1. Use of the *Wolff* Rearrangement of Diazo Ketones from Amino Acids as a Synthetic Method for the Formation of Oligonucleo-peptides: A Novel Approach to Chimeric Biomolecules

by **Christel Guibourdenche**[1]) and **Dieter Seebach***

Laboratorium für Organische Chemie der Eidgenössischen Technischen Hochschule, ETH-Zentrum, Universitätstrasse 16, CH–8092 Zürich

and **François Natt**

Ciba-Geigy Ltd., Central Research Laboratories, R-1060.2.14, CH–4002 Basel

(16.X.96)

Photolysis and Ag-benzoate-catalyzed decomposition of the diazo ketones **2** and **4** derived from Z-Ala-OH and Z-Ala-Ala-OH in the presence of oligonucleotide derivatives bearing at the 5′-terminus an NH_2 instead of the OH group, or an aminohexyl phosphate group lead to Z-protected 3-aminobutanoyl and to Z-Ala-β-HAla derivatives, respectively (conjugates **12**, **13**, and **17–23**, *Schemes 3–5*). In solution, this amide-forming acylation reaction could be realized only with oligomers containing up to 8 unprotected nucleotide building blocks (*Schemes 3* and *4*). With the analogous polymer-bound and protected oligonucleotide derivatives as amino nucleophiles, excellent yields were obtained with all chain lengths tested (up to 15mer, *Scheme 5*). The products were purified by reversed-phase HPLC and characterized by MALDI-TOF mass spectrometry (*Figs. 2–4*, *Table 2*) and by capillary gel electrophoresis (*Fig. 2*).

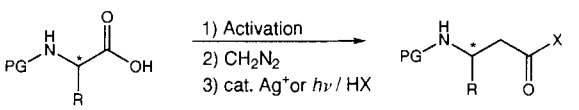
1. Introduction. – Owing to the potential of oligonucleotides or peptides as therapeutic agents and the tremendous efforts put into the chemical development of these biopolymers over the past 25 years, conjugates such as those derived from oligonucleotides conjugated with peptides (also called oligonucleo-peptides) are attracting increasing interest. These conjugates offer a new chemical way to broaden the spectrum of properties of synthetic oligonucleotides [1]. For example, conjugation of either cationic peptides such as poly-L-Lys or poly-L-Arg or of hydrophobic groups such as tryptophan with an oligonucleotide can increase its cellular uptake [2]. Oligonucleotides have also been conjugated to peptide signal sequences [3] and α-helical peptides [4]. Recently, nucleopeptides were proposed as potential artificial nucleases [5] [6]. Furthermore, a lysine-rich peptide coupled to a DNA sequence was reported to show a 48 000 fold acceleration over its unmodified counterpart during duplex formation [7]. The combination of know-how acquired in both fields of peptide and oligonucleotide synthesis has triggered different synthetic strategies. Linear solid-phase syntheses of these hybrid molecules are described which required special protecting schemes due to chemical incompatibilities of standard oligonucleotide or peptide reactivities and properties [8]. 5′-Peptide-oligonucleotide conjugates were reported to be accessible in a convergent manner by reaction of a phosphoramidite derived from a suitably protected peptide containing an OH group. For example,

[1]) Postdoctoral research fellow, ETH Zürich, 1995 (stipend from *Sandoz Pharma AG*, Basel).

van Boom et al. prepared 5′-peptide-oligonucleotides *via* reaction of 2-cyanoethyl phosphoramidites derived from di- or tripeptides containing a serine or tyrosine residue with a support-bound oligodeoxyribonucleotide [9] or an oligoribonucleotide [10]. In solution, the condensation of mercapto-oligonucleotides with either *N*-(bromoacetyl)-peptides [11] or with maleido-peptides [12] is commonly used. Condensation of active esters or isothiocyanates with amino-functionalized oligonucleotides (although possible in solution) are usually performed on solid support [1].

As part of our synthetic investigations into peptide backbone modifications, we have prepared β-amino acids from α-amino acids and incorporated them into peptides [13]. β-Amino acids, although less abundant than α-amino acids, are components of a variety of natural products having antitumor activity [14] (*e.g.* taxol) or antibiotic activity (*e.g.* β-lactams) [15]. Furthermore, their incorporation in peptides might induce particular turns and special secondary structures [16]. The preparation of such compounds has been reviewed [17]. We focused on the *Arndt-Eistert* reaction which presents, as its main advantage, the use of the broad (and well-known) α-amino-acid 'library' as starting materials (*Scheme 1*).

Scheme 1



In an earlier paper [18], we pointed out a surprising selectivity in the acylation by ketenes (obtained from the *Wolff* rearrangement of amino-acid-derived diazo ketones) of molecules bearing multiple nucleophilic sites, and we were (by encouraging results with model compounds) prompted to test these observations on deoxyribo- or ribonucleosides. These molecules bear a single primary OH group at C(5′) which can compete with one or two secondary OH groups, as well as with nucleophilic positions in the base component (see *Fig. 1*). Still, no acylation of the bases has been observed. In the case of simple model compounds, the functional-group selectivity between primary amines, and primary or secondary alcohols was found to favor almost completely the amino group. This motivated us to extend this reaction to more complex molecules such as oligonucleotides (*Fig. 1*) or amino-functionalized oligonucleotides (which would offer as only reactive site a 5′-terminal nucleophilic amino group). Therefore, both reactions of unprotected oligonucleotides in solution as well as of protected support-bound oligonucleotides with the amino-acid-derived ketenes appeared worthy of investigation.

We report here our results on the synthesis of chimeric biomolecules by means of the *Wolff* rearrangement of diazo ketones derived from α-amino acids or peptides in the presence of oligonucleotides, in solution or on solid phase.

