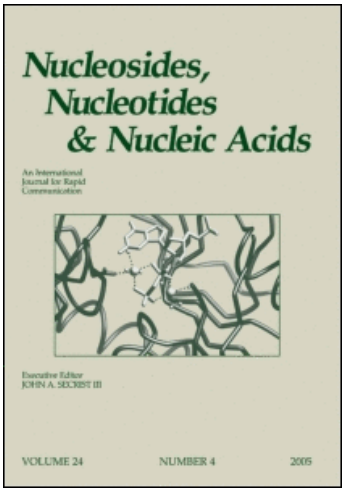

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Synthesis of Cytidylyl(3'→5')cytidylyl(3'→5')-(2')3'-O-[L$^{\alpha}$-alanyl]adenosine.

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*Abstract : A new synthesis of protected C-C-A-[L$^{\alpha}$-Ala] **14** is reported using a new set of complementary groups such as 2-phenylsulfonylethoxycarbonyl (PSEC) for the protection of exocyclic amino functions, o-chlorophenyl (o-ClPh) for the internucleotide phosphotriester, 3-methoxy-1,5-dicarbomethoxypentan-3-yl (MDMP) and the 4-monomethoxytrityl (MMTr) for the protection of the α-amino fuction of the amino acid. **14** could be deprotected in two steps by treatments with 1,1,3,3-tetramethylguanidinium oximate under a dry condition and then by neat trifluoroacetic acid. Treatment with neat trifluoroacetic acid produced a stable salt : [C-C-A-Ala-N$^{\alpha}H_3^+$ $CF_3CO_2^-$] and did not promote any internucleotide phosphate migration or degradation of the oligomeric molecule. This salt was considerably more stable than C-C-A-Ala conjugate with a free α-amino group, and, therefore, it could be easily purified on a silica gel column and was isolated in 82 % yield. This strategy should be useful for the synthesis of longer oligonucleotide-aminoacyl conjugate.*

The 3'-terminus of transfer ribonucleic acid (t-RNA) contains the common 5'-(C-C-A)-3' sequence and its 3'-OH end is attached by an ester linkage to an α-L-amino acid, each amino acid that is involved in protein biosynthesis has thus an amino acid-specific aminoacyl t-RNA (aa-tRNA). Enzymatic degradation of aa-tRNA showed the presence of this conserved aminoacylated C-C-A sequence at the 3'-end[1] in all known isoacceptor species of tRNA. It could be isolated only in very limited quantities for biological studies and, therefore, it has been considered important to design its efficient synthetic route by chemical methods[4,5,6]. These compounds are important tools for studying the role of the 3'-terminus of the aa-tRNA in protein biosynthesis[2]. The aminoacylated C-C-A is also an important probe to study the modes of stereochemical interactions of aa-tRNA with peptidyl transferase and elongation factor T_u which are involved in protein biosynthesis cycle and are integral parts of the large ribosomal subunit[3]. Such studies may help in elucidating the stereochemical control of the 3'-terminus of aa-tRNA in the ribosome-mediated protein biosynthesis cycle[2,3].

Development of the synthesis of 3'-O-aminoacyl-oligonucleotides[4,5,6] encounters the problem of two sensitive functions, the 3'-aminoacyl bond and the phosphotriester linkage, which have the reverse sensitivities during deblocking of the fully-protected C-C-A-amino acid conjugate. Additionally, the need for selective deprotection of the exocyclic amino protecting groups of cytosine and adenine moieties under a mild condition, without any scission of the 3′-O-aminoacyl bond puts up a serious demand in the design of the synthetic route leading to C-C-A-3′-O-amino acid conjugate.These problems make it a challenging task to design a set of complementary protecting groups in order to produce the final target compound, such as **14**, in a high overall yield.

