Solid-Phase Synthesis of a Monofunctional *trans*-a$_2$Pt^{II} Complex Tethered to a Single-Stranded Oligonucleotide**

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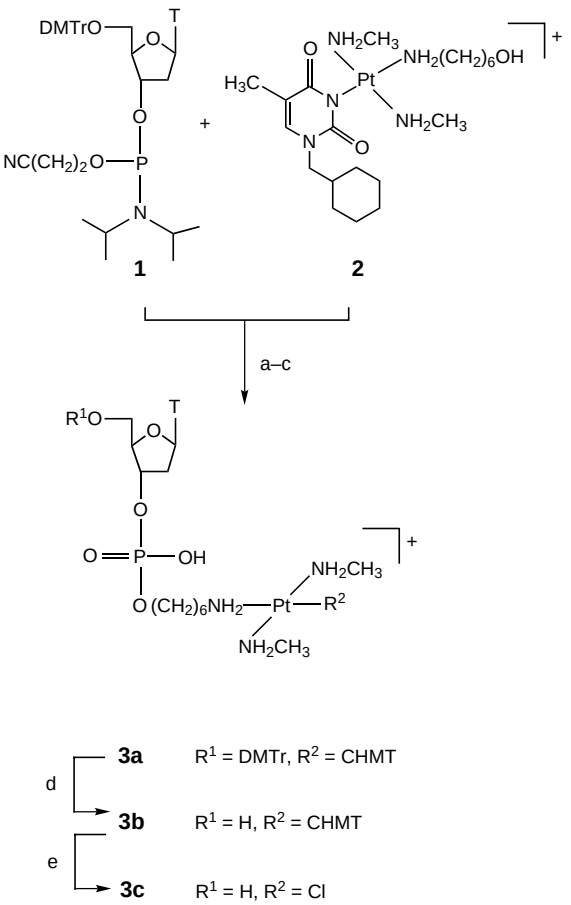
Single-stranded oligonucleotides bind with high selectivity to complementary sequences within DNA or RNA. This important feature allows regulation of gene expression at the stage of transcription (antigene strategy) or translation (antisense strategy).[1] Effectuation of this idea in combating cancer and AIDS seems promising.[2, 3] So far the general applicability of this concept is, however, hampered by the observation that the adducts of oligonucleotides with DNA or RNA do not fully survive the replication and transcription machinery.[4, 5]

Model studies indicated that cross-linking of an oligonucleotide and its target by a *trans*-a$_2$Pt^{II} species (a = NH_3 or amine) is a potential solution to this problem.[6] The implementation of this concept in the antigene and antisense strategy is limited due to the lack of an efficient synthesis of selectively platinated oligonucleotides. For instance, the reaction of an oligonucleotide with a Pt^{II} species does not proceed regioselectively, resulting in tedious and time-consuming purification of the end product. On the other hand, incorporation of preplatinated nucleotide building blocks in an oligonucleotide by solid-phase DNA synthesis appears to be more rewarding in achieving specific and sequence-independent platination.[7, 8] Unfortunately, the preparation of *trans*-a$_2$Pt^{II}-modified oligonucleotides by this method is accompanied by the formation of an inactivated species, which excludes cross-linking with target molecules.[8]

Here we report a novel solid-phase approach to an oligonucleotide-tethered monofunctional *trans*-Pt^{II} complex with cross-linking ability. The newly synthesized *trans*-Pt^{II} building unit **2** (Scheme 1), in which the Pt^{II} center is inter alia coordinated to 1-amino-hexan-6-ol (AHOL) and 1-*N*-cyclohexylmethylthyminate (CHMT), was designed to execute essential chemical manipulations en route to the target compound (i.e., **9** in Scheme 2). Thus, the primary hydroxyl group of the AHOL ligand served to tether **2** through a phosphate bond to the 5′-terminus of immobilized DNA.



Scheme 1. a) *o*-NPT; b) I_2/collidine; c) 25% aq. NH_3; d) 80% aq. AcOH; e) DCl (pD 2.2). DMTr = 4,4′-dimethoxytrityl.

Moreover, the presence of the cyclohexylmethyl moiety, instead of a methyl group,[9] would enhance the solubility of **2** in organic solvents. Apart from this, it was essential to establish whether the Pt^{II} center in **2** would be compatible with the solid-phase synthesis of DNA. The latter prerequisite is of primary importance in effecting exchange of the CHMT ligand by a chloro ligand,[9] thus giving access to a monofunctionally platinated, single-stranded oligonucleotide in the final stage of the synthesis.