2. Results and Discussion. – 2.1. *Rearrangement of Diazo Ketones in Various Solvent Systems.* Diazo ketones **1**–**4** (*Scheme 2*) were prepared from the corresponding amino acids as reported previously [16–18]. Before studying the reactivity of oligonucleotides as nucleophiles in the *Wolff* rearrangement of these diazo compounds, we undertook a model study in different solvent systems commonly used to dissolve ionic, hydrophilic, and high-molecular-weight compounds such as oligonucleotides. We focused on MeCN, DMF, and *N*,*N*-dimethylacetamide (DMA) and studied the influence of lithium salts

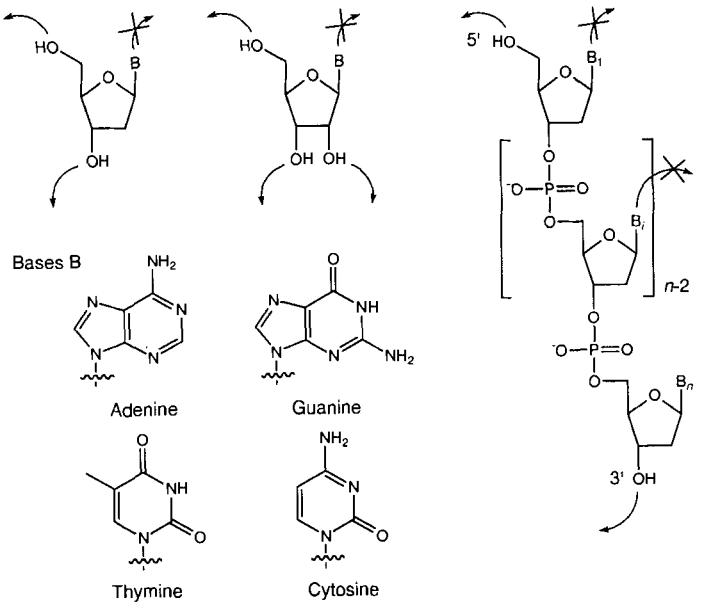
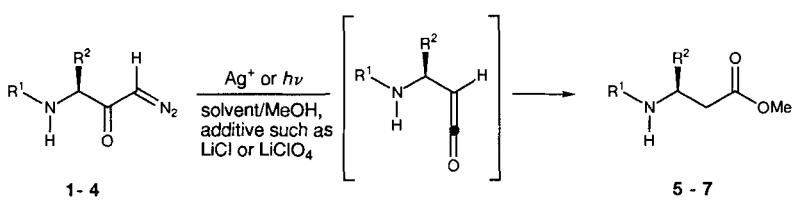


Fig. 1. *Potential nucleophilic sites of nucleosides and oligonucleotides for acylations by ketenes from the* Wolff *rearrangement*

(LiCl or $LiClO_4$) on the *Wolff* rearrangement of diazo ketones derived from α-amino acids. Lithium salts are known to contribute to the solvation of hydrophilic molecules such as peptides [19], nucleotides [20], or cellulose [21] in aprotic solvents. We performed the *Wolff* rearrangement of diazo ketones **1–3** derived from Z-L-valine, Z-L-alanine and Boc-L-alanine (Z = (benzyloxycarbonyl), Boc = (*tert*-butoxy)carbonyl), respectively, in the presence of MeOH as nucleophile and *via* two different cleavage procedures: silver benzoate catalysis or photolysis (*Scheme 2*). The results are summarized in *Table 1*.

Scheme 2. *Diazo Ketones* **1–4**, *and the* Wolff *Rearrangement of* **1–3** *in the Presence of MeOH and of Additives* (see *Table 1*)



R^1	R^2	Diazo ketone	Ester
BnOCO (Z)	*i*-Pr	**1**	**5**
BnOCO (Z)	Me	**2**	**6**
t-BuOCO (Boc)	Me	**3**	**7**
Z-Ala	Me	**4**	–

Table 1. *Influence of Solvent and of Added Lithium Salts in the* Wolff *Rearrangement of the Diazo Ketones* **1**–**3** *in the Presence of MeOH to Give the Esters* **5**–**7** (see *Scheme 2*). DMF = *N*,*N*-dimethylformamide, DMA = *N*,*N*-dimethylacetamide.

Entry	Diazo ketone	Solvent	Salt additive	Method of decomposition	Product	
					No.	Yield [%]
1	**1**	MeCN	–	Ag^+	**5**	93
2	**1**	MeCN	LiCl	Ag^+	**5**	–
3	**2**	MeCN	LiCl	*hν*	**6**	93
4	**3**	MeCN	$LiClO_4$	Ag^+	**7**	93
5	**3**	DMF	–	Ag^+	**7**	92
6	**3**	DMF	$LiClO_4$	Ag^+	**7**	92
7	**2**	DMA	LiCl	*hν*	**6**	75
8	**2**	DMA	$LiClO_4$	Ag^+	**6**	80

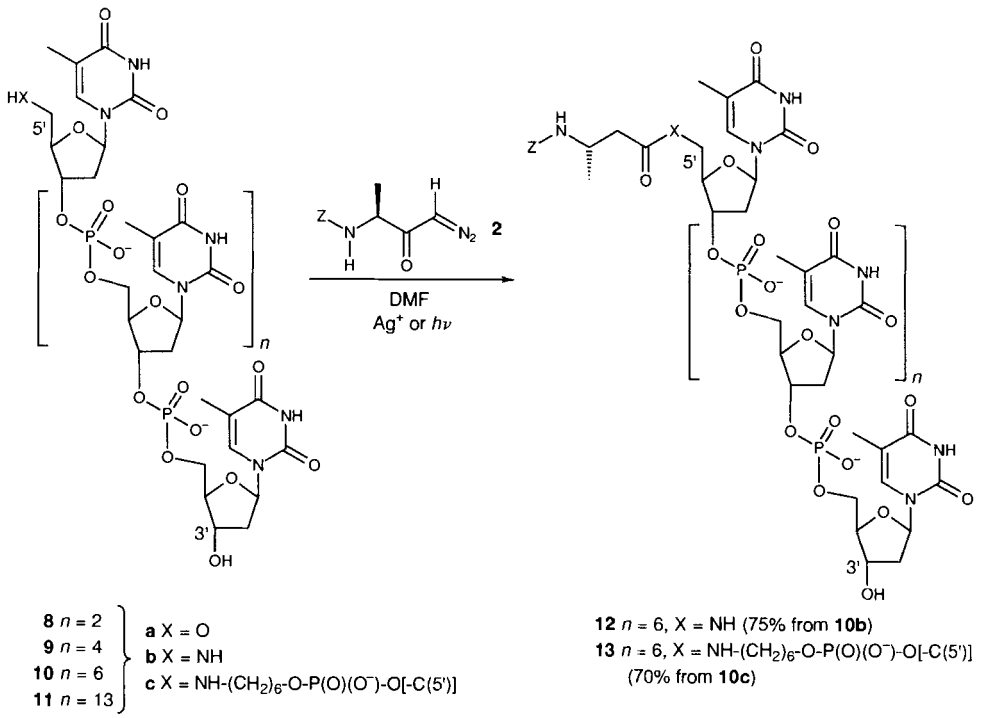
With Ag^+ activation, rearrangement occurred to give the *β*-amino esters **5**–**7**, in MeCN and DMF, with or without added $LiClO_4$, as well as with the DMA/$LiClO_4$ couple, in good to excellent yields (80–93%; *Entries 1*, *4*–*6*, and *8* of *Table 1*). The presence of LiCl inhibited the reaction with Ag^+ (by formation of AgCl, *Entry 2*). In the photochemically induced reactions, there was no interference with the rearrangement by the amide solvents, by MeCN, or by the lithium salts. The ester **6** was obtained in 75 and 93% yield (*Entries 3* and *7* of *Table 1*). Consequently, all solvent systems and additives were suitable for the decomposition of diazo ketones, the *Wolff* rearrangement, and the trapping of the generated ketenes by hydroxylated nucleophiles.

