energy.[18] The ring inversion barrier is somewhat higher in **5** than in **4** (see Table II). This could be due to the restraint caused by the double bond. For cyclooctene, experiments[19] and force-field calculations[20] resulted in a smaller activation barrier for the ring inversion ($\Delta G^{\ddagger}$ = 8.2 kcal/mol).

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Solid-Phase Synthesis of Hentriacontanucleotide

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Solid-phase synthesis is very attractive when preparing polynucleotides of defined sequences, since the synthesis of polypeptides (~30 amino acids) has been very successful on various polymer supports.[1] There were some difficulties in bringing fruitful results with solid-phase synthesis in the polynucleotide field. This relative lack of success was probably due to inefficient coupling methods in forming an internucleotidic phosphate bond between two nucleoside derivatives. Although the classical phosphodiester method to make phosphate bonds is powerful and accurate,[2] it has certain inherent disadvantages, including low yields in the coupling reaction. Accordingly, solid-phase synthesis of polynucleotides by the phosphodiester method was not successful.[3] Recent improvements by several groups in the phosphotriester approach have changed this situation drastically.[4] When a slight excess of one coupling unit is used, it is practical to drive a coupling reaction almost to completion by a liquid-phase synthesis, forming a phosphotriester bond.[5] Very recently we introduced a new strategy, a block coupling phosphotriester approach on a polymer support to synthesize oligodeoxyribonucleotides of defined sequences.[6] We now report the synthesis of a hentriacontanucleotide, d(TGGTGCACCTGACTCCTGAGGAGAAGTCTGC), on the poly(acrylyl morpholidate) support **6b** by using a similar strategy. The essential features of the approach are very simple: (a) sequential addition of appropriately protected trinucleotide blocks **7** to the solid-support **6b** in the presence of a coupling reagent, 2,4,6-triisopropylbenzenesulfonyl tetrazolide (TPSTe), (b) masking of any unreacted 5'-hydroxyl group with acetic anhydride, and (c) removal of the dimethoxytrityl group from the polynucleotides bound to the support to afford a new 5'-hydroxyl function for the next coupling reaction.

Table I

step	solvent or reagent	amount, mL	shaking, min	no. of operations
1	2% BSA	10	0.5	1
2	$CHCl_3$-MeOH (7:3 v/v)	10	1	2
3	pyridine	10	1	2
4	trimer (5 equiv) in pyridine	10	coevaporation	2
5	TPSTe (15 equiv) and pyridine	8	180	1
6	pyridine	10	1	2
7	10% Ac_2O in pyridine	10	60	1
8	pyridine	10	1	2
9	$CHCl_3$-MeOH (7:3 v/v)	10	1	2

In Scheme I, the outline of the approach is described. The commercially available Enzacryl Gel K-2 **1** (Aldrich) was derivatized with ethylenediamine in ethylene glycol to the amino support **2** (0.20 mmol/g of the amino function) as published.[7] 5'-*O*-Dimethoxytrityl deoxynucleoside **3** was reacted with succinic anhydride (1.5 mol equiv) in the presence of 4-(dimethylamino)pyridine (1.5 mol equiv) in pyridine at room temperature overnight to give the monosuccinate derivative **4** in ~80% yield. When **4** was treated with pentachlorophenol (1.1 mol equiv) and dicyclohexylcarbodiimide (3 mol equiv) in dimethylformamide (DMF) at room temperature for 20 h, the activated ester **5** was obtained in ~90% yield. Treatment of the amino support **2** with this ester **5** (2.5 mol equiv) and triethylamine (2.75 mol equiv) in DMF, shaking at room temperature for 20 h, gave the dimethoxytrityl support **6a**. Any unreacted amino group **2** was masked by treatment with phenyl isocyanate (10% solution in pyridine) at room temperature for 1 h and the dimethoxytrityl group was removed by treatment with a 2% solution of benzenesulfonic acid (BSA) in $CHCl_3$-MeOH (7:3 v/v) at room temperature for 30 s. The amount of released dimethoxytrityl group from the support **6a** was estimated by an absorption spectrum in a 1% BSA solution in $CHCl_3$ [λ_{max} 507 nm, ϵ_{max} 92 100 M^{-1} cm^{-1}] and is in agreement with that of the nucleoside liberated from the support **6b** by treatment with aqueous ammonia (28%) at 50 °C overnight (0.177 mmol/g of the nucleoside). Each trinucleotide addition cycle started from step 4 (Table I), coevaporation of the support **6b** (0.80 g) and the trinucleotide **7** (5 mol equiv) in pyridine twice to remove hydroxylic solvents. TPSTe[8] (15 mol equiv) and anhydrous pyridine (8 mL) were added to the residue, and the reaction mixture was shaken for 3 h (step 5) and filtered. The support was washed with pyridine twice (step 6) and treated with a 10% solution of acetic anhydride in pyridine for 1 h to mask the unreacted 5'-hydroxyl group (step 7). The mixture was filtered and washed successively with pyridine (step 8) and $CHCl_3$-MeOH (7:3 v/v, step 9). The dimethoxytrityl function was removed from the polynucleotide bound to the support by treatment with a 2% BSA solution in $CHCl_3$-MeOH (7:3 v/v, step 1) for 30 s at room temperature. The new coupling cycle was resumed after washing the support with $CHCl_3$-MeOH (step 2) and pyridine (step 3). The first coupling unit, a derivative of the trinucleotide **7** (B_1 = C^{Bz}, B_2 = T, and B_3 = $G^{i\text{-}Bu}$ in Scheme I), was coupled to the 5'-hydroxyl N-benzoylated deoxycytosine polymer **6b**, and nine other trinucleotides with the desired sequences ($A^{Bz}G^{i\text{-}Bu}T$, $A^{Bz}G^{i\text{-}Bu}A^{Bz}$, $A^{Bz}G^{i\text{-}Bu}G^{i\text{-}Bu}$, $C^{Bz}TG^{i\text{-}Bu}$, $C^{Bz}TC^{Bz}$, $TG^{i\text{-}Bu}A^{Bz}$, $A^{Bz}C^{Bz}C^{Bz}$, $TG^{i\text{-}Bu}C^{Bz}$, and $TG^{i\text{-}Bu}G^{i\text{-}Bu}$) were sequentially used to synthesize the 31-mer. The average coupling yield estimated by the absorption spectrum of the dimethoxytrityl