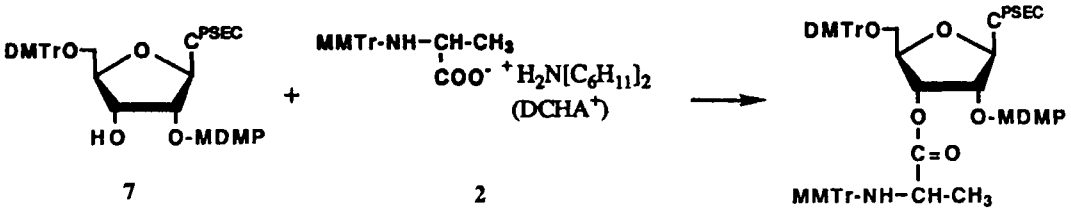
Chladek[5] employed the phosphotriester methodology[7] with the following protecting groups: the benzoyl for heterocyclic amino functions, 2-chlorophenyl (2-ClPh)[7] for the internucleotide phosphotriester, 4-monomethoxytrityl (MMTr) for the 5'-OH, 3-methoxytetrahydropyranyl (MTHP) for the 2'-OH and the N-benzyloxycarbonyl for the α-amino acid. The main disadvantage of this approach was that, during the deprotection of the benzoyl group by hydrazine hydrate, the cleavage of the 3′-O-aminoacyl bond took place which lowered the yield of the final deprotected aminoacyl-oligonucleotide conjugate. A new combination of protecting groups was therefore employed to circumvent the above problem[6]: 9-fluorenyl-methoxycarbonyl (FMOC)[8] for the NH_2 functions of adenine and cytosine moieties, the 2-ClPh for the internucleotide phosphate, 4,4-dimethoxytrityl (DMTr) for the 5'-OH, MTHP for the 2'-OH and 2-(4-biphenyl)isopropyloxycarbonyl (Bpoc)[10,11] for the blocking of the α-amino acid. The protected C-C-A was synthesized in a stepwise fashion and quantitatively aminoacylated with the aid of 1-mesitylenesulfonyl-tetrazole (MST). The deprotection was carried out in only two steps by successive treatments with the oximate ion and diluted formic acid to obtain the target molecule C-C-A-amino acid conjugate in ca 30% yield.

These works clearly suggest that the success in the chemical synthesis of C-C-A-amino acid conjugate completely depends upon a judicious choice of a set of complementary protecting groups particularly for the reactive functions such as the exocyclic amino groups, the internucleotide phosphodiester, the α-amino group of the aminoacyl moiety and the 2′-hydroxyl functions.

We have recently proposed a set of 2-arenesulfonylethoxycarbonyl groups[13] for the protection of amino groups of adenine, cytosine and guanine moieties of β-D-nucleosides. Our preliminary experiments showed[13] that such β-eliminating group protected nucleosides are stable under a variety of conditions and can be chemoselectively removed, as the Fmoc group[8], by a brief treatment (5 -10 min) of 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) or 1,1,3,3-tetramethylguanidine [TMG] or by 1,1,3,3-tetramethylguanidinium-*syn*-4-nitro benzaldoximate [TMG-oximate] at 20 °C. In our present work, we have therefore synthesized a 3'-aminoacyl-oligonucleotide conjugate, 5′-(C-C-A)-3′-O-[L$^{\alpha}$-Ala] **15**,

employing the following protecting groups: 2-phenylsulfonylethoxycarbonyl group (PSEC)[13] for the exocyclic amino protection, 3-methoxy-1,5-dicarbomethoxypentan-3-yl (MDMP)[12] for the 2'-OH group, the DMTr for the 5'-OH group and MMTr group for the α–amino protection of L-alanine[14]. The reason we chose the MMTr group for the protection of α-amino function of the amino acid, as in **2**, unlike Chladek's use[6] of the Bpoc group[10,11], was due to the fact that N^{α}-(MMTr) group should be removable at a much faster rate [trityl is removable almost 8 times faster than the Bpoc group in 80% acetic acid at room temperature][11]. This would therefore save the C-C-A-[L^{α}-amino acid] conjugate from being subjected in an acidic medium for a more prolonged time than actually necessary.