2.2. *(β-Aminoacyl)ations of Oligonucleotides in Solution.* We studied two types of nucleophiles (see *Scheme 3*): oligonucleotides (series **a**) and 5′-amino-5′-deoxy- (series **b**) or $O^{P5'}$-(aminoalkyl)-oligonucleotides (series **c**) (see *Scheme 3*). The *Wolff* rearrangement of diazo ketones in the presence of oligonucleotides **a** should eventually lead to biodegradable ester-linked conjugates where the peptide part would act as a 'carrier' molecule [22]. Reactions involving 5′-amino-functionalized oligonucleotides **b** and **c** would provide amide-linked conjugates, both parts of which could play a role in the bioactivity of the molecule [1]. We first tested the rearrangement of the diazo ketone **2** derived from Z-L-alanine in the presence of homogeneous sequences such as polythymidines **8**–**11** of various lengths and with different 5′-terminal functions (**a**–**c**) using the two decomposition procedures (Ag^+ and *hν*). The reaction mixtures were analyzed by matrix-assisted laser-desorption time-of-flight mass spectroscopy (MALDI-TOF MS) [23], and the purity of isolated conjugates was assayed by capillary gel electrophoresis (CGE).

No acylated products derived from the oligonucleotides **8a**, **9a**, and **10a** with 4, 6, and 8 thymidine units were detected. When silver benzoate was used, the formation of a complex between Ag^+ and the oligonucleotide was observed by MALDI-TOF MS with the detection of a peak at $[M + 107]^+$. Treatment of this complex with NaI led to removal of Ag^+ and recovery of the oligonucleotide (as shown by MALDI-TOF MS). We first thought that the lack of reactivity might be caused by the NH_4^+ counter ions of the oligonucleotide phosphodiester groups, which might interact with the reactive intermediate. However, when the reaction was performed with different salt forms of polythymidine (sodium or potassium) at various temperatures (−20°, +5°, and room temperature) the result was the same: the starting oligonucleotide was recovered in quantitative

yield. When the diazo ketone **2** was photolyzed in the presence of the oligonucleotides **8a**, **9a**, and **10a**, again, no ester formation was observed. In both reactions, a TLC analysis of the reaction mixture indicated complete decomposition of the diazo compound[2]).

Scheme 3. *Attempted (β-Aminoacyl)ations of Polythymidine Derivatives* **8**–**11** *by the Ketene Intermediate Formed from* **2**. *Formation of Z-Protected β-Amino Acid Amides* **12** *and* **13**



For the 5′-amino-functionalized oligonucleotides **10b** and **10c**, we expected a strong preference for amide *vs.* ester formation [18]. In experiments in which the silver catalyst was used at −20°, +5°, and room temperature, again, none of the desired product was detected. The formation of a complex with Ag^+ was again observed in these cases. However, the diazo-ketone photolysis (**2**, at room temperature, overnight) gave the desired conjugates **12** and **13**; no starting material was present in the crude product mixture (by MALDI-TOF MS analysis). The products **12** and **13** were isolated in 75 and 70% yield, respectively, after a reversed-phase HPLC (see *Scheme 3*, *Fig. 2*, and *Table 2*). It is interesting to note that, under the conditions used, there was no difference of reactivity between the amine functions attached to the 5′-terminal thymidine directly or through the linker.

---

[2]) Presence of H_2O in the reaction mixture containing the hygroscopic oligonucleotides might have prevented the desired reaction.

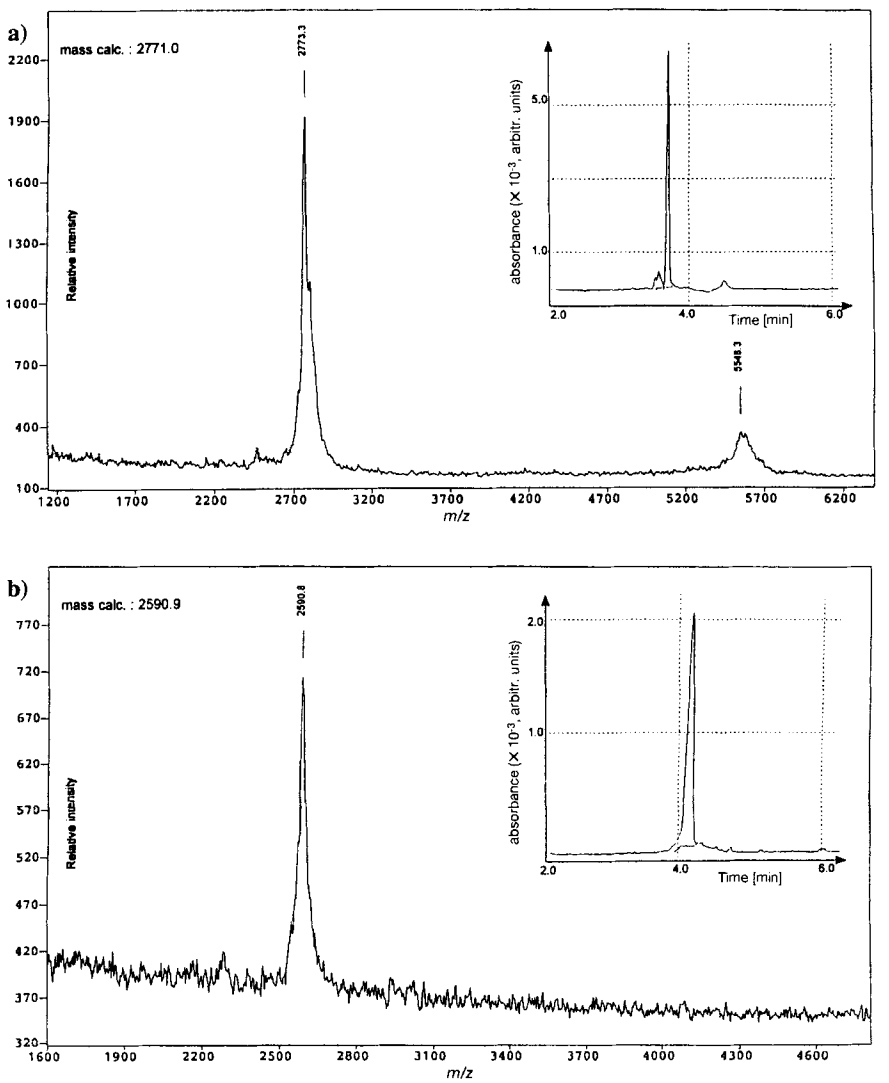


Fig. 2. *MALDI-TOF Mass spectra* a) *of purified Z-protected 5′-(3-aminobutanoylamino)-5′-deoxyoctathymidine derivative* **12** ([*M* – H]⁻ at 2773.3, [2 *M* – H]⁻ at 5548.3) *and* b) *of the crude conjugate* **13** ([*M* – H]⁻ at 2590.8), *as well as CGE* (insets) *of the purified conjugates:* a) **12** *and* b) **13**

