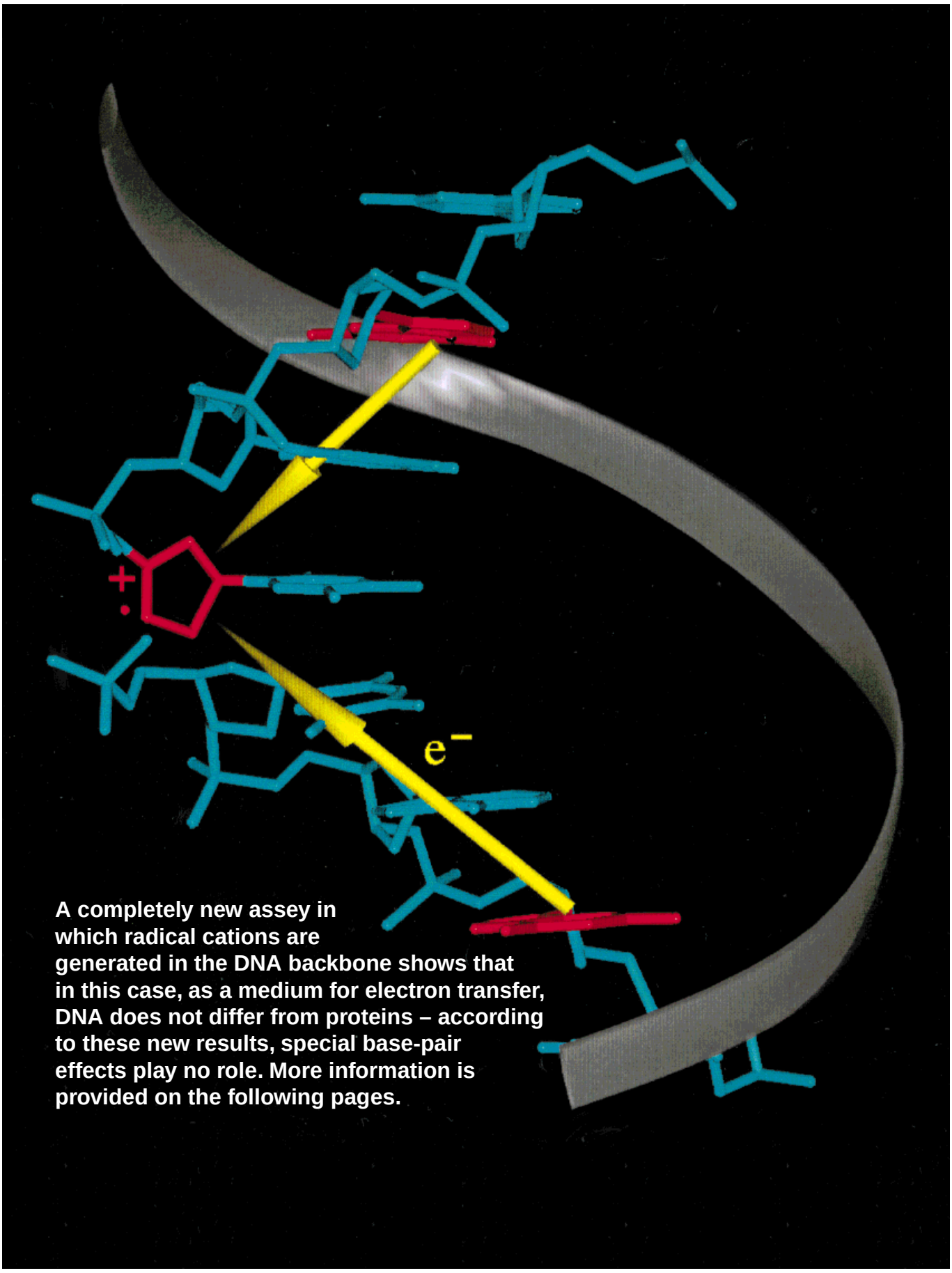

A completely new assey in which radical cations are generated in the DNA backbone shows that in this case, as a medium for electron transfer, DNA does not differ from proteins – according to these new results, special base-pair effects play no role. More information is provided on the following pages.