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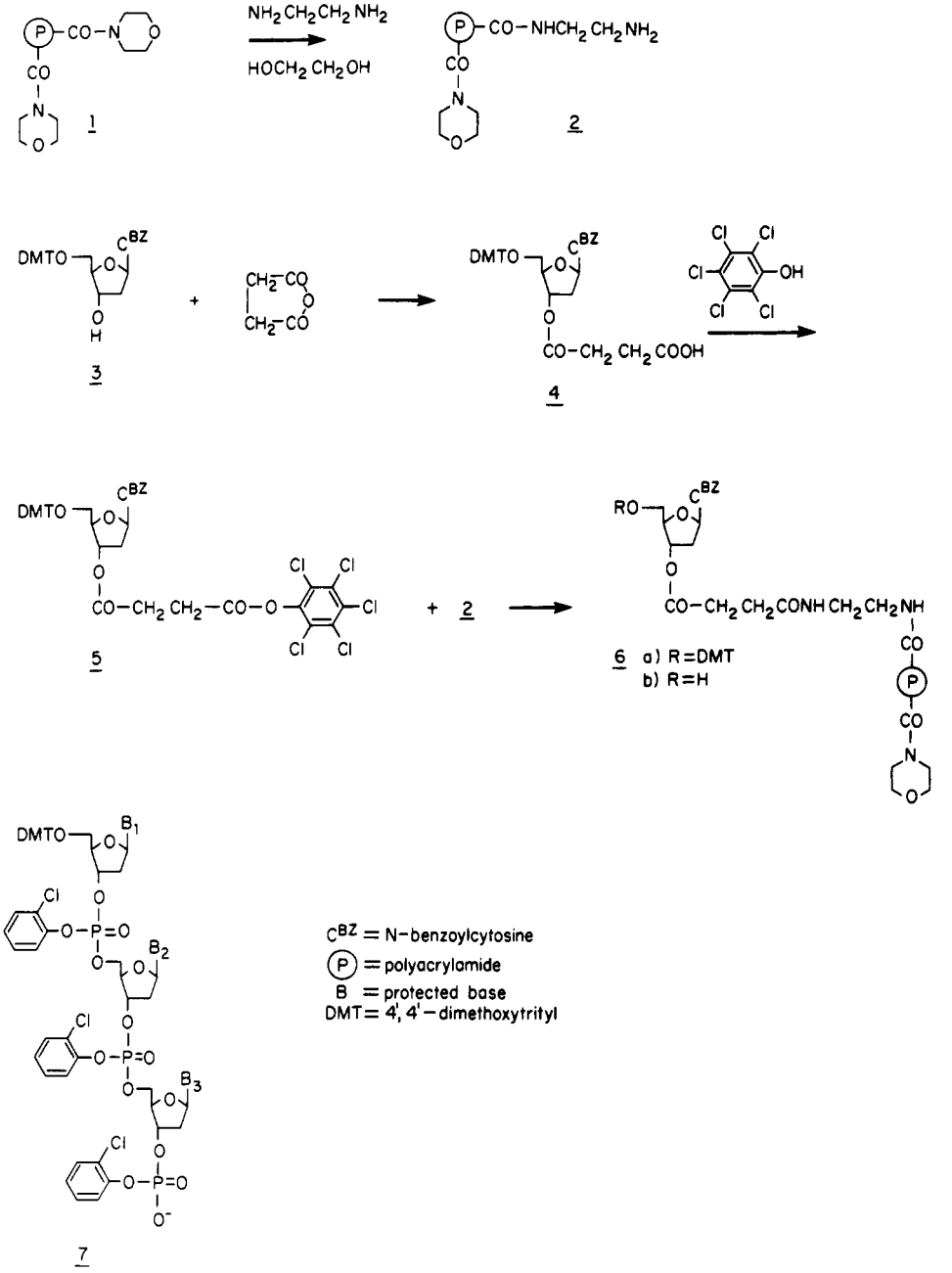
Scheme I