Thus, it turns out that the photoactivation is the method of choice for coupling in solution of 5′-amino-functionalized oligonucleotides with the ketenes generated from diazo ketones of type **1**–**4**. We next investigated the photoinduced rearrangement of diazo ketones **2** and **4** derived from the amino acid Z-L-alanine and from the dipeptide Z-Ala-Ala-OH in the presence of $O^{P5'}$-(aminohexyl)-oligonucleotides containing thymidine of various lengths (**10c**, **11c**) or various mixed sequences (**14**–**16**) (*Scheme 4*). The reaction of

Table 2. *Characterization by HPLC and MS, and Yields of the Z-β-HAla Derivative* **12** *and of the Z-β-HAla Derivatives* **13** *and* **17**–**23** *of Aminohexyl-5′-Phosphates, Obtained from Octa-* (**10b**, **10c**, **14**, **15**), *Dodeca-* (**16**), *and Pentadecanucleotides* (**11c**)

Starting materials[a])		Products (Z-Ala-NH or Z-Ala-β-HAla-NH derivatives)				
		No.	Yield [%]	HPLC[b]) t_R [min]	Molecular ion [Da] (by MALDI-TOF)[c])	
					calc.	found
2	**10b**	**12**	75	11.22	2590.9	2590.8
2	PB-**10b**-PG	**12**	85	11.28		2592.2
2	**10c**	**13**	70	13.05	2771.0	2773.3
2	PB-**10c**-PG	**13**	86	13.04		2770.9
4	**10c**	**17**	35	13.22	2842.1	2845.3
4	PB-**10c**-PG	**17**	84	13.16		2842.9
2	**14**	**18**	20	10.90	2809.0	2810.4
2	PB-**14**-PG	**18**				2808.8
2	**15**	**19**	40	11.15	2839.0	2839.5
2	PB-**15**-PG	**19**				2838.4
4	PB-**14**-PG	**20**	87	12.35	2880.1	2880.4
4	PB-**15**-PG	**21**	94	12.00	2910.1	2906.4
4	PB-**11c**-PG	**22**	92	12.43	4971.5	4968.6
4	PB-**16**-PG	**23**	79	12.37	4115.9	4112.0

[a]) PB-**n**-PG stands for *p*olymer-*b*ound oligonucleotides with *p*rotecting *g*roups on the internal phosphate group and on the bases (see *Scheme 5*).

[b]) The retention times were determined as described in the *General Procedure GP 2*.

[c]) The mass spectra were measured as described in *GP 2*, some molecular-ion peaks are shown in *Figs. 2–4*.

the diazo ketone **2** derived from Z-L-alanine with mixed octamer sequences **14** and **15** gave the desired amides **18** and **19** in 20 and 40% yield, respectively, after reversed-phase HPLC purification (*Table 2*). The difference of yields obtained with these two sequences might be due to the presence of cytidine[3]) in **14**. The reaction of diazo ketone **4** derived from Z-Ala-Ala-OH with octathymidine derivative **10c** as nucleophile gave the conjugate **17** in 35% yield (*Fig. 3*). On the other hand, the mixed sequences **14** and **15** did not lead to the desired products, even after prolonged reaction times, with the dipeptide derivative **4**. Attempted reactions of the ketene generated from the diazo ketone **2** with the longer homogeneous and mixed aminoalkylated oligonucleotide sequences **11c** and **16** were unsuccessful: The solubility of these oligonucleotides in DMF was insufficient, even after heating at 60° or after sonication. Inspite of the fact that the use of LiCl/DMA (5% *w*/*v*) led to complete and immediate dissolution of the nucleotides, no product formation was observed.

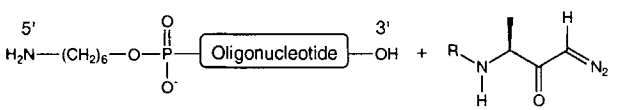
More complex oligonucleotide derivatives can, obviously, no longer be acylated by this method. One reason for this failure may be the multitude of unprotected bases in the larger oligonucleotides (an especially problematic base being cytosine[3])). Another reason for the acylating ketene not to 'find' the nucleophilic NH_2 group may be the presence of too many competing H_2O molecules which have not completely been removed from the

[3]) We described previously the difficulties encountered with acylations in the presence of cytidine [18].

hygroscopic oligonucleotides used in the reaction[4]). We, therefore, turned to amino-alkyl phosphodiesters of solid-support-bound oligonucleotides, in which the bases and the phosphodiester groups are protected.

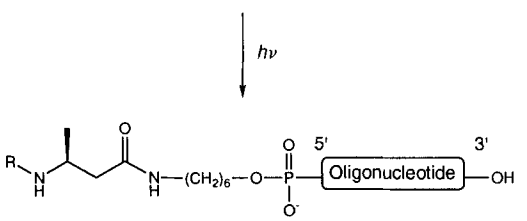
2.3. *(β-Aminoacyl)ations of Support-Bound and Protected Amino-Modified Oligonucleotides.* After attachment of the 5′-amino modifier to the support-bound oligonucleotide and deprotection of the terminal amino function (→ 5′-(aminoalkyl phosphate)), the support-bound substrate presents only one nucleophilic site: The phosphodiester and the exocyclic amino groups on the base components are still protected in the usual way (cyanoethyl, benzoyl, isobutyryl) (see *Scheme 5*). We thought that the silver-benzoate mode of diazo-ketone decomposition would offer the best chance of success: The porous character of the support used (polystyrene or controlled pore glass (CPG)) might have caused a reduced photolysis rate by partial absorption of the light. We decomposed the diazo ketone **2** derived from Z-L-alanine and the diazo ketone **4** from Z-Ala-Ala-OH (20 equiv., MeCN, $PhCO_2Ag/Et_3N$) in the presence of a broad spectrum of amino-modified oligonucleotide sequences, and the results are shown in *Scheme 5*.

