J. prakt. Chem. 336 (1994) 233 - 236

Journal für praktische Chemie
Chemiker-Zeitung


# The tert.-Butyloxycarbonyl-(Boc) Group, a Suitable 2'-OH-Protecting Group in Oligoribonucleotide Solid-Phase Synthesis

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Received March 5th, 1993 respectively June 16th, 1993

**Abstract.** The solid-phase synthesis of the dodecaribonucleotide ACCACUAAAGCG is described using the tert. butyloxycarbonyl (Boc) as 2'-OH-protecting group. The synthesis was carried out on a filter disc support Whatman 3 MM by the phosphotriester method. Under these conditions, the 2'-OH-Boc-group proved to be absolutely stable. Moreover, it is quantitatively and selectively removable by 4n HCl/dioxane. Thus, the group renders to be an excellent persistent 2'-OH-protection in ribonucleotide synthesis.

## Introduction

In our recent investigations using model experiments, the Boc group was demonstrated to be a promising persistent protecting group for the 2'-OH position in ribonucleotide synthesis [1, 2]. Furthermore, we have developed convenient synthetic pathways to the corresponding building units, namely the N-protected 2'-Boc-5'-Dmtr-nucleosides [2]. In this paper we illustrate the practical advantage of this type of protecting group on the synthesis of the dodecaribonucleotide ACCACUAAAGCG from the terminal region (66–77) of yeast $tRNA^{Val}$.

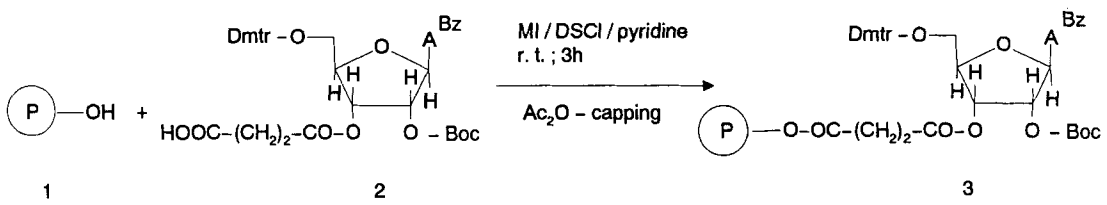
## Results and Discussion

The synthesis of the sequence was achieved on a filter disc support Whatman 3 MM, whereby the cellulose was functionalized via a succinate spacer according to scheme 1 [3, 4].

Using the phosphotriester method and durenesulfonylchloride (DSCl) as the condensation reagent [5], the internucleotide linkage was performed.

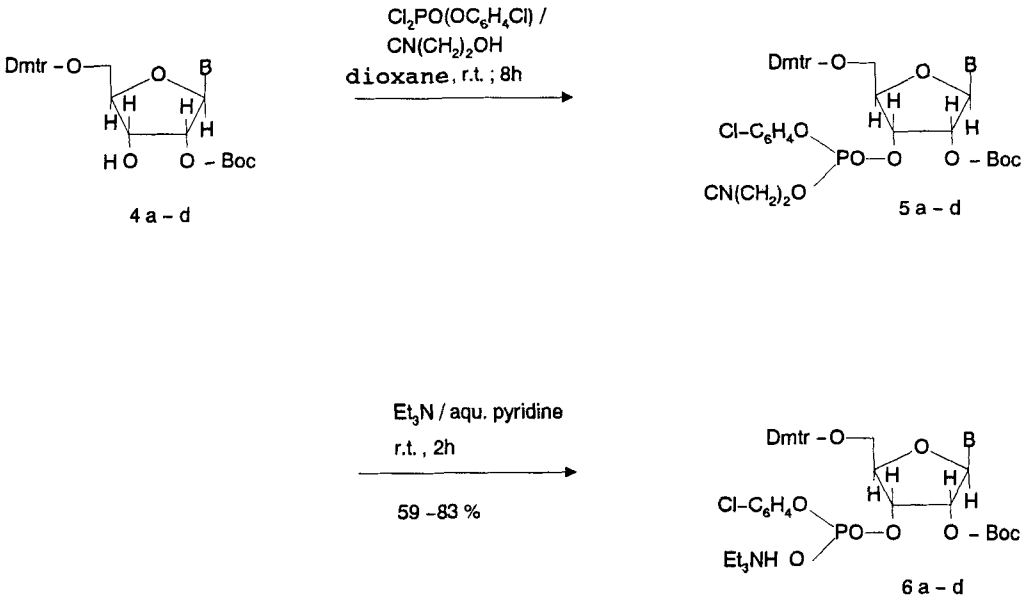
The 3'-O-phosphate components needed for this purpose were prepared from the N-protected 2'-Boc-5'-Dmtr-nucleotides **4a–d** [1]. They were transformed to the corresponding 2-chlorophenyl-2-cyanoethyl-phosphoric acid-derivatives (**5a–d**) [6] and subsequently saponified to give the triethylammonium salts **6a–d** (scheme 2, table 1).