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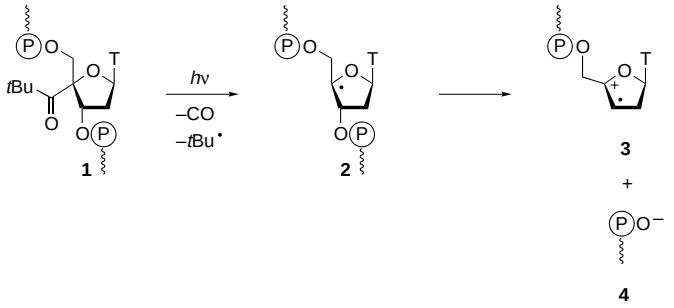
Electron Transfer through DNA in the Course of Radical-Induced Strand Cleavage**

Eric Meggers, Dirk Kusch, Martin Spichty, Uta Wille, and Bernd Giese*

In electron transfer (ET) reactions in proteins the transfer rates decrease by a factor of 10 for every 1.5 – 2.5 Å separation between reaction centers.[1] In sharp contrast to this generally accepted distance effect, studies on ET through the DNA have given contradictory results and raised a controversial discussion.[2] Depending upon the experimental probe either a minute[3] or a substantial[4] distance influence on the ET rate through DNA double strands was observed. In most of these studies the rates were determined by fluorescence quenching of photoactivated metal complexes or aromatic compounds attached to the DNA. In this communication we present a completely different assay that mimics the natural conditions of DNA reactions in a cell, and in which the ET through the DNA is measured by the chemical yield of a reduction step. The experimental method utilizes the spontaneous cleavage of a 4′-DNA radical **2**, which affords the radical cation **3** as a strong oxidant (Scheme 1).[5] We have observed that deoxy-



Scheme 1.

guanosine nucleotides within the DNA double strand can reduce this oxidized DNA fragment **3** and yield enol ether **5** by an ET step,[6] which mechanistically should be considered as a hole-transfer process.[4b–c, 7] The efficiency of this charge transfer depends upon the distance between the radical cation **3** and the guanine (G) base. It is important to note that in contrast to photoinduced ET studies, in our ground state experiments the ET is irreversible: there is no back transfer.

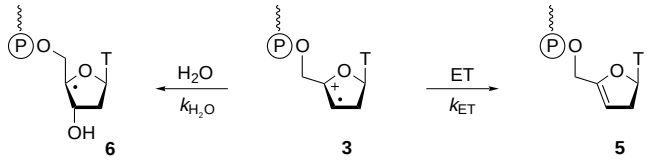
The experiments were carried out with DNA double strands in which one of the two DNA strands contained one 4′-acylated thymidine (T*) as in **1** that leads to radical cation **3** by Norrish type I cleavage (**1** → **2**) and subsequent heterolysis (**2** → **3** + **4**).[5b] The yield of phosphorylated oligonucleotide **4**, formed to the same extent as **3** in this reaction, is 71 – 89 %. The enol ether **5** was not detected when the double strands contained no G (Table 1, entries 1 and 2), but the yield reached 67 % if three guanosines were direct neighbors in the 5′- and 3′-direction (Table 1, entry 10).[8]

Table 1. Distance dependence of the electron transfer rates in single- and double-stranded DNA.

Entry	Sequence[a]	Δr [Å]	Yield of **5** [%][b,c]	$k_{ET,rel} \times 10^2$ [c]	Yield of **4** [%][b,c]
1	5′-dT$_5$T*T$_9$	-	-	-	81
2	5′-dT$_2$A$_3$T*T$_9$	-	-	-	78
3	5′-dG$_3$T$_2$T*T$_9$	14.1	< 0.2 (18)	< 0.25 (31)	80 (77)
4	5′-dT$_5$T*T$_2$G$_3$T$_4$	13.2	0.3	0.4	89
5	5′-dTG$_3$TT*T$_9$	11.1	1.2 (22)	1.6 (41)	76 (77)
6	5′-dT$_5$T*TG$_3$T$_5$	9.7	2.2	2.9	77
7	5′-dT$_2$C$_3$T*T$_9$	9.6	7.0	10	77
8	5′-dT$_2$G$_3$T*T$_9$	7.3	42 (30)	94 (45)	86 (96)
9	5′-dT$_5$T*G$_3$T$_6$	7.0	57 (36)	250 (92)	79 (75)
10	5′-dT$_2$G$_3$T*G$_3$T$_6$	-	67	310	89
11	5′-dT$_4$GT*T$_9$	7.3	24 (19)	52 (32)	71 (77)
12	5′-dT$_5$T*GT$_8$	7.0	41 (32)	100 (64)	83 (81)

[a] Sequence of the modified oligonucleotide; T* represents the 4′-acylated thymidine as in **1**; hybridization to the double strands was performed with the complementary, unmodified oligonucleotides. [b] Yields were obtained by HPLC. [c] Data for single strands in parentheses.

