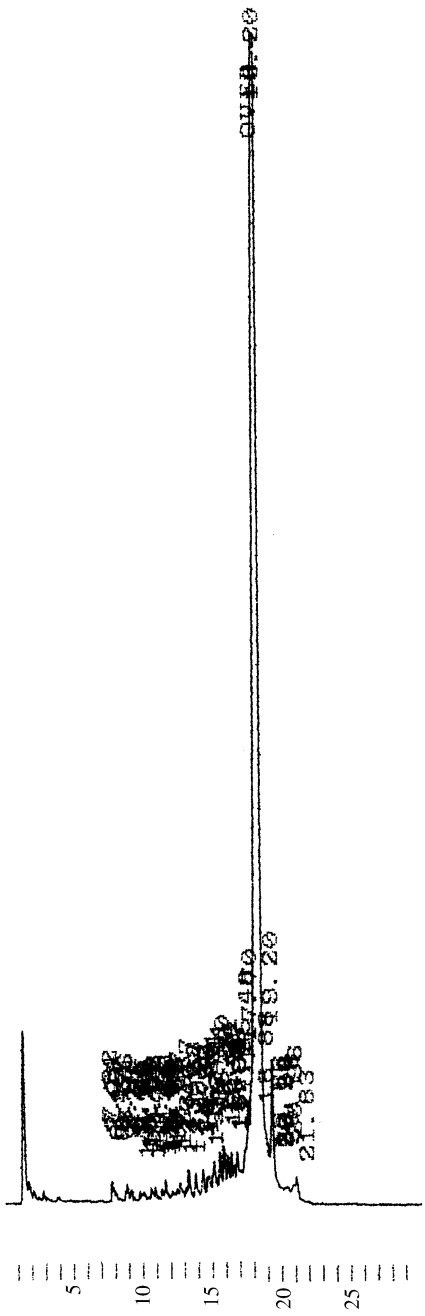


Figure 4. IE HPLC traces of 5′-GCGAAGCTTTGGAGAGTGGCAT-GAAGAAA-3′ synthesized on the $\mathbf{A_f}$-support and treated with 2 M NH_3/MeOH, 1 h at 20°C, followed by 32% ammonium hydroxide, 4 h at 55°C. Time is in minutes.

Similar experiments were carried out with a longer sequence oligo-T_{50}, which was assembled on $\mathbf{A_f}$- and $\mathbf{A_{pac}}$-supports and commercial **T**-CPG. Fig. 3 shows traces of a crude oligo-T_{50} sample, synthesized on $\mathbf{A_f}$-support, after treatment with 2 M NH_3/MeOH (20°C, 1 h); followed by the addition of an equal volume of 32% ammonium hydroxide (20°C, 1 h). The relative yields of oligo-T_{50}, synthesized on different solid supports, are summarized in Table 3.

Table 3. Relative yield of oligo-T_{50}, synthesized on different solid supports. The yield of oligo-T_{50} obtained from the **T** bound support was taken as 100%

Solid support	Relative yield[a] of oligo-T_{50} (%)	Purity (%)[b]
$\mathbf{A_f}$	48	62
$\mathbf{A_p}$	27	56
T	100	61

[a] Yield of oligo-T_{50} resulting from the commercial **T**-CPG support was taken as 100%. Yield of oligomer, resulting from **A**-support, was calculated relative to the yield from **T**-CPG.

[b] Purity was assessed using IE HPLC by integrating peaks at 260 nm.

The preliminary experiments with **A**-supports clearly demonstrated that they may be used as a universal solid phase, allowing for the fast and easy cleavage of sufficient amounts of oligothymidilates.