Scheme 4. *Diazo-Ketone Decomposition with* Wolff *Rearrangement and Ketene Trapping by* $O^{P5'}$*-Aminoalkyl-Substituted Oligonucleotides* **10c**, **11c**, *and* **14–16**



10c, 11c, 14 - 16

2 R = Z
4 R = Z-Ala



17 R = Z-Ala (35% from **10c** and **4**)
18 R = Z (20% from **14** and **2**)
19 R = Z (40% from **15** and **2**)

	Oligonucleotide sequence 5' to 3'
10c, 17	d(TTT TTT TT) (*cf. Scheme 3*)
11c	d(TTT TTT TTT TTT TTT) (*cf. Scheme 3*)
14, 18	d(TCG AAG CT)
15, 19	d(TTG AAG TT)
16	d(TCG ATG CAA GCT)

[4]) A third reason might be that the phosphate anions, when increasing in number, compete successfully with the amino group (the mixed anhydrides thus formed may not be available as actual acylating sites when the oligonucleotides are too large?).

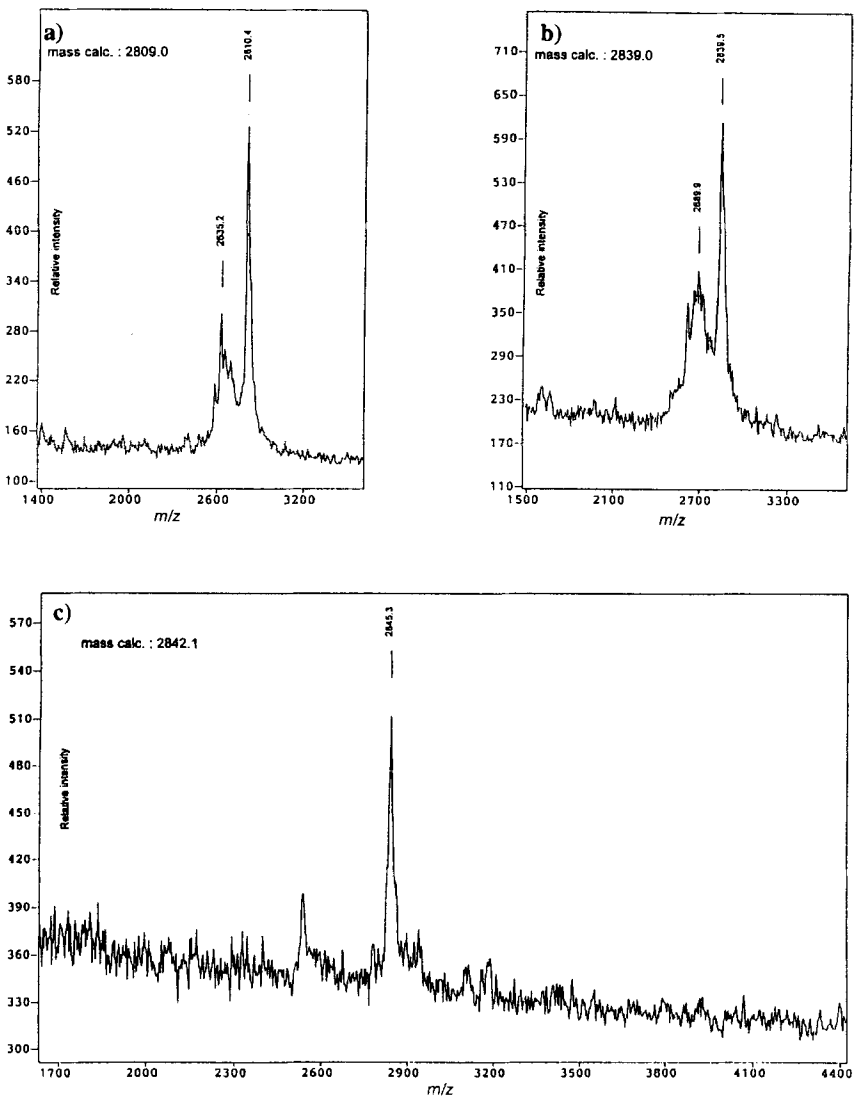
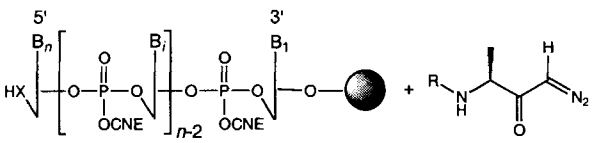


Fig. 3. *MALDI-TOF Mass spectra* a) *of the crude conjugate* **18** ([*M* – H]$^-$ at 2810.4), b) *the crude conjugate* **19** ([*M* – H]$^-$ at 2839.5) *and* c) *of the purified conjugate* **17** ([*M* – H]$^-$ at 2845.3)

The reaction of diazo ketone **2** with the amino-functionalized octathymidine derivatives (polymer-bound and protected **10b** and **10c** with a 5′-amino and 5′-(aminohexyl phosphate) group, resp.) as well as with oligonucleotide derivatives (containing the mixed sequences of **14** and **15**) afforded the corresponding (*β*-aminoacyl)amino-substituted compounds **12**, **13**, **18**, and **19**. When the dipeptide-derived diazo ketone **4** was decomposed in the presence of the same nucleophiles derived from **10c**, **14**, and **15**, the conjugates **17**, **20**, and **21** were obtained with yields ranging from 84 to 94%. Application

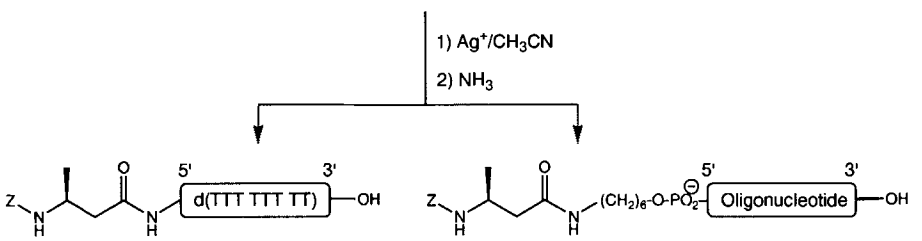
of the reaction to longer polymer-bound oligonucleotide derivatives (of the homonucleotide **11c** and of the mixed sequence **16**, containing 15 and 12 residues, respectively) gave the desired nucleodipeptides **22** and **23** in excellent yields of 92 and 79% (see *Scheme 5* and *Fig. 4*) after the one-step cleavage from the polymer and deprotection by treatment with 32% aqueous NH_3. As can be seen from the formation of products **18**, **20**, and **23**, the cytosine base is compatible with the reaction carried out under these conditions. All nucleo-amide and nucleo-peptide conjugates were characterized by mass spectroscopy either in the crude protected mixture or after reversed-phase HPLC purification.