Our strategy for the synthesis of fully-protected C-C-A-[L^{α}-Ala] **14** was based upon first a regiospecific synthesis[12] of the partially-protected C-C-A block, such as **13**, and then a high-yielding condensation with N^{α}–(MMTr)-L-alanine **2**. The N^{α}-(MMTr)-Ala was prepared as the dicyclohexylamine (DCHA) salt **2**, in 70% yield by the method devised by Theodoropoulos[14]. 1-(mesitylenesulfonyl)-tetrazole [MST][15] and 1-methylimidazole promoted condensation[6] of a model compound **7** with **2** in *dry pyridine* solution at RT was complete within 12 h. The building block **6** was prepared from intermediate **5** which can be conveniently obtained in a satisfactory yield from **4**. After each step, we employed aqueous ammonium bicarbonate work up since the PSEC group was found to be not quite stable in the usual condition of saturated sodium bicarbonate work up. The 5′-O-DMTr derivative **7** (73%) was then converted to its 3′-O-phosphate **8** in 97% yield upon treatment of **7** with o-chlorophosphoro-bis(1,2,4-triazolide)[16] in dry pyridine solution. The chain was elongated in 5′ → 3′ direction by the condensation of the 5′-O-protected building block **8** with a 3′, 5′-dihydroxy block[17] **6** in dry pyridine solution in presence of an excess of MST[15] as the condensing agent to give desired partially protected dinucleoside monophosphate **9** in 79% yield. We did not observe formation of any 3′ → 3′ symmetrical product in the latter condensation reaction[17]. The 3′-hydroxyl group of compound **9** was subsequently phosphorylated in the usual manner *(vide supra)*[16] giving **10** in 89% yield. The condensation of **10** with **12** [obtained in two steps from **11**] was carried out also under a similar condition, as described above, producing compound **13** in 94% yield. The partially protected trimer **13**, with a free 3′-hydroxyl function, was aminoacylated by condensing with an excess of a N^{α}-(MMTr)-Ala **2** using an excess of MST[15]. The fully protected aminoacylated-trimer **14** was deprotected in two steps: (1) a treatment with *dry* TMG-oximate[18,19,6] at RT for 3 h, the lipophilic side products and excess of base were removed by precipitation from diethyl ether (precipitate showed a homogeneous spot upon tlc examination); (2) the dried precipitate was taken up directly in neat trifluoroacetic acid and stirred at 0 °C for 2 h, (tlc examination under a 254 nm UV lamp showed again the formation of only one ninhydrine positive product), this was then precipitated directly in diethyl ether at 0 °C. The solid residue thus obtained was loaded on a silica gel column and eluted with n-butanol-ethanol-H_2O (16:2:10, v/v/v). Appropriate fractions containing the target compound were collected, filtered and concentrated



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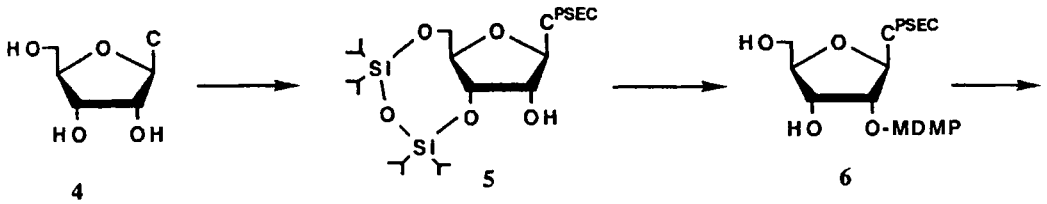

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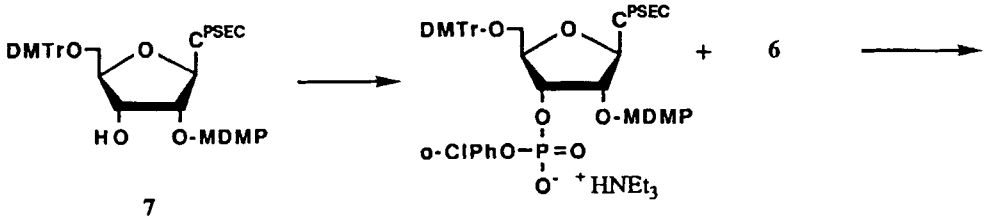