In order to investigate the applicability of **A**-supports for the preparation of oligodeoxyribonucleotides of mixed

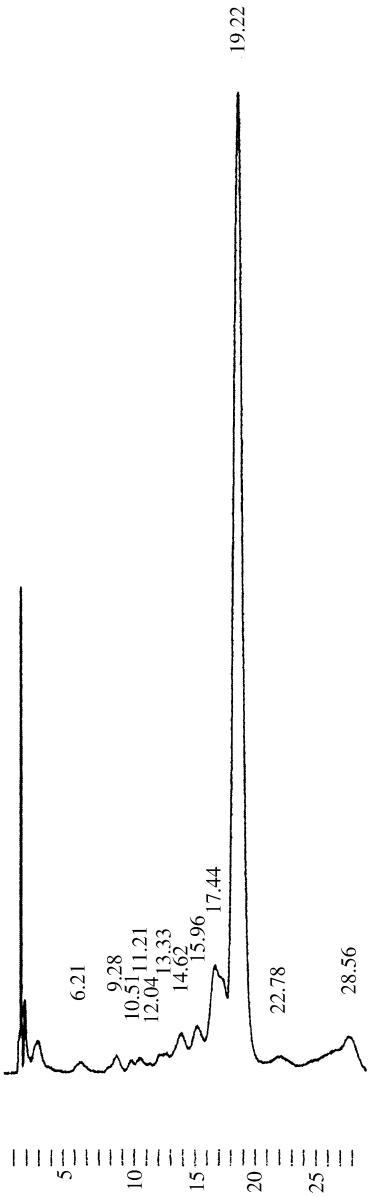


Figure 5. Ion exchange HPLC traces of 5′-TGGCGTCTTCCATTT-3′ phosphorothioate synthesized on the $\mathbf{A_f}$-support and treated with 2 M NH_3/MeOH, 1 h at 20°C, followed by 32% ammonium hydroxide, 4 h at 55°C. Time is in minutes.

sequences, seven longer oligonucleotides were synthesized on a 1 μmol scale:

5′-TGGCGTCTTCCATTT-3′ (15 mer),
5′-GTGGAATTCCAGCAGCAGAAAGAGCTCATC-3′ (30 mer),
5′-TAT GGATCC TCAGCTGCAAATGAGGG-3′ (26 mer),
5′-GTGGAATTCATGAAGAAAGAGATGATCATG-3′ (30 mer),
5′-TATGGTACCTCAGCCGTCCGTGCTGCTT-3′ (28 mer),
5′-GCGAAGCTTTGGAGAGTGGCATGAAGAAA-3′ (29 mer),
5′-TATGGATCCAACCATTCAACATGGTGGAC-3′ (29 mer).

Fig. 4 shows the typical analysis of crude 29 mer 5′-GCGAAGCTTTGGAGAGTGGCATGAAGAAA-3′ assembled on the $\mathbf{A_f}$-support and cleaved with 2 M ammonia in methanol (20°C, 1 h), and finally deprotected by adding an equal amount of 32% ammonium hydroxide and heating at 55°C for an additional 4 h. The structures of long oligomers were confirmed by electrospray ionization mass spectrometry[7] (data not shown) after chromatographic purification (compounds were assembled on a 1 μmol scale; yields were 50–75 OD_{260}) All oligonucleotides appeared to be good primers, and performed well in PCR experiments (data not shown).

The oligonucleotide phosphorothioate 5′-TGGCGTCTTCCATTT-3′ was synthesized on commercial a **T**-CPG support and support $\mathbf{A_f}$, following the recommended protocol. No differences in coupling efficiencies (>98% as determined from trityl assay) were detected between support $\mathbf{A_f}$ and the **T**-CPG. The support-bound oligonucleotide phosphorothioate was cleaved and deprotected as described above, and then analyzed by IE HPLC. Fig. 5 shows an analysis of crude oligonucleotide phosphorothioate prepared on $\mathbf{A_f}$-support. This oligomer was identical to the phosphoprothioate prepared on the **T**-bound solid phase.

3. Conclusion

In summary, we have developed new universal solid supports that are compatible for preparing common types of oligodeoxyribonucleotides. These resins allow for fast elimination of the 3′-terminal phosphodiester or phosphorothioate function with ammonia in methanol at room temperature. We believe that these inexpensive universal solid phases may become extremely useful for the preparation of oligonucleotides from both conventional and parallel industrial 96- or 192-well synthesizers.