The viability of our strategy was explored first by executing the following solution-phase experiment (Scheme 1): Compound **2** was condensed with 5′-*O*-DMTr-dT-3′-phosphoramidite (**1**) under the influence of 5-(*ortho*-nitrophenyl)-1-*H*-tetrazole (*o*-NPT) to give, after oxidation of the intermediate phosphite, the corresponding phosphotriester derivative. Removal of the β-cyanoethyl group by treatment with concentrated aqueous ammonia resulted, after purification by reverse-phase HPLC, in the isolation of the homogeneous compound **3a**. The identity of **3a** was firmly established by ^{1}H, ^{31}P, and ^{195}Pt NMR spectroscopy as well as LC-MS. It was also verified that **3a** survived the forced ammonolysis conditions (6 h at 50 °C) usually applied for the removal of N-acyl protecting groups of deoxynucleoside building units, clearly indicating that **2** is fully compatible with the DNA synthesis protocol. Transformation of **3a** into the required chloro derivative **3c** could be realized by the following two-step

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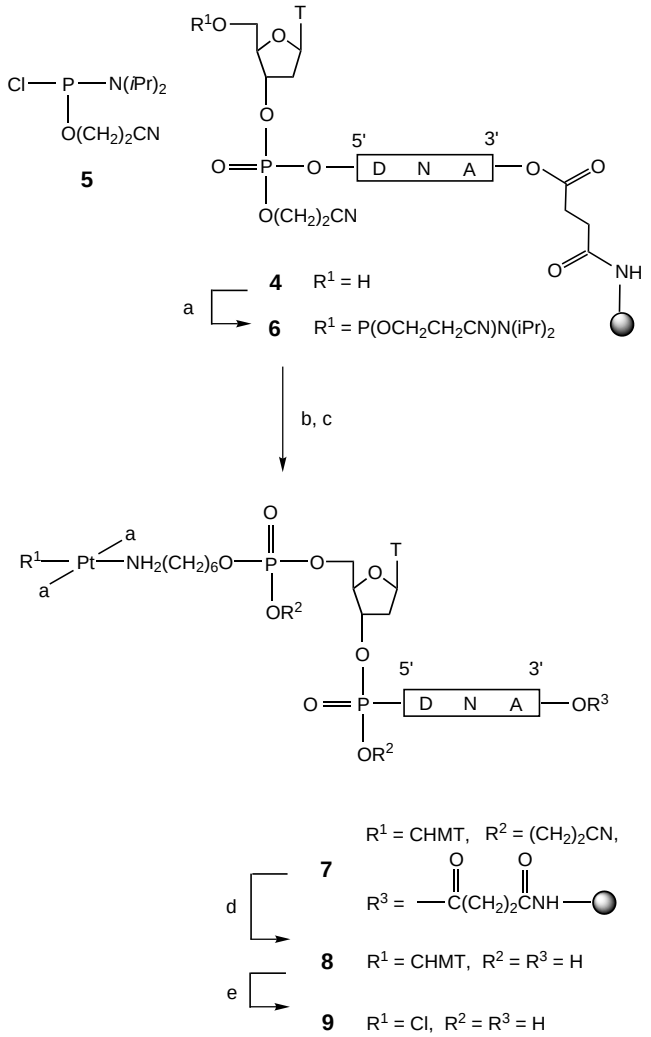
process: Detritylation of **3a** under the influence of aqueous acetic acid gave, after purification, **3b** in a nearly quantitative yield. Exchange[9] of the CHMT ligand of **3b** with diluted DCl (pD 2.2, 96 h, 40 °C) proceeded smoothly and quantitatively to afford homogeneous **3c**, as evidenced by ^{1}H NMR and LC-MS.