group liberated from the support was ca. 80%. After the last coupling reaction, the phosphotriester products on the support were treated with (i) 0.3 M N^1,N^2,N^3,N^3-tetramethylguanidinium pyridine-2-carboxaldoximate in dioxane–water (1:1 v/v) at 20 °C for 24 h to remove the phosphotriester protecting group and (ii) aqueous ammonia (28%) at 50 °C overnight to deblock the amino protecting groups. After filtration, the filtrate was concentrated and the residue was dissolved in 0.1 M triethylammonium bicarbonate (TEAB) buffer and extracted three times with ether. The aqueous solution was passed through a Sephadex G-50 column (0.1 M TEAB buffer, pH 7.5) and the excluded fraction was collected. The product was purified by high-performance liquid chromatography on a μBondapak C_{18} column (Waters) by using a linear gradient of acetonitrile (10–20%) at pH 7.8. Peak I (Figure 1) contained 5′-hydroxyl nucleotides and the peak II dimethoxytritylated products were collected and evaporated. The residue was treated with 80% acetic acid at room temperature for 15 min to remove the dimethoxytrityl group and the desired product was further purified by electrophoresis on an acrylamide gel in the presence of 7 M urea. The slowest moving band was isolated by electroelution in a 0.25% overall yield from the first nucleoside bound to the resin **6b**.[9] After labeling of the 5′-hydroxyl group with γ[^{32}P]-ATP,[10] the purity of the desired product was analyzed by electrophoresis on an acrylamide gel, which showed one band. The sequence of the hentriacontanucleotide was confirmed by the Maxam–Gilbert sequence analysis.[10]

Solid-phase synthesis is now realistic and practical for the preparation of polydeoxyribonucleotides and, to our knowledge, the hentriacontamer is the longest chemically synthesized deox-

(9) If the deblocking reaction of the dimethoxytrityl groups from the growing polynucleotide chains goes to completion, only the final product should have that group since any unreacted 5′-hydroxyl group is masked after each coupling reaction. However, the electrophoretical analysis of the detritylated product of peak II shows that ca. 50% of peak II is the desired product. In this synthesis, a mild detritylation condition (2% BSA, 30 s) was used in order to avoid a depurination reaction.

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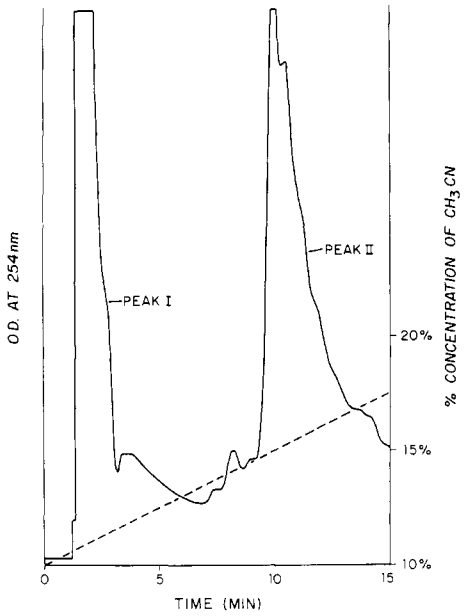

Figure 1. Absorption spectrum of the dimethoxytrityl group.

yribonucleotide with a defined sequence.

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Cyclization Dynamics of Polymers. 4. Electron Exchange between Chromophores on the Ends of Alkyl Chains: Diffusion-Controlled or Conformationally Controlled Cyclization?