4. Experimental

4.1. General

(±)-3-Amino-1,2-propanediol was purchased from Fluka, acetic anhydride, benzoyl chloride, pivaloyl chloride, 2,6-lutidine, succinic anhydride, trifluoroacetic acid, and solvents were from Aldrich, 4,4′-dimethoxytrityl chloride was purchased from ChemGenes, lcaaCPG was purchased from Sigma or Glen Research and hydroquinone-*O*,*O*′-diacetic acid was purchased from Lancaster. Reagents (solvents, activators, etc.) for oligonucleotide synthesis were purchased from Glen Research. Column flash chromatography was performed on Silica gel 60 (available from Merck). NMR spectra were recorded on a Bruker Avance DRX 500 spectrometer with tetramethylsilane as an internal standard (s=singlet; d=doublet; dd=doublet of doublets; m=multiplet; br.s=broad signal). All compounds bearing free hydroxy- and amino-groups were co-evaporated with CD_3OD before measurements. Electrospray ionization mass spectra (ESI MS) were aquired using a LCQ quadrupole ion trap mass spectrometer equipped with an electrospray ionization source (Finnigan MAT). IR spectra were taken with a Perkin–Elmer 983 spectrometer.

4.2. Oligodeoxyribonucleotide synthesis

The protected oligonucleotides were assembled on an Applied Biosystems 392 DNA Synthesizer using phosphoroamidite chemistry and recommended DMTr-off protocols for 40 nmol, 0.2 μmol and 1 μmol scales.

4.3. HPLC techniques

Oligonucleotides containing phosphodiester functions were analyzed by ion exchange HPLC (column: Dionex DNAPac PA-100, 4×250 mm; buffer A: 0.1 M NaAc in 20% MeCN pH 8, buffer B: 0.1 M NaAc and 0.4 M $NaClO_4$ in 20% MeCN pH 8; flow rate 1 ml min^{-1}; on a linear gradient from 0–15% B over 20 min for oligo-T_6 and modified oligo-T_6 oligonucleotides, and from 5 to 45% B over 30 min for longer oligomers). Phosphorothioate oligonucleotides were analyzed by ion exchange HPLC (column: Poly LP PolyWax 4.6×100 mm, 5 μm, 300 Å; buffer A: 0.05 M KH_2PO_4 in 50% formamide, pH 6.7; buffer B: 0.05 M KH_2PO_4 and 1.5 M NaBr in 50% formamide, pH 6.7; flow rate 1 ml min^{-1}; on a linear gradient from 5 to 50% B over 30 min).

4.3.1. (±)-3-Trifluoroacetamido-1,2-propanediol (1). (±)-3-Amino-1,2-propanediol (2.73 g, 30 mmol) was dissolved in methyl trifluoroacetate (30 ml, 300 mmol) and left overnight at 20°C. The reaction mixture was evaporated and dried by coevaporation with toluene (3×100 ml) to give 5.44 g (97%) of **1** as a colorless oil: ν_{max} (KBr) 3360–3120 (OH and NH), 2960 (CH aliph.), 1720 (C═O), 1570 (NH), 1440 (CH_2), 1350 (C–N), 1220–1060 (C–F and C–O); δ_H (500 MHz $CDCl_3$) 6.82 (br.s, 1H, N*H*), 3.92 (m, 1H, C*H*OH), 3.73 (m, 1H, C$H_a$$H_b$OH), 3.62 (m, 1H, C$H_a$$H_b$NH), 3.57 (m, 1H, CH$_a$$H_b$OH), 3.42 (m, 1H, CH$_a$$H_b$NH); ESI MS: found 187.0. $C_5H_8F_3NO_3$ requires 187.0; anal. calcd for $C_5H_8F_3NO_3$: C, 32.09; H, 4.31; N, 7.49%. Found: C, 31.89; H, 4.47; N, 7.40.

4.3.2. (±)-3-Acetamido-1,2-propanediol (2). (±)-3-Amino-1,2-propanediol (0.91 g, 10 mmol) was dissolved in 20 ml of dry pyridine, and acetic anhydride (4.7 ml, 50 mmol) was added. The mixture was left overnight at 20°C and evaporated. The oily residue was treated with 25% aqueous ammonia (30 ml) for 1 h at 20°C. After evaporation and drying by coevaporation with toluene (3×50 ml), compound **2** was used for 4,4′-dimethoxytritylation without further purification.