In scheme 3 the yields achieved in each prolongation step in relation to the degree of substitution of the carrier are summarized. Excess of the coupling compound and 1 hour reaction time were applied. Moreover, figures 1a–f show the HPLC profiles which were obtained after cleavage of aliquote samples of the sequence from the support by using $NH_3$/pyridine as 2'-Boc-5'-Dmtr-derivatives.




1 2 3

**Scheme 1**



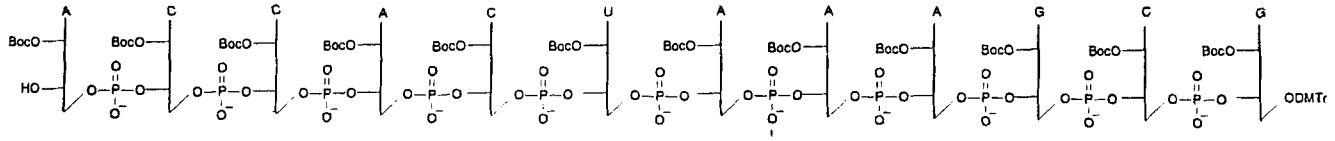

4 a - d

5 a - d

6 a - d

| 4-6 | B |
|---|---|
| a | $^{A^{Bz}}$ |
| b | $^{G^{Bz}}$ |
| c | $^{C^{Bz}}$ |
| d | U |

**Scheme 2**




| loading | | | | | | | | | | | | yield [%] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 32 ± 0.3 μmol/g | 87 | 92 | 98 | 99 | 93 | 95 | 96 | 95 | 92 | 94 | 98 | 53.0[a] |
| 30 ± 3.0 μmol/g | 92 | 94 | 96 | 95 | 98 | 92 | 92 | 94 | 92 | 95 | 93 | 48.8[b] |

[a] Obtained on a HPLC column RP18/Hypersil (4.6 × 250 mm), linear gradient (0–30 min of 0–100 % ammonium acetate (0,05 m), flow 1 ml/min; [b] Determined by the trityl-method [10].

**Scheme 3**

**Table 1** 2′-O-Boc-protected phosphate components for the solid-phase synthesis of oligoribonucleotides

| Product | yield[a] (%) | m.p.[b] (°C) | Molecular Formula[c] | $R_f$[d] | $R_t$[e] | $^{31}$P-NMR[f] ($CH_2Cl_2$/TMS) |
|---|---|---|---|---|---|---|
| **6a** | 67 | 114–116 | $C_{55}H_{62}ClN_6O_{12}P$ (1064.5) | 0.36 | 30.1 | –6.04 |
| **6b** | 59 | 125–126 | $C_{55}H_{62}ClN_6O_{13}P$ (1080.5) | 0.54 | 31.0 | –6.86 |
| **6c** | 69 | 110–111 | $C_{54}H_{63}ClN_4O_{13}P$ (1041.5) | 0.41 | 27.0 | –6.25 |
| **6d** | 83 | 105–107 | $C_{47}H_{57}ClN_3O_{13}P$ (937.5) | 0.49 | 28.9 | –6.11 |

[a] Purification by column chromatography on silica gel 60 (0.063–0.20 mm) Merck using $CH_2Cl_2$ (linear MeOH gradient 0–5 %, 1 % $Et_3N$

[b] Corrected, measured with a Boëtius apparatus.