The yield of the enol ether **5** decreased from 57 to 0.3 % when the distance Δr[9,10] for the ET step increased from 7.0 to 13.2 Å (Table 1, entries 9 and 4). At a distance of 14.1 Å the reduction product **5** was not detectable: the ET reaction can no longer compete with other trapping reactions of the radical cation **3**. The main competition reaction is the addition of water (**3** → **6**, Scheme 2) that leads to further cleavage of the DNA.[11] Because of first-order or pseudo first-order conditions (excess of H_2O) the ratio between the rates of the ET reaction (k_{ET}) and all other trapping reactions (k_{trap})



Scheme 2.

like the water addition (k_{H_2O}) is given by the concentration ratio [**5**]/([**3**] – [**5**]).[12] Under the assumption that the competing reactions are not dependent upon the nucleotide sequence, we used the ratio k_{ET}/k_{trap} as the relative rate $k_{ET,rel}$ of the ET step (Table 1). In Figure 1 these relative rates are plotted against the distances Δr of the ET step according to Equation (1).[1]

$$k_{ET,rel} = A\, e^{-\beta \Delta r} \quad (1)$$

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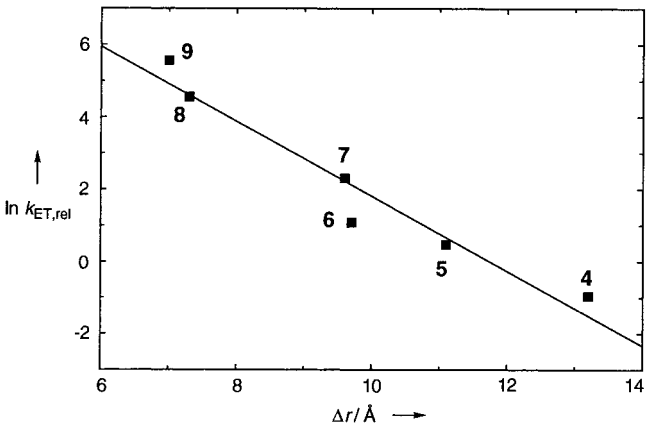

Figure 1. Distance dependence of electron transfer rates in double stranded DNA. The numbers at the data points correlate with the numbers at the arrows in Figure 2 and the entries in Table 1.

A linear dependence (correlation coefficient 0.97) of the rate ln $k_{ET,rel}$ upon the distance Δr of the ET reaction is observed. It is remarkable that the correlation holds even if the ET starts from the complementary strand (Table 1, entry 7) or from the cleaved strand (Table 1, entries 4, 6, and 9), and whether G is adjacent to the radical cation or separated by T bases. Figure 2 gives a graphical representation of this situation. The slope β of 1.0 ± 0.15 Å^{-1} in Figure 1 demonstrates that the ET rate decreases by a factor of 10 every 2.5 Å. Because this distance dependence is similar to ET reactions in proteins, it can be concluded that the hole transfer from the radical cation of the sugar phosphate backbone to the G base in DNA double strands does not benefit from base stacking effects (Figure 2).