4.3.3. (±)-3-Benzoylamido-1,2-propanediol (3). Benzoyl chloride (0.7 ml, 6 mmol) in 1,4-dioxane (5 ml) was added dropwise to a cold (0°C) stirred solution of (±)-3-amino-1,2-propanediol (0.55 g, 6 mmol) in 1,4-dioxane–water (5:1; 20 ml). The reaction mixture was warmed to 20°C within 2 h, solvents were removed under vacuum and derivative **3** was isolated by flash chromatography (isocratic, 7% methanol in dichloromethane) as a colorless oil (0.83 g, 71%): ν_{max} (KBr) 3800–3010 (OH, NH and CH arom.), 2960 (CH aliph.), 1650 (C═O), 1580 (NH), 1540–1490 (CH_2 and C═C arom.), 1400 (C–N), 1260–1050 (C–O), 930–710 (CH arom.); δ_H (500 MHz $CDCl_3$) 10.05 (br.s, 1H, N*H*), 8.06–7.13 (m, 5H, Ph), 3.93 (m, 1H, C*H*OH), 3.60 (m, 4H, C$H_a$$H_bOH, CH_a$$H_bOH, CH_a$$H_bNH, CH_a$$H_b$NH); ESI MS: found 195,1, $C_{10}H_{13}NO_3$ requires 195.1; anal. calcd for $C_{10}H_{13}NO_3$: C, 61.53; H, 6.71; N, 7.18%. Found: C, 61.39; H, 6.83; N, 7.02.

4.3.4. (±)-3-Pivaloylamido-1,2-propanediol (4). Pivaloyl chloride (0.74 ml, 6 mmol) dissolved in 5 ml of 1,4-dioxane was added dropwise to a cold stirred solution (0°C) of (±)-3-amino-1,2-propanediol (0.55 g, 6 mmol) in 1,4-dioxane–water (5:1; 20 ml). The reaction mixture warmed to 20°C within 2 h, solvents were removed under vacuum and derivative **4** was isolated by flash chromatography (isocratic, 7% of methanol in dichloromethane) as a colorless oil (0.8 g, yield 77%): ν_{max} (KBr) 3360–3080 (OH and NH), 2960–2880 (CH aliph.), 1640 (C═O), 1540 (NH), 1480–1430 (CH_2), 1400 (C–N), 1370 (CH_3), 1220–1050 (C–O); δ_H (500 MHz $CDCl_3$) 6.96 (br.s, 1H, N*H*), 3.78 (m, 1H, C*H*OH), 3.67 (m, 1H, C$H_a$$H_b$OH), 3.47 (m, 1H, C$H_a$$H_b$NH), 3.40 (m, 1H, CH$_a$$H_b$OH), 3.40 (m, 1H, CH$_a$$H_b$NH), 1.20 (s, 9H, Me_3C); ESI MS: found 175.2, $C_8H_{17}NO_3$ requires 175.1; anal. calcd for $C_8H_{17}NO_3$: C, 54.84; H, 9.78; N, 7.99%. Found: C, 54.71; H, 9,91; N, 7.83.

4.4. (±)-3-Trifluoroacetamido-1-(4,4′-dimethoxytriphenylmethyl)-2-propanediol (1a), (±)-3-acetamido-1(4,4′-dimethoxytriphenylmethyl)-2-propanediol (2a), (±)-3-benzoylamido-1-(4,4′-dimethoxytriphenylmethyl)-2-propanediol (3a) and (±)-3-pivaloylamido-1-(4,4′-dimethoxytriphenylmethyl)-2-propanediol (4a). General procedure