[c] Satisfactory microanalyses obtained: C ± 0.29, H ± 0.17, N ± 0.15.

[d] Obtained on Merck silica gel $60F_{254}$ using $CH_2Cl_2$/MeOH (90:10) as eluent, developer: $H_2SO_4$/MeOH (20:80), UV-detection at 254 nm.

[e] Obtained on a HPLC column RP18/Hypersil (4.6×250 mm), linear gradient (0–30 min) of 0–100 % ammonium acetate (0.05 m) in 70 % acetonitrile/ammonium acetate (0.05 m), flow 1 ml/min.

[f] Recorded on a Bruker WH 90/DS spectrometer.

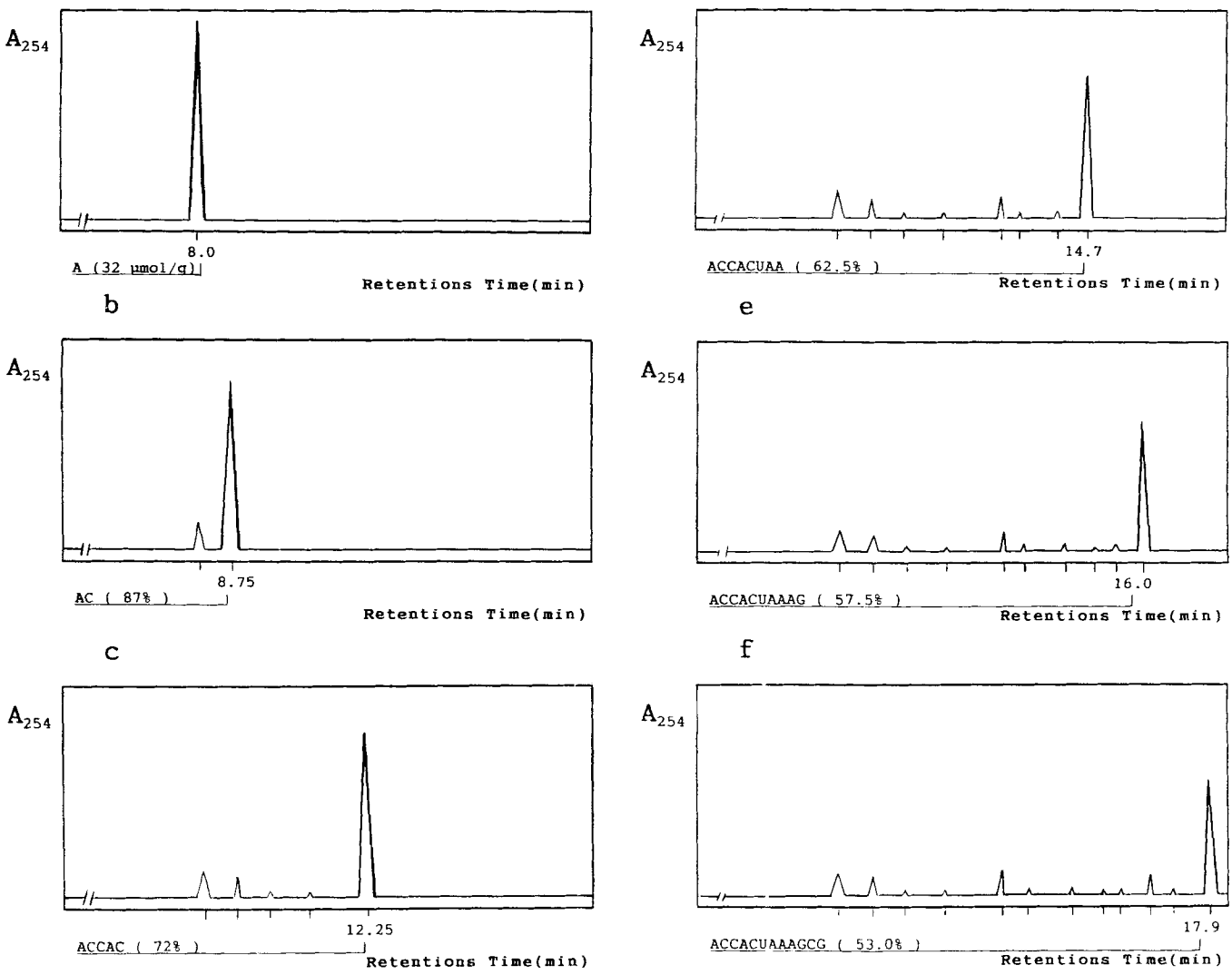



**Fig. 1a–f** HPLC profiles [a] following the condensation course to the dodecanucleotide ACCACUAAAGCG. The determination is performed after cleavage from the carrier by $NH_3$/pyridine (V:V/2:1) as 2′-Boc-5′-Dmtr-derivative in each case.
[a] Obtained on a HPLC column RP 18/Hypersil (4.6 × 250 mm), linear gradient (0–30 min) of 0–100 % ammonium acetate (0.05 m) in 70 % acetonitrile/ammonium acetate (0.05 m), flow 1 ml/min.

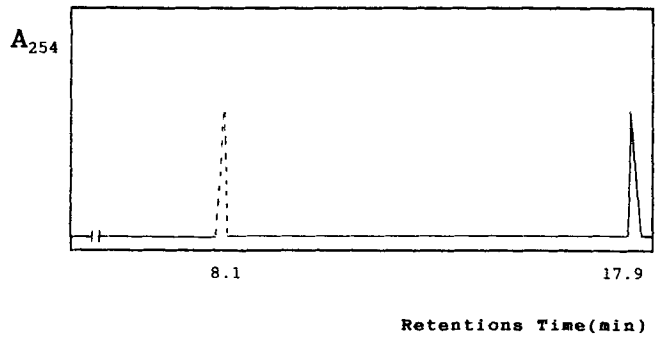



**Fig. 2** —— HPLC profile[a] of the 2′-Boc-5′-Dmtr-dodecanucleotide-derivative after separation from the crude product. - - - - HPLC profile[a] of the dodecanucloetide after cleavage of the protecting groups by 4n HCl/dioxane (60 min).
[a] Obtained on a HPLC column RP 18/Hypersil (4.6 × 250 mm), linear gradient (0–30 min) of 0–100 % ammonium acetate (0.05 m) in 70 % acetonitrile/ammonium acetate (0.05 m), flow 1 ml/min.