At this stage, tetrathymidylic acid **4** (Scheme 2) immobilized by a 3′-*O*-succinyl bond to controlled pore glass (CPG) was phosphitylated[10] with the bifunctional reagent **5**. The newly formed 5′-amidite **6** was then coupled with **2** under the influence of *o*-NPT to afford, after oxidation (I_2/collidine), immobilized **7**. Subjecting **7** to concentrated aqueous ammonia led to decyanoethylation and concomitant release from the solid support to give **8** in an estimated yield of 34%, as gauged by HPLC in combination with electrospray-ionization mass spectrometry. The structure of **8** was substantiated independently by transformation into the corresponding chloro-coordinated product **9**. Thus, treatment of purified (reverse-phase HPLC) **8** with diluted hydrochloric acid (pH 2.3, 40 °C, 48 h) led to the exclusive formation of the monofunctional *trans*-Pt^{II} oligonucleotide **9**, as evidenced by LC-MS.



Scheme 2. a) Bifunctional reagent **5**, triethylamine; b) **2**, *o*-NPT; c) I_2/collidine; d) 25% aq. NH_3; e) aq. HCl (pH 2.3). DNA = d(TTT), T = thymine.

The methodology described here shows for the first time[11] that a monofunctional *trans*-Pt^{II} complex tethered to a homopyrimidine oligodeoxynucleotide can be constructed by a solid-phase approach, thus opening the way to the future design of a new class of useful chemical nucleic acids probes and antigene reagents. Although the yield of the condensation of **2** with the immobilized DNA fragment **4** (i.e., conversion of **4** into **7** in Scheme 2) is not yet fully optimized, sufficient material can be attained to examine in detail the cross-linking ability of the monofunctional *trans*-Pt^{II} oligonucleotide complex with DNA. Moreover, the possible occurrence of depurination[12] in the acid-mediated (HCl, pH 2.3) exchange process (i.e., conversion of **8** into **9** in Scheme 2) may be prevented using a slightly more acid-labile temporary ligand. Both aspects are currently under investigation and will be reported in due course.

Experimental Section

2: 1-*N*-Cyclohexylmethylthymine was prepared according to a slightly modified published procedure[13] and converted into its potassium salt.[14] *trans*-[$(NH_2CH_3)_2$PtCl(CHMT)], obtained by a slight modification of a published procedure,[15] was stirred with 1-amino-hexan-6-ol (1 equiv) in methanol/water (1/1) at 60 °C for 72 h to give crystalline **2** in 60% yield.

3a: To a solution of **2** (13.5 μmol) in dioxane was added amidite **1** (13 μmol) and *o*-NPT (53.5 μmol) in acetonitrile. After 10 min an aqueous solution of I_2/collidine was added. The solvents were removed and the residue dissolved in 1000 μL of methanol containing 200 μL of concentrated aqueous ammonia. After 10 h at 20 °C, the sample was freeze-dried and purified by reverse-phase HPLC (Alltima C 18 column, 50 mmol of aqueous ammonium bicarbonate/acetonitrile buffer, gradient: 40 → 70% acetonitrile) to give **3a**. ^{195}Pt NMR (CD_3OD): $\delta = -2621$; ^{31}P NMR (CD_3OD): $\delta = 0.18$; the complete assignment of the nonexchangeable protons was corroborated by a ^{1}H,^{1}H COSY experiment; ESI-MS: *m*/*z*: 1201.5 [M^+].

Automated DNA synthesis: Immobilized d(TTTT) (0.2 μmol) was synthesized on a Gene Assembler (Pharmacia) following a standard protocol,[16] phosphitylated,[10] and allowed to react with **2** (15 μmol, 0.05 M solution in dioxane/acetonitrile (1/1)) in the presence of *o*-NPT (30 μmol, 0.2 M solution in acetonitrile). Decyanoethylation and release from the solid support were effected by treatment of crude **7** with concentrated aqueous ammonia for 12 h at 20 °C.

LC-MS analysis was carried out on a PE Sciex API 165 mass unit connected to a Jasco HPLC system. HPLC analysis was performed using a Merck LiChrosphere 100 RP18 column (pore size 5 μm, diameter 4 mm, length 250 mm) and 10 mM aqueous ammonium acetate/acetonitrile (gradient: 5 → 50% acetonitrile) buffer. Apart from **8** (*m*/*z*: 1812 [M^+], 906 [M^{2+}]; t_r = 12.50 min), also tetrathymidylic acid (*m*/*z*: 1155.6 [M^+]; t_r = 7.10 min) and its corresponding 5′-amidate (*m*/*z*: 1318.5 [M^+]; t_r = 10.13 min) were present in nearly equal amounts (33% each).