4,4′-Dimethoxytrityl chloride (1.7 g, 5 mmol) was added to a 5 mmol stirred solution of propanediol (**1**, **2**, **3** or **4**) in dry pyridine (20 ml). The mixture was agitated overnight at 20°C and the reaction was stopped by the addition of 2 ml methanol. Solvents were removed by evaporation, and the residue was dissolved in 50 ml of ethylacetate, then washed with saturated sodium hydrocarbonate solution (3×30 ml) and water (2×30 ml). The organic solution was dried over Na_2SO_4 and evaporated to dryness. (±)-3-Acylamido-1-(4,4′-dimethoxytriphenylmethyl)-2-propanediols (**1a**, **2a**, **3a** and **4a**) were isolated by flash chromatography (step gradient from 0 to 2% methanol in dichloromethane in the presence of 0.1% of pyridine, for acetamido-1(4,4′-dimethoxytriphenylmethyl)-2-propanediol (**2a**) and from 0 to 5% methanol in dichloromethane in the presence of 0.1% pyridine) as pale yellow oils.

The yield of **1a** was 1.98 g (81%): ν_{max} (KBr) 3510 (OH), 3060–2840 (NH and CH aliph.), 1730 (C═O), 1610 (C═C arom.), 1580 (NH), 1460 and 1440 (CH_2), 1420 (C–N), 1310–1040 (C–O methoxy, C–F and C0–O), 950–710 (CH arom.); δ_H (500 MHz $CDCl_3$)8.52 (br.s, 1H, N*H*), 7.43–6.81 (m, 13H, arom.), 3.92 (m, C*H*OH), 3.79 (s, 6H, 2×C$H_3$$OC_6H_4$), 3.61 (m, 1H, C$H_a$$H_b$NH), 3.31 (dd, 1H, CH$_a$$H_b$NH), 3.25(dd, 1H, *J*=7.6, 9.7 Hz, C$H_a$$H_b$ODMTr), 3.15 (dd, *J*=7.6, 9.7 Hz, CH$_a$$H_b$ODMTr); ESI MS: found 489.3, $C_{26}H_{26}F_3NO_5$ requires 489.2; anal. calcd for $C_{26}H_{26}F_3NO_5$: C, 63.80; H, 5.35; N, 2.86%. Found: C, 64.06; H, 5.39; N, 2.70.

The yield of **2a** was 1.7 g (78%): ν_{max} (KBr) 3390 (OH, NH and CH arom.), 2990 (CH aliph.), 1660 (C═O), 1620 (C═C arom.), 1590 (NH), 1530–1520 (CH_2 and C═C arom.), 1470 and 1450 (CH_2), 1400 (C–N), 1370 (CH_3),

1260–1040 (C–O methoxy and C–O), 990–710 (CH arom.); δ_H (500 MHz $CDCl_3$) 7.40–6.80 (m, 13H, arom.), 6.12 (br.s, 1H, N*H*), 3.88 (m, C*H*OH), 3.79 (s, 6H, 2×CH_3OC$_6$H$_4$), 3.52 (m, 1H, CH_aH$_b$NH), 3.38 (m, 1H, CH$_a$$H_b$NH), 3.28 (m, 1H, C$H_aH_b$ODMTr), 3.19 (m, 1H, CH$_a$$H_b$ODMTr), 2.01 (s, 3H, C$H_3$CONH). ESI MS: found 435.1, $C_{26}H_{29}NO_5$ requires 435.2; anal. calcd for $C_{26}H_{29}NO_5$: C, 71.70; H, 6.71; N, 3.22%. Found: C, 71.51; H, 6.92; N, 3.01.

The yield of **3a** was 2.1 g (85%): ν_{max} (KBr) 3800–3020 (OH, NH and CH arom.), 2970 (CH aliph.), 1670 (C═O), 1580 (NH), 1540–1490 (CH_2 and C═C arom.), 1460 and 1440 (CH_2), 1400 (C–N), 1370 (CH_3), 1260–1050 (C–O methoxy C–O), 980–710 (CH arom.); δ_H (500 MHz $CDCl_3$) 7.64–6.80 (m, 18H, arom.) 6.48 (br.s, 1H, N*H*); 3.86 (m, 1H, C*H*OH), 3.77 (s, 6H, 2×CH_3OC$_6$H$_4$), 3.60 (m, 3H, CH_aH$_b$NH, CH$_a$$H_bNH, CH_aH_b$ODMTr), 3.46 (m, 1H, CH$_a$$H_b$ODMTr); ESI MS: found 487.3, $C_{31}H_{31}NO_5$ requires 497.2; anal. calcd for $C_{31}H_{31}NO_5$: C, 74.83; H, 6.28; N, 2.81%. Found: C, 75.06; H, 6.30; N, 2.69.