Scheme 5. *Ag^+-Induced Decomposition of Excess Diazo Ketones* **2** *and* **4** *in the Presence of Solid-Support-Bound and Protected Oligonucleotide Derivatives and Cleavage/Deprotection to Give the Conjugates* **12**, **13**, *and* **17–23**[a])



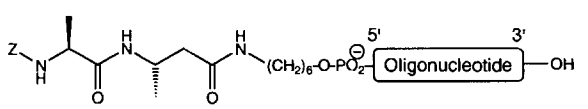
polymer bound and protected form of oligonucliotides **10b**, **10c**, **11c**, **14** - **16** (see *Schemes 3* and *4*)

2 R = Z
4 R = Z-Ala
(see *Scheme 2*)



12 (see *Scheme 3*)

13, **18**, **19** (see *Schemes 3* and *4*)



17, **20** - **23** (see *Scheme 3* and accompanying table)

Polymer-bound and protected oligonucleotide derivative	**10b**	**10c**	**10c**	**11c**	**14**	**14**	**15**	**15**	**16**
Product No	**12**	**13**	**17**	**22**	**18**	**20**	**19**	**21**	**23**
R	Z	Z	Z-Ala	Z-Ala	Z	Z-Ala	Z	Z-Ala	Z-Ala
Yield[%]	85	86	84	92	n.d.[b])	87	n.d.[b])	94	79

[a]) For specifications, see *Schemes 2–4*. The internal phosphate groups were protected as cyanoethyl (CNE) esters, the bases as benzoyl and isobutyryl derivatives. [b]) The yields of **18** and **19** were not determined (these compounds have also been obtained in solution!).

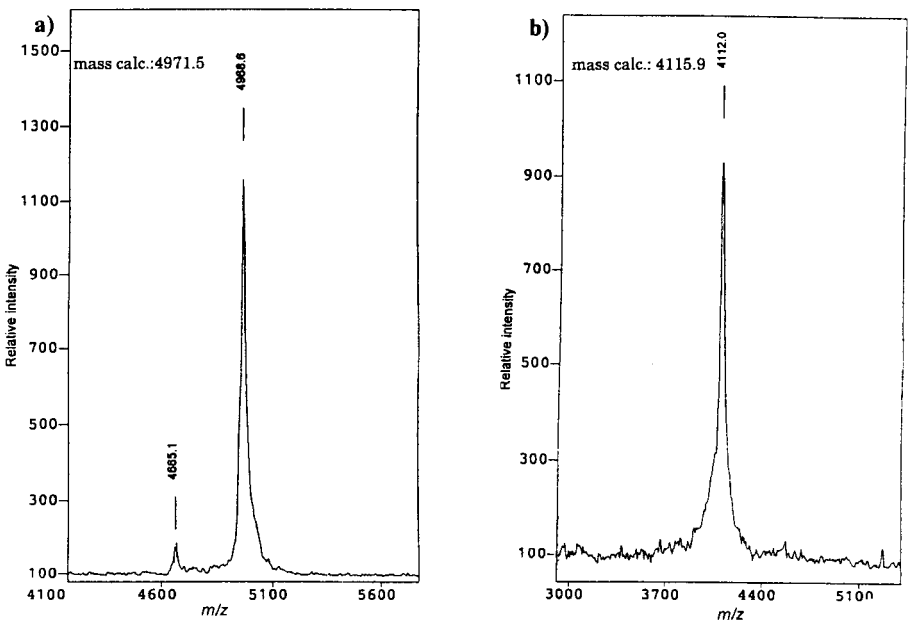


Fig. 4. *MALDI-TOF Mass spectra of purified nucleo-dipeptide derivatives:* a) **22** ([*M* – H]$^-$ at 4968.6) *and* b) **23** ([*M* – H]$^-$ at 4112.0)

3. Conclusion. – The use of amino-modified oligonucleotides as nucleophiles for trapping the acylating intermediates from the *Wolff* rearrangement of diazo-ketones derived from the amino-acid L-alanine and from the dipeptide Z-Ala-Ala-OH led to the formation of conjugates in moderate to excellent yields. In solution, the method appears to be limited to – unprotected – oligonucleotides containing up to eight residues. The solid-phase modification, on the other hand, is length- and sequence-independent up to at least 15 nucleoside units and is the method of choice for the synthesis of oligonucleo-dipeptides by the *Wolff* rearrangement from the corresponding $O^{P5'}$-(aminoalkyl)-oligo-nucleotides and diazo ketones. The conjugates thus available may have biological activity and the conjugation with β-peptides can be envisaged.

We thank Dr. *R. Häner* for helpful discussions, Dr. *A. Waldner* for the gift of 5′-[(monomethoxytrityl)amino]-thymidine 3′-[(2-cyanoethyl)diisopropylphosphoramidite], and *A. K. Beck*, Dr. *J. L. Matthews*, *I. Naumann*, and *S. Sigrist* for their help during the final preparations of this manuscript.

Experimental Part

1. *General.* Abbreviations: DMA (*N*,*N*-dimethylacetamide), EDTA (ethylenediaminetetraacetic acid), *GP* (*General Procedure*), h.v. (high vacuum, 0.01–0.1 Torr). Common three-letter amino-acid and one-letter nucleoside abbreviations are used [24]. All solvents for reactions were used as purchased from *Fluka*. Crude solvents for chromatography and for workup were distilled from *Sikkon* (Et_2O from KOH/$FeSO_4$). Amino-acid derivatives were purchased from *Bachem*, *Senn*, and *Degussa*. Lithium salts were heated at 60° under h.v. for 15 min and cooled under h.v. before use. Et_3N was distilled from CaH_2. The diazo ketones **1**–**4** were prepared in accordance with [18]. Moisture-sensitive reactions were performed in dried vessels (140°, 24 h) under Ar using syringe techniques.