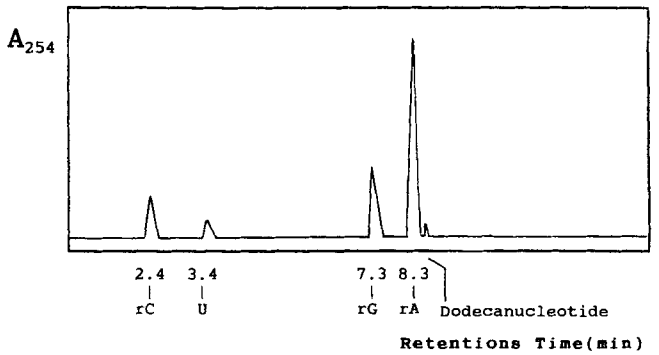



**Fig. 3** HPLC profiles[a] of the cleavage products of the dodecanucleotide after enzymatic degradation by snake toxin (vipera russelli).
[a] Obtained on a HPLC column RP 18/Hypersil (4.6 × 150 mm), linear gradient (5–15 min) of 0–100 % methanol in $H_2O$, flow 1 ml/min.

In agreement with scheme 3, the data clearly indicate that only insignificant amounts of truncated sequences are formed besides the desired main product. Side products, which could result from the loss of the Boc-group, can be excluded.

After detachement of the final dodecaribonucleotide from the carrier by using $NH_3$/pyridine, the oligoribonucleotide was purified and isolated by reverse phase chromatography on a preparative RP18-column. Subsequently, the Boc-group was removed using absolute 4n HCl/dioxane in 1 h. As shown in figure 2, the deprotection proceeded completely, whereas no attack on the aminal linkages of the purine and pyrimidine bases did occur.

To ascertain the correct 3′ → 5′-phosphate linkages, the deprotected nucleotide was degradated by phosphatase containing phosphodiesterase from vipera russelli toxin [7]. A quantitative interpretation of the HPLC-graphs of the degradation products revealed the theoretically expected base composition A(4.80) C(4.00) G(2.07) U(1.05). Artefacts of any kind could not be observed (figure 3).