The yield of **4a** was 1.76 g (74%): ν_{max} (KBr) 3660–3010 (OH, NH and CH arom.), 2960–2880 (CH aliph.), 1650 (C═O), 1620 (C═C arom.), 1540 (NH), 1520–1430 (CH_2 and C═C arom.), 1450 (CH_2), 1400 (C–N), 1370 (CH_3), 1270–1020 (C–O methoxy and C–O), 980–710 (CH arom.); δ_H (500 MHz $CDCl_3$) 7.41–6.81 (m, 13H, arom.), 5.94 (br.s, 1H, N*H*), 3.93 (m, 1H, C*H*OH), 3.77 (s, 6H, 2×CH_3OC$_6$H$_4$), 3.51 (m, 1H, CH_aH$_b$NH), 3.42 (m, 1H, CH$_a$$H_b$NH), 3.28 (m, 1H, C$H_aH_b$ODMTr), 3.11 (m, 1H, CH$_a$$H_b$ODMTr), 1.11 (s, *Me*$_3$CCONH). ESI MS: found 477.2, $C_{29}H_{35}NO_5$ requires 477.3; anal. calcd for $C_{29}H_{35}NO_5$: C, 72.93; H, 7.48; N, 2.93%. Found: C, 73.12; H, 7.70; N, 2.79.

4.4.1. (±)-3-Amino-1-(4,4′-dimethoxytriphenylmethyl)-2-propanediol (5). Compound **1a** (2.5 g) was dissolved in a 9 M solution of ammonia in methanol (75 ml), left overnight at 20°C and finally evaporated to dryness to give a colorless oil. Crude **5** was used for the preparation of **A**-supports without additional purification, ν_{max} (KBr) 3480–3020 (OH, NH and CH arom.), 2970–2840 (CH aliph.), 1620–1520 (CH_2 and C═C arom.), 1490–1420 (CH_2), 1300 (C–N), 1260–1040 (C–O methoxy and C–O), 900–710 (CH arom.); δ_H (500 MHz $CDCl_3$) 7.88–6.75 (m, 13H, arom.), 3.97 (m, 1H, C*H*OH), 3.78 (s, 6H, 2×CH_3OC$_6$H$_4$), 3.14 (m, 2H, CH_aH$_b$ODMTr, CH$_a$$H_b$ODMTr), 3.03 (dd, 1H, *J* 3.1, 12.9 Hz, CH_aH$_b$NH$_2$), 2.91 (dd, 1H, *J*=9.8, 12.9 Hz, CH$_a$$H_bNH_2$). ESI MS: found 393.2, $C_{24}H_{27}NO_4$ requires 393.2.

4.5. Coupling of hydroquinone-*O*,*O*′-diacetic acid to (±)-3-acylamido-1-(4,4′-dimethoxytriphenylmethyl)-2-propanediols 1a–4a to give compounds 1b, 2b, 3b and 4b. General procedure

Hydroquinone-*O*,*O*′-diacetic acid (0.452 g, 2 mmol) was dissolved in 5 ml of dry pyridine–dimethylformamide (4:1), *N*,*N*′-di-isopropylcarbodiimide (0.16 ml, 1 mmol) was added and the mixture was stirred for 1 h at 20°C. The resulting solution was added to a solution of (±)-3-acylamido-1-(4,4′-dimethoxytriphenylmethyl)-2-propanediol (1 mmol, **1a**, **2a**, **3a** or **4a**) and 4-*N*,*N*-dimethylaminopyridine (10 mg) in dry pyridine (3 ml) and the reaction mixture was stirred overnight at 20°C. Water (1 ml) was then added and the resulting mixture was evaporated to dryness. Compounds **1b**, **3b** and **4b** were isolated by flash chromatography (step gradient from 0 to 5% methanol in dichloromethane in the presence of 0.1% pyridine for **1b**, **3b** and **4b**) as pale yellow oils. Crude **2b** was dried under high vacuum to give 0.37 g of a pale yellow oil, which was used for $\mathbf{A_Q}$**-2**-support preparation without additional purification.