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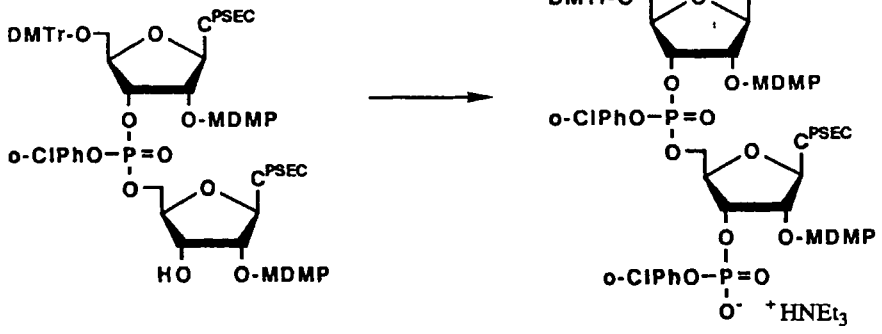

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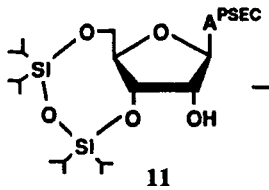

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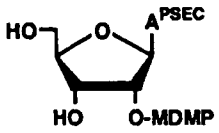

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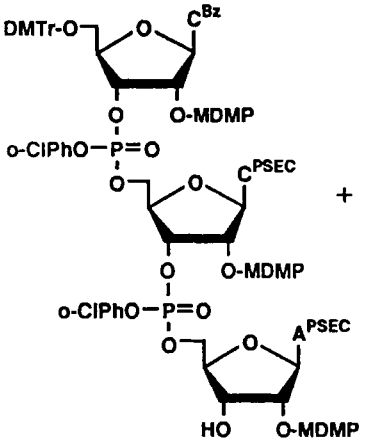

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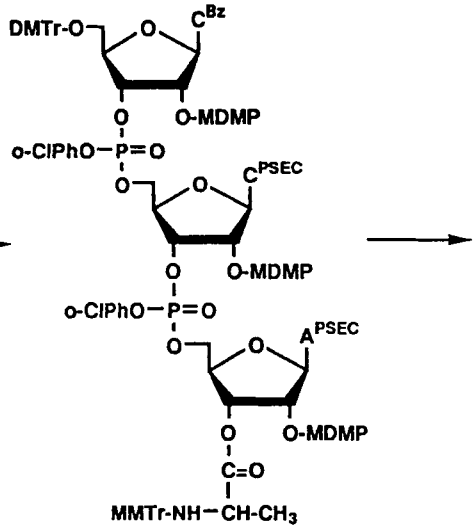

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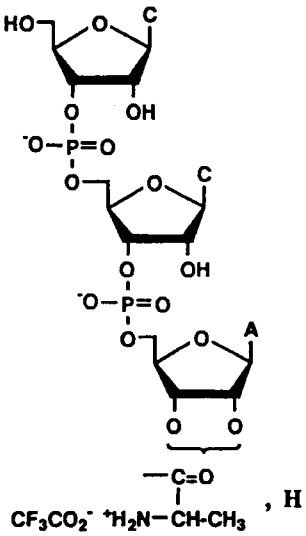

[2′- & 3′- isomers]

15

into a half volume, the clear solution was then liophilized twice from water. The lyophilized powder of **15** [343 A_{260} o.d units, 24% hyperchromicity, dry weight: 29 mg, 82% yield] was homogeneous on tlc and showed a positive ninhydrine test.

The C-C-A-Ala **15**, which is presumably an equilibrium isomeric mixture of 2′- and 3′-aminoacyl isomers, was characterized by alkaline hydrolysis (2% aqueous ammonia at RT) to C-C-A and L-Ala. More stringent alkaline treatment (0.1M KOH at ~ 97 °C) gave a mixture of 2'/3'-cytidylic acid and adenosine (ratio Cp/A = 2.1); complete digestion with snake venom phosphodiesterase showed cytidine (1.07 parts) + 5'-CMP (0.96 parts) + 5'-AMP (0.92 parts). Digestion with RNase A produced 3'-cytidylic acid and adenosine (ratio 2.09). In the above hydrolysates, L-alanine was identified separately on Tlc by ninhydrine spray.These complete enzymatic digestions suggest that both 3' → 5' phosphodiester linkages were specifically introduced and the synthetic material was free of by-products containing 2' → 5' internucleotidyl phosphate linkage.