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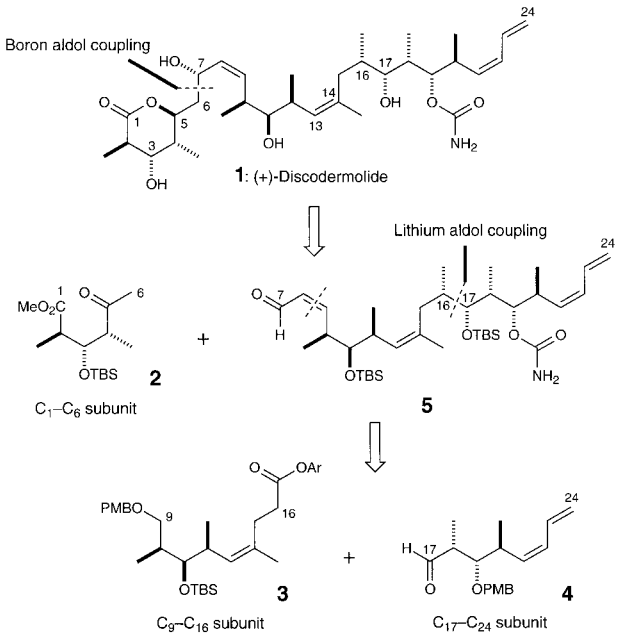

Scheme 1. Retrosynthetic analysis. Ar = 2,6-dimethylphenyl.

Total Synthesis of the Antimicrotubule Agent (+)-Discodermolide Using Boron-Mediated Aldol Reactions of Chiral Ketones**

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Discodermolide (**1**) is a unique polyketide isolated in 1990 by Gunasekera et al. at the Harbor Branch Oceanographic Institute, Florida, from the Caribbean sponge *Discodermia dissoluta*.[1] Structurally (Scheme 1), it bears 13 stereogenic centers, a tetrasubstituted δ-lactone (C_1-C_5), one di- and one tri-substituted *Z*-alkene, a carbamate moiety, and a terminal *Z*-diene. Discodermolide displays potent activity as an antimitotic agent, with a similar mechanism of action to taxol (paclitaxel), namely by stabilizing microtubules and promoting the polymerization of tubulin.[2] It inhibits the growth of human breast cancer cells in vitro, as well as paclitaxel-resistant ovarian and colon cancer cells, and other multidrug resistant cells.[3] Hence, discodermolide is a particularly promising candidate for development in cancer chemotherapy, but its use is severely limited by its scarce supply (0.002% w/w isolation yield)[1] from the rare sponge source.

To date, two total syntheses of the natural (+)-discodermolide and three syntheses of the antipodal (−)-discodermolide have been reported,[4] together with various synthetic approaches.[5] Schreiber and co-workers[4a] have synthesized both antipodes and established the absolute configuration, as well as preparing a number of structural analogues.[4b] Despite these impressive efforts, there is still a pressing demand for developing a more practical and efficient synthetic route to (+)-discodermolide.

Herein, we describe a highly convergent total synthesis, which has the potential to provide useful quantities of (+)-discodermolide. Notably, our route is entirely different from earlier syntheses and is based on a novel aldol-coupling strategy, which also employs aldol reactions of chiral ketones to construct the three key subunits **2**, **3**, and **4** (Scheme 1). Our retrosynthetic analysis is based on a C_6-C_7 disconnection which leads back to the (C_1-C_6) methyl ketone **2** and the enal **5**. Further disassembly of **5** gives the (C_9-C_{16}) ester **3** and the (C_{17}-C_{24}) aldehyde **4**.

As shown in Scheme 2 the synthesis of the C_1-C_6 subunit **2** began with a boron-mediated *anti*-selective aldol reaction between the readily available[6] ethyl ketone (*S*)-**6** and acetaldehyde. The intermediate aldolate was reduced in situ with $LiBH_4$ to give diol **7** (98%, >97% diastereoselectivity (ds)).[6a] Protection as the bis-*tert*-butyldimethylsilyl (TBS) ether **8**, followed by methanolysis using catalytic CSA in $MeOH/CH_2Cl_2$ at 0 °C, gave C_5-alcohol **9** in 70% yield, together with starting material and diol **7**, which were recycled accordingly. After debenzylation, diol **10** was converted into subunit **2** by sequential double Swern oxidation, $NaClO_2$ oxidation, and esterification. This three-step operation was carried out without chromatography in 93% yield. The

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