The yield of **1b** was 0.45 g (65%); ν_{max} (KBr) 3460 (OH), 2950 (NH), 1720 (C═O), 1670 (C═O), 1610–1590 (C═C arom., NH, COO^-), 1510 (C═C arom.), 1470 and 1450 (CH_2), 1400 (C–N and COO^-), 1300–1040 (C–F, C–O methoxy and C–O), 900–710 (CH arom.); δ_H (500 MHz $CDCl_3$–CD_3OD, 4:1) 7.45–6.82 (m, 17H, arom.), 5.17 (br.s, 1H, N*H*), 4.58 (br.s, 4H, 2×OCOCH_2O), 3.79 (s, 6H, 2×CH_3OC$_6$H$_4$), 3.77 (m, 1H, C*H*OH), 3.48 (m, 2H, CH_aH$_b$NH, CH$_a$$H_b$NH), 3.26 (m, 2H, C$H_aH_b$ODMTr, CH$_a$$H_b$ODMTr). ESI MS: found 697.3, $C_{36}H_{34}F_3NO_{10}$ requires 697.2; anal. calcd for $C_{36}H_{34}F_3NO_{10}$: C, 61.98; H, 4.91; N, 2.01%. Found: C, 62.21; H, 4.99; N, 1.89.

The yield of **3b** was 0.48 g (69%); ν_{max} (KBr) 3800–3020 (OH, NH and CH arom.), 2980 (CH aliph.), 1720 (C═O), 1670 (C═O), 1620–1580 (C═C arom., NH, COO^-), 1540–1490 (CH_2 and C═C arom.), 1400 (C–N and COO^-), 1460 and 1440 (CH_2), 1400 (C–N), 1370 (CH_3), 1260–1050 (C–O methoxy C–O), 980–710 (CH arom.); δ_H (500 MHz $CDCl_3$–CD_3OD, 4:1) 7.88–6.81 (m, 22H, arom.), 4.56 (br.s, 4H, 2×OCOCH_2O), 3.97 (m, 1H, C*H*OH), 3.79 (s, 6H, 2×CH_3OC$_6$H$_4$), 3.68 (m, 3H, CH_aH$_b$NH, CH$_a$$H_bNH, CH_aH_b$ODMTr), 3.37 (m, 1H, CH$_a$$H_b$ODMTr). ESI MS: found 705.2, $C_{41}H_{39}NO_{10}$ requires 705.2; anal. calcd for $C_{41}H_{39}NO_{10}$: C, 74.83; H, 6.28; N, 2.81%. Found: C, 74.58; H, 6.33; N, 2.63.

The yield of **4b** was 0.43 g (62%); ν_{max} (KBr) 3650–3010 (OH, NH and CH arom.), 1720 (C═O), 1650 (C═O), 1620–1540 (C═C arom., NH, COO^-), 1520–1430 (CH_2 and C═C arom.), 1400 (C–N and COO^-), 1370 (CH_3), 1270–1020 (C–O methoxy and C–O), 980–710 (CH arom.); δ_H (500 MHz $CDCl_3$–CD_3OD, 4:1) 7.37–6.82 (m, 17H, arom.), 6.37 (br.s, 1H, N*H*), 4.55 (br.s, 4H, 2×OCOCH_2O), 3.91 (m, 1H, C*H*OH), 3.79 (s, 6H, 2×CH_3OC$_6$H$_4$), 3.53 (m, 1H, CH_aH$_b$NH), 3.44 (m, 1H, CH$_a$$H_b$NH), 3.23 (m, 2H, C$H_aH_b$ODMTr, CH$_a$$H_b$ODMTr), 1.07 (s, 9H, Me$_3$CCONH). ESI MS: found 685.1, $C_{39}H_{43}NO_{10}$ requires 685.3; anal. calcd for $C_{39}H_{43}NO_{10}$: C, 68.31; H, 6.32; N, 2.04%. Found: C, 68.53; H, 6.49; N, 1.89.