In summary, our present C-C-A-[L^{α}-Ala] synthesis with the complementary of protecting groups on nucleosides (PSEC for protection of exocyclic amino functions, DMTr and MDMP for protections of 5′- and 2′-OH groups respectively), internucleotide phosphate (o-ClPh) and the α-amino fuction of the amino acid (MMTr) produced the fully-protected C-C-A-[L^{α}-Ala] **14** which could be deprotected only in two steps, first, a brief treatment with 1,1,3,3-teramethylguanidinium oximate under a dry condition followed by a treatment of neat trifluoroacetic acid. Treatment of neat trifluoroacetic acid did not cause either any internucleotide phosphate migration or degradation of the oligomeric C-C-A-[L^{α}-Ala]; such an acidic treatment however had the advantage over a formic acid treatment due to the fact that the resultant product [C-C-A-Ala-$N^{\alpha}H_3^+$ $CF_3CO_2^-$] was a stable salt of amino acyl - nucleoside conjugate. This salt was considerably more stable than C-C-A-Ala with a free α-amino group, and, therefore, it could be easily purified on a silica gel column. This strategy should be useful for the synthesis of longer oligonucleotide-aminoacyl conjugate.

Experimental:

^{1}H-NMR spectra were measured at 90 MHz with Jeol FX 90 Q spectrometer using tetramethylsilane as an internal standard (δ scale). ^{31}P-NMR spectra were recorded at 36 MHz in the same solvent mixture as for ^{1}H-NMR using 85% phosphoric acid as an external standard (δ scale). UV absorption spectra were recorded with a Cary 2200 spectrometer. Reactions were monitored using Merck pre-coated silica gel 60 F_{254}. High performance liquid chromatography (Hplc) was performed with the LDC equipments, model II pumps, UV III monitor and gradient master. The short column chromatographic separations were carried out using Merck G60 silica gel column eluted with a linear gradient of mixtures of MeOH-

CH_2Cl_2-pyridine and with n-butanol-ethanol-H_2O (16:2:10, v/v/v). The following thin layer chromatographic systems were used: *for silica gel plates (F_{254})* S_1: 7% EtOH-CH_2Cl_2; S_2: n-ButOH-EtOH-H_2O (16:2:5, v/v/v); S_3: n-ButOH-EtOH-AcOH (5:3:2, v/v/v); S_4: n-ButOH-EtOH-H_2O (16:2:10, v/v/v); S_5: n-PrOH-NH_4OH-H_2O (11:7:2, v/v/v); *for cellulose plates (PEI)*: S_6: 1M aq. NaCl.

4-N-(PSEC)-3',5'-O-(tertaisopropyl-1,3-disiloxane-1,3-diyl)-cytidine (5).

Cytidine (2.16 g, 5 mmol), was coevaporated two times with dry pyridine, then taken up in the same solvent (50 ml). Trimethylchlorosilane (5.7 ml, 45 mmol) was added and stirred at RT for 2 h, followed by addition of PSEC-Cl (1.49 g, 6 mmol), the solution mixture was kept at RT overnight. It was worked up by aqueous NH_4^+ HCO_3^- (5%, 50 ml) extraction from CH_2Cl_2 and reextracted with H_2O (50 ml). The organic layer was collected and dried on $MgSO_4$, then evaporated, coevaporated with toluene. The crude material was purified on a silica gel column using a mixture of CH_2Cl_2-MeOH (95:5, v/v). Appropriate fractions were pooled, evaporated and coevaporated with dry pyridine twice. The residue was redissolved in dry pyridine and 1,3-dichloro-1,1,3,3-tetraisopropyl-1,3-disiloxane (1.1 equiv.) was added. The reaction mixture was stirred at RT for 2 h and worked up and purified as above using CH_2Cl_2-MeOH (99:1, v/v) for column chromatographic purification. The total yield for two steps 3.24 g (93%) ^{1}H-NMR ($CDCl_3$ + CD_3OD) : 8.16 (d, J = 7.4 Hz, 1H) H-6; 8.0 - 7.5 (m, 5H) arom.; 7.06 (d, J = 7.4 Hz, 1H) H-5; 5.80 (s, 1H) H-1′; 4.54 (t, 6.2 Hz, 2H); 4.5 - 3.6 (m, 5H); 3.55 (t, 6.2 Hz, 2H); 1.3 - 0.7 (m, 28H).