Caution: The generation and the handling of diazomethane requires special precautions [25]. Reagents for oligonucleotide synthesis were purchased from *Cruachem* and *Glen Research* (aminohexyl modifier). Oligonucleotide syntheses and modifications were performed on a *Applied-Biosystems-394B* DNA synthesizer. Flash column chromatography (FC): *Merck* silica gel *60* (230–400 mesh). TLC: precoated plates, silica gel *60* F_{254}, *Merck*; detection by UV visualization or cerium molybdate soln. (phosphomolybdic acid (25 g), $Ce(SO_4)_2 \cdot H_2O$ (10 g), conc. H_2SO_4 (60 ml), H_2O (940 ml)), I_2/KI soln. (I_2 (18.8 g), KI (1.25 g), H_2O (125 ml), EtOH (125 ml)). Reversed-phase (RP)-HPLC: *Shandon-C_{18}ODS-Hypersil* column (5 μm, 125 mm × 4.6 mm) on a *Beckman Gold* liquid chromatography system. Capillary gel electrophoresis (CGE): *Beckman* capillary electrophoresis system, model *P/ACE 5010*, equipped with *P/ACE 3.0* and 'Caesar' software for data analysis. NMR: *Bruker Avance DPX 400* (^{1}H 400 MHz; ^{13}C 100 MHz); δ in ppm rel. to $SiMe_4$ (= 0 ppm); coupling constants J in Hz; for ^{13}C, center of $CDCl_3$ signal (δ(C) 77.5) as internal standard. Mass spectra: MALDI-TOF MS, *LDI 1700* instrument (*Linear Scientific Inc.*).

2. *General Procedures for the Synthesis of Homologated Amino-Acid Derivatives. GP 1a*: The diazo ketone was dissolved in the appropriate solvent (0.1M), with the aid of a lithium salt (5 equiv.) where necessary, under Ar with the exclusion of light. MeOH (100 equiv.) and a soln. of silver benzoate (0.11 equiv.) in Et_3N (2.9 equiv.) were then added at r.t., and the mixture was stirred for 3 h. The solvent was evaporated and the residue dissolved in AcOEt. After aq. workup by extraction with sat. $NaHCO_3$, NH_4Cl, and NaCl solns., the org. phase was dried ($MgSO_4$) and evaporated. FC afforded the β-amino methyl ester.

GP 1b: The diazo ketone was dissolved in the appropriate solvent (3.6 mM), with the aid of a lithium salt (5 equiv.) where necessary, and placed in a quartz apparatus with 10% (*v*/*v*) MeOH. The mixture was irradiated with a Hg lamp for 8 h. The solvent was evaporated and the residue taken up in AcOEt. Workup and FC as in *GP 1a* yielded the β-amino methyl ester.

3. *Homologations of Diazo Ketones Derived from Simple Amino Acids. Methyl (*R*)-3-(Benzyloxycarbonylamino)-4-methylpentanoate* (**5**). With Z-Val-CHN_2 (280 mg, 1 mmol) in MeCN according to *GP 1a*. FC (AcOEt/pentane 1:4) yielded **5** (260 mg, 93%). ^{1}H-NMR (400 MHz, $CDCl_3$): 0.92 (*d*, J = 6.8, Me_2C); 1.73–1.90 (*m*, H–C(4)); 2.48–2.60 (*m*, H–C(2)); 3.63 (*s*, MeO); 3.79–3.90 (*m*, H–C(3)); 5.09–5.20 (*m*, PhCH_2, NH, overlapped); 7.29–7.36 (*m*, Ph). ^{13}C-NMR (100 MHz, $CDCl_3$): 18.50, 19.27 (2*q*, C(5), Me); 31.72 (*d*, C(4)); 36.88 (*t*, C(2)); 51.69 (*d*, MeO); 55.62 (*d*, C(3)); 66.58 (*t*, PhCH_2); 128.03, 128.13, 128.31, 128.48, 128.58, 136.65 (5*d*, *s*, Ph); 156.06 (*s*, CONH); 172.19 (*s*, C(1)).

*Methyl (*S*)-3-(Benzyloxycarbonylamino)butanoate* (**6**). With Z-Ala-CHN_2 (125 mg, 5 mmol) in DMA and $LiClO_4$ according to *GP 1a*. FC (AcOEt/pentane 1:3) yielded **6** (100 mg, 80%). Following *GP 1b* with MeCN as solvent and LiCl also yielded **6** (116 mg, 93%). Following *GP 1b* with DMA as solvent and LiCl yielded **6** (94 mg, 75%). NMR: corresponding to that described in [13].

*Methyl (*S*)-3-[(*tert*-Butoxy)carbonylamino]butanoate* (**7**). With Boc-Ala-CHN_2 (106 mg, 5 mmol) in MeCN and $LiClO_4$ according to *GP 1a*. FC (AcOEt/pentane 1:3) yielded **7** (100 mg, 93%). Following *GP 1a* with DMF as solvent, **7** (99 mg, 92%) was formed. Following *GP 1a* with DMF as solvent and $LiClO_4$, **7** was formed (99 mg, 92%). NMR: corresponding with that described in [26].

4. *Preparation of Oligonucleotides*. Syntheses were performed using the 2-cyanoethyl diisopropylphosphoramidite strategy on controlled pore glass (CPG) or polystyrene supports. For the soln.-phase (β-aminoacyl)ations, the oligonucleotides were treated with 32% aq. ammonia overnight at 55° (1 ml for 1 μmol on a synthesis scale), and the crude ammonia mixtures were desalted by size-exclusion chromatography (*NAP-10*™, *Pharmacia*), lyophilized, and used without further purification.

5. *(β-Aminoacyl)ations of Amino-modified Oligonucleotides in Solution; Preparation of Products* **12**, **13**, *and* **17**–**19** (*cf. Schemes 3* and *4*). *General Procedure 2* (*GP 2*). The amino-modified oligonucleotide or the oligonucleotide (10 A_{260} units; 0.05 to 0.1 μmol) and the diazo ketone (4 equiv.) were dissolved in a *Pyrex* tube under Ar, in DMF or in DMA/LiCl (5% *w*/*v*) at a 250 μM concentration. The mixture was irradiated with a Hg lamp for 24 h. The crude mixture was diluted with H_2O (sufficient quantity for 1 ml) and pre-purified by size-exclusion chromatography (*NAP-10*™, *Pharmacia*). The product (conjugate) was purified by RP-HPLC (mobile phase: buffer *A*, 0.05M (Et_3NH)OAc, pH 7.0, and buffer *B*, 70% of MeCN + 0.05M (Et_3NH)OAc, pH 7.0; flow rate 1 ml/min; gradient: 5 min 15% buffer *B*, from 5 to 45 min 15–55% buffer *B*; detection at λ 260 nm). The purity of the products was determined by CGE (10% polyacrylamide capillaries type ssDNA 100 (100 μm i.d., total length 47 cm, effective length 40 cm; *Beckman*; detection at λ 260 nm, injection, electrokinetic, 5 s, voltage of 5 kV; negative polarity; separation at 550 V/cm; running buffer, 100 mM *Tris*-borate, 2 mM EDTA, 7M urea, pH 8.3 at 40°). MALDI-TOF MS (2,4,6-trihydroxyacetophenone in i-PrOH and ammonium citrate as matrix, neg. mode): *Table 2*.