4.6. A_Q-Supports. General procedure

A mixture of lcaa-CPG (0.3 g), hydroxybenzotriazole (HOBT) (30 mg, 0.2 mmol), *N*,*N*′-di-isopropylcarbodiimide (35 μl, 0.2 mmol), conjugate of (±)-3-acylamido-1-(4,4′-dimethoxytriphenylmethyl)-2-propanediol and hydroquinone-*O*,*O*′-diacetic acid (**1b**, **2b**, **3b** or **4b**, 0.15 mmol) in pyridine (5 ml) was agitated overnight at 20°C. Solids were filtered off, washed with pyridine (5 ml), tetrahydrofuran

(5 ml), diethyl ether (10 ml) and dried. The resulting support was suspended in a mixture of 1-methylimodazole–tetrahydrofuran (4:21; 5 ml) and acetic anhydride:2,6-lutidine–tetrahydrofuran (1:1:8; 5 ml) and agitated for 45 min at 20°C. The filtrate was washed with tetrahydrofuran (5 ml), acetonitrile (10 ml), diethyl ether (10 ml) and dried. The supports contained the following amount of DMTr-groups of per gram of CPG: **A_Q-1**—100 μmol, **A_Q-2**—50 μmol, **A_Q-3**—40 μmol and **A_Q-4**—40 μmol.

4.6.1. A-Supports. A mixture of lcaa-CPG (1 g), succinic anhydride (0.75 g, 7.5 mmol), pyridine (5 ml) and 1-methylimidazole–tetrahydrofuran (4:21; 5 ml) was agitated overnight at 20°C. Solids were filtered off, washed with pyridine (5 ml) and then tetrahydrofuran (5 ml). The resulting support **6** was suspended in a mixture of 1-methylimodazole–tetrahydrofuran (4:21; 5 ml) and acetic anhydride:2,6-lutidine–tetrahydrofuran (1:1:8; 5 ml) and agitated for 45 min at 20°C. The filtrate was washed with tetrahydrofuran (5 ml), 10% aqueous pyridine (10 ml), pyridine (5 ml), acetonitrile(10 ml) and finally dried. A mixture of derivatized CPG, compound 5 (1.2 g, 3 mmol), 1-hydroxybenzotriazole (0.46 g, 3 mmol), pyridine (7.5 ml) and *N*,*N*′-diisopropylcarbodiimide (0.47 ml, 3 mmol) was agitated overnight at 20°C. Solids were filtered off, washed with acetonitrile (5 ml), re-suspended in a mixture of triethylamine–water–acetonitrile (1:1:3; 5 ml) and agitated for 45 min at 20°C. The filtrate was washed with acetonitrile (10 ml) and finally dried.

The resulting support **7** (0.42 g) was suspended in a mixture of 2,6-lutidine (3 ml), tetrahydrofuran (8 ml) and acylated by a mixture (3×0.5 ml over 3 h period) prepared from formic acid (1.13 ml, 30 mmol) and acetic anhydride (1.9 ml, 20 mmol). The resulting suspension was agitated overnight at 20°C. The resulting filtrate was washed with acetonitrile (10 ml) and finally dried. Support **A_f** contained 100 μmol of DMTr-groups per gram of CPG.

Alternatively, the resulting support **7** (0.5 g) was suspended in a mixture of the corresponding carboxylic acid (4 mmol), *N*,*N*′-diisopropylcarbodiimide (0.32 ml, 2 mmol), pyridine (5 ml) and 4-*N*,*N*-dimethyaminopyridine (0.05 g, 0.04 mmol) and agitated overnight at 20°C. Solids were filtered off, washed with acetonitrile (10 ml) and finally dried. Support **A_p** contained 100 μmol of DMTr-groups per gram of CPG; **A_{mac}**—82 μmol of DMTr-groups per gram of CPG; **A_{mpac}**—86 μmol of DMTr-groups per gram of CPG; **A_{ac}**—97 μmol of DMTr-groups per gram of CPG; **A_{dcac}**—90 μmol of DMTr-groups per gram of CPG; **A_{tcac}**—73μmol of DMTr-groups per gram of CPG.

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