4-N-(PSEC)-2′-O-(MDMP)-cytidine (6).

Compound **5** (0.62 g, 0.89 mmol) was coevaporated twice in dry toluene, then dissolved in dry dioxane (1 ml), 3-methoxy-1,5-dicarbomethoxy-2-pentene (3.84 ml, 17.7 mmol) and benzenesulfonic acid (0.05 g, 0.3 mmol) were added at RT. The reaction mixture was stirred at RT for 2 h, and then it was quenched by addition of pyridine (1 ml), which was worked up by extraction with aqueous NH_4HCO_3 in the usual way. The residue was treated with n-tetra-butylammonium fluoride (3 eq, 0.05 M in THF-pyr-H_2O, 8:1:1, v/v/v) for 20 min. It was worked up and purified as described for compound **5**. Yield 0.45 g (67%). ^{1}H-NMR ($CDCl_3$ + CD_3OD) : 8.22 (d, 8.1 Hz, 1H) H-6; 8.0 - 7.5 (m, 5H) arom.; 7.14 (d, 8.1 Hz, 1H) H-5; 5.82 (d, 5.4 Hz, 1H) H-1′; 4.71 (m, 1H); 4.54 (t, 6.3 Hz, 2H); 4.3 - 3.6 (m, 4H); 3.68 (s, 6H); 3.55 (t, 6.3 Hz, 2H); 3.2 - 1.8 (m, 8H); 3.08 (s, 3H).

5′-O-(DMTr)-2′-O-(MDMP)-cytidine (7).

Compound **6** (0.39 g, 0.57 mmol) was dried by coevaporation with pyridine, then taken up in the same solvent, DMTr-Cl (0.22 g, 0.63 mmol) was added and the reaction mixture was stirred at RT overnight. This was then worked up as described above. The product was

purified on a silica gel column by elution with a mixture of MeOH-pyr-CH_2Cl_2 (1:1:98, v/v/v) Yield 0.4 g (73%). ^{1}H-NMR ($CDCl_3$ + pyridine-d_5) : 8.38 (d, 8.1 Hz, 1H) H-6; 8.0 - 6.9 (m, 18H) arom.; 6.84 (d, 8.1 Hz, 1H); 6.14 (d, 1.2 Hz, 1H) H-1′; 4.54 (m, 2H); 4.6 - 2.4 (m, 5H); 3.80 (s, 6H); 3.56 (m, 2H); 3.67 (s, 3H); 3.64 (s, 3H); 2.35 (s, 3H); 2.3 - 1.8 (m, 8H).

4-N-(PSEC)-5′-O-(DMTr)-2′-O-(MDMP)-cytidine-3′-O-[(o-chlorophenyl) triethylammonium phosphate] (8).

Compound **7** (0.62 g, 0.64 mmol) was coevaporated with pyridine and dissolved in the same solvent (16 ml) o-chlorophenylphosphorobis-1,2,4-triazolide (10.3 ml, 1.28 mmol) in dry acetonitrile (0.25 M solution) was added and stirred at RT for 20 min. The reaction mixture was quenched with 0.5M triethylammonium bicarbonate (TEAB) solution [pH = 7.5], stirred for a few minutes, and then worked up in a similar manner as above. Yield 0.74 g (97%). ^{31}P-NMR ($CDCl_3$ + CD_3OD) : -5.91

4-N-(PSEC)-5′-O-(DMTr)-2′-O-(MDMP)-cytidylyl-(3′→5′)-4-N-(PSEC)-2′-O-(MDMP)-cytidine (9).