6. *(β-Aminoacyl)ations on Solid Support; Preparation of the Products* **12**, **13**, *and* **17**–**23** (*cf. Scheme 5*). *General Procedure* (*GP 3*). The support-bound amino-modified oligonucleotide (10 mg, *ca.* 0.3 μmol) was suspended in dry MeCN (350 μl). The diazo ketone (20 equiv.) in dry MeCN (50 μl) was added. A soln. of silver benzoate (2 equiv.) in Et_3N (60 equiv.) was added with exclusion of light. The suspension was stirred for 1–3 d. The mixture was centrifugated and the supernatant removed. The solid support was washed with EtOH (2 ×) and H_2O (1 ×). The cleavage from the solid support and the deprotection of the product was realized by treatment with 32% aq. ammonia (500 μl) at 55° overnight. The ammonia soln. was lyophilized, the remaining pellet dissolved in H_2O (500 μl), and the product prepurified by size-exclusion chromatography (*NAP-10*™, *Pharmacia*). The purification and analyses were performed as described in *GP 2*. HPLC and MS: *Table 2*.

REFERENCES

[1] J. Goodchild, *Bioconjugate Chem.* **1990,** *1,* 165.

[2] Z. Wei, C.-H. Tung, T. Zhu, S. Stein, *Bioconjugate Chem.* **1994,** *5,* 468; M. Lemaitre, B. Bayard, B. Lebleu, *Proc. Natl. Acad. Sci. U.S.A.* **1987,** *84,* 648.

[3] R. Eritja, A. Pons, M. Escarceller, E. Giralt, F. Albericio, *Tetrahedron* **1991,** *47,* 4113; B.G. De la Torre, A. Avino, G. Tarrason, J. Piulats, F. Albericio, R. Eritja, *Tetrahedron Lett.* **1994,** *35,* 2733.

[4] J.-C. Truffert, O. Lorthioir, U. Asseline, N.T. Thuong, A. Brack, *Tetrahedron Lett.* **1994,** *35,* 2353; N.J. Ede, G.W. Tregear, J. Haralambidis, *Bioconjugate Chem.* **1994,** *5,* 373.

[5] J.-C. Truffert, U. Asseline, A. Brack, N.T. Thuong, *Tetrahedron* **1996,** *52,* 3005.

[6] See also: J. Hall, D. Hüsken, U. Pieles, H.E. Moser, R. Häner, *Chem. Biol.* **1994,** *1,* 185.

[7] D.R. Corey, *J. Am. Chem. Soc.* **1995,** *117,* 9373.

[8] F. Bergmann, W. Bannwarth, *Tetrahedron Lett.* **1995,** *36,* 1839.

[9] C.M. Dreef-Tromp, J.C.M. van der Maarel, H. van den Elst, G.A. van der Marel, J.H. van Boom, *Nucleic Acids Res.* **1992,** *20,* 4015.

[10] C.M. Dreef-Tromp, H. van den Elst, J.E. van den Boogaart, G.A. van der Marel, J.H. van Boom, *Nucleic Acids Res.* **1992,** *20,* 2435.

[11] K. Arar, A.-M. Aubertin, A.-C. Roche, M. Monsigny, R. Mayer, *Bioconjugate Chem.* **1995,** *6,* 573.

[12] K. Arar, M. Monsigny, R. Mayer, *Tetrahedron Lett.* **1993,** *34,* 8087.

[13] J. Podlech, D. Seebach, *Liebigs Ann.* **1995,** 1217.

[14] K.C. Nicolaou, W.-M. Dai, R.K. Guy, *Angew. Chem.* **1994,** *106,* 38.

[15] 'The Organic Chemistry of β-Lactams', Ed. G.I. Georg, Verlag Chemie, New York, 1993.

[16] D. Seebach, M. Overhand, F.N.M. Kühnle, B. Martinoni, L. Oberer, U. Hommel, H. Widmer, *Helv. Chim. Acta* **1996,** *79,* 913; D. Seebach, P.E. Ciceri, M. Overhand, B. Jaun, D. Rigo, L. Oberer, U. Hommel, R. Amstutz, H. Widmer, *Helv. Chim. Acta* **1996,** *79,* 2043.

[17] E. Juaristi, D. Quintana, J. Escalante, *Aldrichim. Acta* **1994,** *27,* 3; D.C. Cole, *Tetrahedron* **1994,** *50,* 9517; 'Enantioselective Synthesis of β-Amino Acids', Ed. E. Juaristi, Wiley-VCH, New York, 1997, in press.

[18] C. Guibourdenche, J. Podlech, D. Seebach, *Liebigs Ann.* **1996,** 1121.

[19] D. Seebach, A. Thaler, A.K. Beck, *Helv. Chim. Acta* **1989,** *72,* 857; D. Seebach, A.K. Beck, A. Studer, in 'Modern Synthetic Methods 1995', Eds. B. Ernst and C. Leumann, VCH Weinheim, 1995, Vol. 7, p. 1.

[20] M.L. Appleton, C.E. Cottrell, E.J. Behrman, *Carbohydrate Res.* **1990,** *206,* 373; K. Tomita, K. Imahori, *J. Appl. Biochem.* **1982,** *4,* 234.

[21] D.C. Johnson, in 'Cellulose Chemistry and Its Applications', Eds. T.P. Nevell and S.H. Zeronian, Wiley, New York, 1985, p. 181.

[22] N.N. Polushin, J.S. Cohen, *Nucleic Acids Res.* **1994,** *22,* 5492.

[23] U. Pieles, W. Zürcher, M. Schär, H.E. Moser, *Nucleic Acids Res.* **1993,** *21,* 3191.

[24] IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN), 'Nomenclature and Symbolism for Amino Acids and Peptides', Recommendations 1983, *Eur. J. Biochem.* **1984,** *138,* 9; 'Abbreviations and Symbols for Nucleic Acids, Polynucleotides and Their Constituents', *Pure Appl. Chem.* **1974,** *40,* 277.

[25] P. Lombardi, *Chem. Ind. (London) Nov. 5* **1990,** 708; S. Moss, *ibid. Feb. 21* **1994,** 122.

[26] P. Casara, C. Danzin, B. Metcalf, M. Jung, *J. Chem. Soc., Perkin Trans. 1* **1985,** 2201.