From competition kinetic experiments with intermolecular electron donors (KI, methionine, selenomethionine, and $K_4[Fe(CN)_6]$) we deduced a rate of 10^6 M^{-1}s^{-1} for the addition of H_2O to the radical cation **3**.[13] With these data the rate coefficient of the electron transfer k_{ET} from G to a 7 Å distant deoxyribose radical cation (Table 1, entries 8 and 9) is 10^8 s^{-1}.[14]

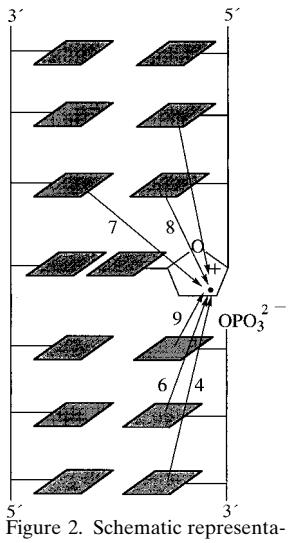

Figure 2. Schematic representation of the electron transfer (arrows) between guanine bases and the radical cation of the DNA sugar–phosphate backbone. The numbers at the arrows correlate with the numbers in Figure 1 and the entries in Table 1.

The β value of 1.0 Å^{-1} demonstrates that radical cation **3**, which is a reactive intermediate in the spontaneous strand cleavage of 4′-DNA radicals, can only be trapped efficiently by ET in DNA double strands if G bases are present in the immediate neighborhood. In sharp contrast the position of the deoxyguanosine has only little influence on the rate of the electron transfer in DNA single strands. As the data of Table 1 (entries 3 and 8) show, when two thymidine residues separate the G base from the deoxyribosyl radical cation **3**, the rate decreases by at least 400 in the double strand, but the same variation of the base sequence reduces the rate of electron transfer in single strands only by a factor of 1.5 (Table 1, data in parentheses). Obviously, single strands are flexible enough to adopt conformations in which the distance Δr between guanosine residues separated even by several nucleotides from the radical cation is short enough for a fast ET step. This assay opens new perspectives for the study of the conformation and flexibility of DNA molecules.

Experimental Section

The oligonucleotides were synthesized on a DNA synthesizer in 1 μmol scale. A standard procedure for 2-cyanoethyl phosphoramidites was used, except that the coupling of the 4′-pivaloyl-modified nucleoside was extended to 15 min. The resin of each synthesis was incubated in 1 mL concentrated ammonium hydroxide at 55°C overnight, and the mixture was subsequently lyophilized to dryness. The extracted crude oligonucleotide mixtures were purified by anion exchange chromatography and reversed phase HPLC. Finally, the oligonucleotides were passed through a sephadex column. The CD spectra of the modified double strands are very similar to those of the unmodified double strands and prove a B-DNA conformation. The UV melting temperatures T_m are decreased by the modification T* by only 0–2°C.

Irradiation experiments were carried out in poly(methyl methacrylate) cuvettes at 15°C by using a 500 W Hg high-pressure lamp with a 320 nm cut-off filter. A mixture of 2.0 nmol 4′-pivaloyl-modified oligonucleotide and 2.4 nmol complementary strand were dissolved in 300 μL buffer (20 mM sodium citrate pH 5.0 with 100 mM NaCl). Annealing of the complementary strand was achieved by heating to 70°C for 1 min and cooling down slowly to room temperature. The solutions were purged with argon for 30 min before photolysis. Single-stranded probes were irradiated for 4 min, double-stranded probes for 3 min. The irradiated solutions were analyzed by HPLC (Waters Symmetry C18, 3.5 μm, 100×4.6 mm) at 260 nm. Elution: Eluent A = 0.1M triethyl ammonium acetate (TEAA) pH 7.0; eluent B = acetonitrile. A typical gradient was 5% B for 2 min, then 5 to 13% B in 60 min; flow rate 1.5 mL min^{-1}, column temperature 30 or 50°C. Evaluation: The peak areas were divided by the calculated extinction coefficients of the corresponding oligonucleotides and quantified with external calibration. The identity of the products was determined by MALDI-TOF mass spectrometry, enzymatic digestion with analysis of the nucleotides, and by comparison of the reversed phase HPLC retention times with reference substances.

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[8] In the course of this ET step G must be oxidized to a radical cation that is known to undergo a fast deprotonation step yielding a very unreactive delocalized G radical. Since we cannot detect any damage at G sites, we assume that the G radical is repaired by hydrogen atom abstraction under anaerobic conditions. For structure, reactivity, and repair of deoxyguanosine radical cations, see: a) S. Steenken, *Chem. Rev.* **1989**, *89*, 503; b) L. P. Candeias, S. Steenken, *J. Am. Chem. Soc.* **1989**, *111*, 1094; c) S. V. Jovanovic, M. G. Simic, *Biochim. Biophys. Acta* **1989**, *1008*, 39.