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Several years ago Shimada and Szwarc[1,2] published an important series of papers investigating the rate of intramolecular electron exchange by ESR techniques in the molecules N-$(CH_2)_m$-$N^{-}\cdot$ and PI-$(CH_2)_m$-$PI^{-}\cdot$, where N is the 1-naphthyl substituent and PI represents the *N*-phthalimidyl moiety. Electron exchange occurs when the two end groups approach within a critical distance of one another. These papers have been frequently cited as representing the first experiments on the *dynamics* of end-to-end cyclization of hydrocarbon chains.[3,4] Dynamically controlled cyclization implies that the reaction occurs on every encounter—or in these examples on every other encounter since the electron can sit on either chromophore. On the other hand,

Scheme I

$$\cdot^{-}N\text{-}(CH_2)_m\text{-}N \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [\bar{N}N] \underset{k_t}{\overset{k_t}{\rightleftharpoons}} [N\bar{N}] \underset{k_t}{\overset{k_{-1}}{\rightleftharpoons}} N\text{-}(CH_2)_m\text{-}N^{-}$$

$$k_{ex}^{(1)} = k_1 k_t/(2k_t + k_{-1})$$

if the electron transfer is inefficient, the reaction is preceded by a conformational equilibrium and is said to be under thermodynamic or conformational control. The purpose of this communication is to point out that for the reactions cited above, *there is no evidence that electron exchange is sensitive to chain dynamics. Rather, all evidence points to these reactions operating under conformational control.*

The mechanism of these reactions can be represented in terms of Scheme I,[1b] where k_1 is the rate constant for diffusion together of the two chain ends, k_{-1} is that for their separation, and k_t is that for the electron exchange. Shimada and Szwarc point out that there are possibly many geometries for electron exchange possessing different intrinsic reactivities; k_t represents an average over these configurations.[1b] For this process to be dynamically (diffusion) controlled, electron transfer must be much faster than separation of the end groups, $k_t \gg k_{-1}$. The rate constant k_t is not infinitely fast. Since electron transfer is accompanied by solvent reorganization as well as changes in the counterion interaction, a nonnegligible activation barrier is anticipated[5] and found[1,2] for these reactions and their corresponding intermolecular counterparts:

$$1\text{-}C_2H_5\text{-}N^{-}\cdot + 1\text{-}C_2H_5\text{-}N \xrightarrow{k_{ex}^{(2)}} 1\text{-}C_2H_5\text{-}N + 1\text{-}C_2H_5\text{-}N^{-}\cdot$$

$$n\text{-}C_4H_9\text{-}PI^{-}\cdot + nC_4H_9\text{-}PI \xrightarrow{k_{ex}^{(2)}} n\text{-}C_4H_9\text{-}PI + n\text{-}C_4H_9\text{-}PI^{-}\cdot$$

In the electron exchange reaction between phthalimides, $k_{ex}^{(2)}$ values are much smaller than the diffusion limit.[2a] In addition, in five solvents $k_{ex}^{(2)}$ is independent of solvent viscosity. These experiments point to a rate-limiting electron-transfer step.

In the corresponding reaction between ethylnaphthalenes, $k_{ex}^{(2)}$ is much smaller than k_{diff},[1a] calculated from the Debye equation, for measurements in DME-2% HMPA, a very fluid solvent. In HMPA, these $k_{ex}^{(2)}$ values are only a factor of 3 smaller than the corresponding k_{diff} values (Table I). Shimada and Szwarc have results that suggest that electron transfer between naphthalenes in HMPA can occur over distances as large as 9 Å.[1] Thus the reaction cross section is much larger than the diffusion cross section for naphthalene in HMPA, and k_{diff} may be an order of magnitude larger than the values shown in Table I. Here again $k_{ex}^{(2)}$ is virtually independent of solvent viscosity.[1a]

The authors of these studies based their conclusion about dynamic control on a simple model calculation.[2] This model compares the experimental effective concentration, $C_{eff} = k_{ex}^{(1)}/k_{ex}^{(2)}$, to the corresponding concentration of two freely diffusing groups in a sphere whose radius is determined by the length r_{max} of the fully extended chain in the molecule from which $k_{ex}^{(1)}$ was determined. The idea is that r_{max} is the largest possible separation in N-$(CH_2)_m$-N. The average $\langle r \rangle$ is less than r_{max} and must lead to a higher effective concentration. These authors state "that the static model can never lead to the inequality $C_{eff} < C_{min}$, where $C_{min} = 1000/(V_{max}N_A)$, V_{max} being the volume of a sphere with radius equal to the length of extended chain. The dynamic model may account for the above inequality, and therefore our findings favor the dynamic model for the studied *intra*molecular electron transfer".[2b]

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