The presented results show that the Boc-group fulfils all necessary requirements for an efficient 2′-OH-protecting group in oligoribonucleotide synthesis promising versatile applications in this field.

The authors are indebted to the Deutsche Forschungsgemeinschaft for financial support.

## Experimental

**Phosphorylation of the 5′-O-Dmtr-2′-O-Boc-N-benzoyl-derivatives (4a–d) to the Triethylammonium Salts (6a–d): General Procedure [6]:**

2-Cyanoethanol (0.1 ml, 1.3 mmol) is slowly added under an Ar atmosphere to a stirred solution of 2-chlorophenylphosphodichloridate (0.2 ml, 1.2 mmol) in dried dioxane (4 ml) at r.t.. After 30 min, triethylamine (0.2 ml, 1.3 mmol) is added and the resulting phosphodiester monochloridate stirred at r.t. for 4 h. The nucleoside derivatives **4a–d** (0.5 mmol, twice coevaporated from dry pyridine) are dissolved in dried dioxane (3 ml) followed by addition of the phosphochloridate agent over an Ar protected frit. After 10 min, 1-methylimidazole (0.25 ml, 3 mmol) is added, the mixture stirred for 3 h at r.t. (TLC control) and the reaction terminated by addition of aqueous pyridine (10 ml) within 15 min. The mixture is then concentrated in vacuo to an oil, which is repeatedly coevaporated with acetonitrile. Subsequently, triethylamine/aqueous pyridine (1:1, 10 ml) is added and the reaction mixture stirred for 2 h at r.t.. The solution is again concentrated in vacuo to an oil, redissolved in 1 m aqueous sodium bicarbonate (10 ml) and then extracted with $CH_2Cl_2$ (3×10 ml). The combined organic extracts are washed with water (10 ml), dried ($MgSO_4$) and evaporated under reduced pressure. The products are purified by column chromatography on silica gel 60 (0.063–0.20 mm) Merck using $CH_2Cl_2$ as eluent (linear MeOH gradient 0–5 %, 1 % $Et_3N$) to give compounds **6a–d** as white solids. Analytical dates see table.

**Dodecaribonucleotide ACCACUAAAGCG of the terminal region (66–77) of yeast $tRNA^{Val}$; General Procedure:**

*1 Functionalisation of the carrier [8]*

What man 3 MM chromatography paper (50 discs of 4 mm diameter) is used and reacted with the adenosine succinate derivative **2** [9] (100 μmol), methylimidazole (900 μmol) and DSCl (300 μmol) in pyridine (10 ml) at r.t. for 3 h (scheme 1). After removing of the reaction solution the carrier is washed with pyridine (10 ml). The capping of still remaining free OH-groups is carried out with a solution of acetanhydride (0.4 ml) and methylimidazole (0.2 ml) in pyridine (5 ml) at r.t. in 10 min. After washing with pyridine (3×10 ml), acetonitrile (2×10 ml) and ether (3×10 ml) the discs are dried ($P_2O_5$) in vacuo. The loading is determined by the trityl method [10] and HPLC (scheme 3).

*2 Oligonucleotide synthesis [9]*

The synthesis is started by stacking the detritylated [1] adenosine loaded discs in an Ar saturated glass column. The condensation reactions with the 3′-phosphate components **6a–d** (10 molequiv) are carried out in the presence of DSCl (10 molequiv) and methylimidazole (MI, 30 molequiv) at r.t. for 60 min, followed by washing with pyridine (3×10 ml) and acetonitrile (3×10 ml). After each coupling step the solution for capping (acetonitrile/methylimidazole/acetic anhydride, 4:1:1, V/V/V) is injected into the column remaining there for 3 min. The excess reagent is subsequently removed by washing with acetonitrile (3×10 ml) and ether (3×10 ml) and the discs are dried.

After cleavage of the dodecanucleotide from the support with $NH_3$/pyridine (2:1 V/V) at 55 °C for 12 h the protecting groups are removed by 4n HCl/dioxane at r.t. in 60 min to afford the deblocked dodecanucleotide.

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