Compound **6** (0.09 g, 0.133 mmol) and **8** (0.24 g, 0.2 mmol) were coevaporated with dry pyridine and taken up in the same solvent (3 ml). MSNT (0.18 g, 0.6 mmol) was added and the reaction mixture stirred at RT for 30 min. The condensation reaction was quenched by addition of water (1 ml) and upon stirring for a further period of 5 min. It was worked up in the usual manner and purified on a silica column eluting **9** with MeOH-pyr-CH_2Cl_2 (2:1:97, v/v/v). Yield 0.18 g (79%). ^{31}P-NMR ($CDCl_3$) : -6.79 & -7.32.

Protected cytidylyl(3′→5′)cytidine-3′-0-[(o-chlorophenyl)triethylammonium phosphate] (10).

Compound **9** (0.18 g, 0.1 mmol) was phosphorylated with o-chlorophenylphosphorobis(1,2,4)-triazolide and worked up in a similar way, as for the monomer **8**, to give **10** (0.17 g, 89%.). ^{31}P-NMR ($CDCl_3$): -5.69 & -7.12.

6-N-(PSEC)-2′-O-(MDMP)-adenosine (12).

Compound **12** was obtained in a similar way, as for compound **6**, in 65% yield (0.13 g). ^{1}H-NMR ($CDCl_3$): 8.75 (s, 1H) H-8; 8.13 (s, 1H) H-2; 8.0 - 7.5 (m, 5H) arom.; 5.88 (d, 7.2 Hz, 1H) H-1′; 5.16 (dd, 5.4 & 7.2 Hz, 1H); 4.63 (t, 6.3 Hz, 2H); 4.6 - 3.5 (m, 4H); 3.69 (s, 3H); 3.59 (s, 3H); 2.71 (s, 3H); 2.4 - 1.4 (m, 8H).

4-N-(PSEC)-5′-O-(DMTr)-2′-O-(MDMP)-3′-citydylyl-(3′→5′)-4-N-(PSEC)-2′-O-(MDMP)-cytidylyl-(3′→5′)–6-N-(PSEC)-2′-O-(MDMP)-adenosine (13)

Compound **12** (0.04 g, 0.06 mmol) and **10** (0.17 g, 0.09 mmol) were condensed using an identical procedure as for the preparation of **9** to give **13**. Yield 0.14 g (94%). ^{31}P-NMR ($CDCl_3$): -6.71, -6.79, -6.96, -6.95, -7.0, -7.22, -7.37, -7.5

Dicyclohexylamine [DCHA] salt of N^{α}-(Monomethoxytrityl)-L-alanine (2).

To a magnetically stirred solution of L-alanine (0.89 g, 10 mmol) in CH_2Cl_2- MeOH (5:1, v/v, 18 ml) trimethylchlorosilane (1.27 ml, 10 mmol) was added. The reaction mixture was refluxed for 2 h and then allowed to cool to the RT. Et_3N was added at a rate sufficient to maintain the gentle reflux, followed by the addition of dichloromethane solution (10 ml) of MMTr-Cl (3.1 g, 10 mmol). The resulting mixture was stirred at RT for 1 h and then excess of MeOH (50 equiv.) was added. Evaporation under reduced pressure left a residue which was partitioned between Et_2O (50 ml) and a precooled solution of aqueous citric acid (5%, 50 ml). The organic phase was collected and worked up by extractions with aqueous NaOH solution (1M, 2 x 20 ml) and water (2 x 10 ml). The combined aqueous layer was washed with Et_2O (3 x 20 ml), chilled to 0 °C and neutralized with acetic acid. The aqueous layer was evaporated, giving a foam (2.53 g, 70%). The foam was dissolved in diethyl ether (20 ml), DCHA (1 equiv.) was added and stirred for a few minutes. Upon evaporation and drying on P_2O_5, the target compound **2** was obtained in a quantitative yield. ^{1}H-NMR ($CDCl_3$): 7.6 - 6.7 (m, 14H); 3.75 (s, 3H); 3.12 (q, 7.2 Hz, 1H); 3.0 - 0.9 (m, 24H); 0.96 (d, 7.2 Hz, 3H)

4-N-(PSEC)-5′-O-DMTr-2′-O-MDMP-3′-cytidylyl-(3′→5′)-4-N-(PSEC)-2′-O-MDMP-cytidylyl-(3′→5′)-6-N-(PSEC)-2'-O-MDMP-adenylyl-3'-O-[N^{α}-(MMTr)-alanine] (14).