[9] G triple units were chosen for the experiments on the distance dependence because they are the most potent electron donating sites in DNA (see ref. [6]). We suppose that the closest G is the initially oxidized base. This assumption is permitted since the change from a single G to a triple G unit in 3′- as well as in 5′-direction has nearly the same ET-acceleration effect of 2.5 and 1.8, respectively (Table 1, entries 8, 11, and 9, 12).

[10] For the determination of the distance Δr, the corresponding DNA duplex sequences were constructed in the B-form with the nucleic acid building tool in the program MacroModel V4.5. In order to mimic the planar enol ether radical cation **3**, the deoxyribose enol ether **5** was modeled into the DNA structure using the AMBER* force field implemented in MacroModel V4.5. Distances Δr were taken between the radical center C3′ of the radical cation and the G carbon atom 5 which has the highest electron density of the HOMO.

[11] Independent experiments with glutathione diethyl ester as a trap for radical **6** demonstrated that, in G-free DNA strands, the addition of water to radical cation **3** (**3** → **6**) occurs in about 70% yield.

[12] We determined the ratio k_{ET}/k_{trap} from the HPLC areas (corrected by the extinction coefficients) of 5′-phosphate **4** (A_4), whose concentration is equal to the overall yield of radical cation **3** (Scheme 1) and enol ether **5** (A_5). The ratio $A_5/(A_4 - A_5)$ is equal to k_{ET}/k_{trap}. Because these ratios turned out to be independent of the conversion, the reactions follow first- or pseudo first-order kinetics.

[13] We used one-electron donors like methionine, selenomethionine, KI, and $K_4[Fe(CN)_6]$ to reduce the radical cation **3** by intermolecular ET in competition to water addition. KI is the fastest ET quencher. Under the assumption that this ET proceeds in a nearly diffusion controlled way, we could determine a rate coefficient of $10^6\,M^{-1}s^{-1}$ by pseudo first-order kinetic experiments with different concentrations of KI.

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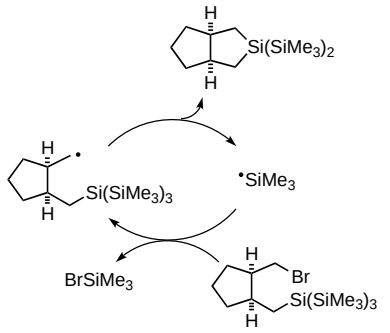
S_Hi Reactions at Silicon as Unimolecular Chain Transfer Steps in the Formation of Cyclic Alkoxysilanes**

Armido Studer*

Intermolecular transfer of bromine, iodine, phenylselenide, and aryltelluride groups between carbon-centered radicals is a well-established procedure for the synthesis of complex organic molecules.[1] In contrast (other than one example[2]), intramolecular transfer involving migration of selenium, tellurium or a halogen has not yet been observed. For the chalcogen group, only a single report describes the 1,4-transfer of a phenylsulfanyl group.[3] However, several reports on the intramolecular migration of stannyl or silyl groups have appeared.[4] The reasons for the facile intramolecular homolytic substitution (S_Hi) reaction at silicon or tin are not well understood.[5]

These S_Hi reactions can be used for the formation of silicon- or tin-containing heterocycles. Giese et al.[6] and Utimoto et al.[7] independently reported intramolecular homolytic substitutions at tris(trimethylsilyl)-substituted Si atoms by C-centered radicals. It was concluded that the trimethylsilyl radical generated during the intramolecular substitution acts as the chain carrier (Scheme 1). According to Curran's terminology,[8] this is an example of a unimolecular chain



Scheme 1. S_Hi reaction at tris(trimethylsilyl)-substituted silanes.

transfer (UMCT) reaction in which the key chain transfer step is a unimolecular process. After consideration of the results of Giese's and Utimoto's groups, we initiated further exploration of intramolecular homolytic substitutions at silicon. It was envisaged that this transformation would be a suitable UMCT step in bimolecular radical addition reactions, where either a silyl or a stannyl radical acts as the chain carrier. In addition, the cyclic silanes formed in these UMCT steps could be transformed into the corresponding alcohol derivatives by Tamao – Fleming oxidation.[9]

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