Compounds **13** (129 mg) and **2** (91.2 mg) were coevaporated with dry pyridine and dissolved in the same solvent and then 1-methylimidazole (68µl) followed by MST (10 eq.) was added in three portions during 4 days and stirred at RT. The reaction mixture was worked up in the usual manner yielding compound **14** (108 mg, 68%).

Trifluoroacetate salt of dicytidylyl-(2′)3'-O-[L-alanyl]adenosine (15).

Compound **14** (80 mg, 28 µmol) was coevaporated with dry toluene, then dissolved in acetonitrile (2.8 ml), *Syn*-4-nitrobenzaldoxime (0.18 g, 1.1 mmol) and 1,1,3,3-tetrame thylguanidine (TMG) (0.12 ml, 0.9 mmol) and morpholine (0.2 mmol) were added and stirred at RT for 3 h. The reaction mixture was poured dropwise into a centrifuge tube containing dry diethyl ether (20 ml). The precipitate was collected and washed twice with dry diethyl ether

(40 ml) to remove the liphophilic matters and the traces of base. The solid residue was dried over P_2O_5 in a desiccator. It was then taken up in neat trifluoracetic acid (1 ml) and stirred at 0 ^oC for 2 h. When the tlc system (S_2) showed that the reaction was complete, dry diethyl ether (20 ml) was added while stirring was continued at 0 ^oC. The precipitate was centrifuged and the solid residue was purified on a silica gel column with the S_4 system. Appropriate fractions were collected and concentrated into a half volume by evaporation. The residue was liophilized twice and dried on P_2O_5 in a desiccator. Yield of **15**: 343 A_{260} o.d units, 24% hyperchromicity, *dry weight*: 29 mg (82%). , which is presumably a mixture of 2′ and 3′-isomers. The R_f of **15** in different tlc systems are: 0.21 (S_4); 0.1 (S_3), 0.75 (S_6). UV (water, pH 2): λ_{max} 279 nm & λ_{min} 244 nm which are identical to the ones reported by Chladek et al.[5,6]. The structure of compound **15** was further identified as follows: (1) Brief alkaline hydrolysis for 20 min with 2% aq. ammonia at RT showed the presence of C-C-A and L-alanine on a silica gel Tlc (system S_2) which were confirmed upon comparison with authentic C-C-A and L-Ala. (2) Alkaline treatment with 0.1 M aq. KOH at ~97 ^oC gave a mixture 2′- & 3′-cytidylic acid and adenosine (ratio Cp/A = 2.1) which were quantitated by Hplc. (3) Digestion with snake venom phosphodiesterase (20 μg) in tris-hydrochloride buffer (0.1 M, pH 9 0, 0.1 M $MgCl_2$, 70 μl) at 37 ^oC for 19 h showed complete digestion to cytidine (1.07 parts), 5′-CMP (0.96 parts) and 5′-AMP (0.92 parts) which were qauantitated by Hplc (4). Digestion with RNase A (20 μg) in ammonium acetate buffer (100 μl, 0.002 M, pH 7, 0.002 M EDTA and containg 0.05% tween 40) at 37 ^oC for 51 h showed complete digestions to 3′-CMP and adenosine (ratio 2.09) [digestion was qantitated by Hplc] [*Conditions for Hplc*: : Spherisorb ODS 10 μ column with 0.0005 M tetrapentylammonium phosphate in water (solvent A) and 0.0005 M tetrapentylammonium phosphate in 20% acetonitrile-water (solvent B) on a linear gradient mode, 0 - 100% of B for 30 min; flow rate 2.0 ml /min.]. All hydrolysates were examined by tlc (system S_5) which showed the presence of L-alanine as visualized by ninhydrine spray.

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