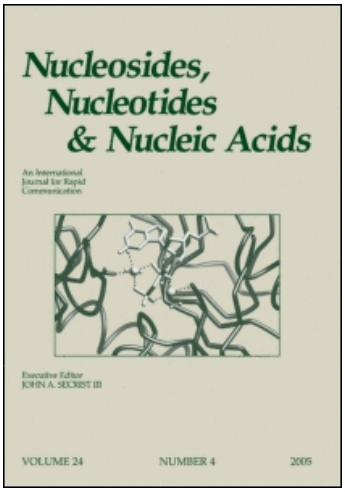

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Kazuo Kamaike[ab]; Takashi Namiki[a]; Etsuko Kawashima[a]
[a] School of Pharmacy, Tokyo University of Pharmacy and Life Science, Hachioji, Tokyo, Japan [b] Tokyo University of Pharmacy and Life Science, Hachioji, Tokyo, Japan

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Novel Base-Labile Protecting Groups for 5′-Hydroxy Function in Solid-Phase Oligonucleotide Synthesis

Kazuo Kamaike,* Takashi Namiki, and Etsuko Kawashima

School of Pharmacy, Tokyo University of Pharmacy and Life Science, Hachioji, Tokyo, Japan

ABSTRACT

The 6-(levulinyloxymethyl)-3-methoxy-2-nitrobenzoyl (LMM*o*NBz) and 2-(levulinyloxymethyl)-5-methoxy-4-nitrobenzoyl (LMM*p*NBz) groups were developed as novel base-labile protection for the 5′-hydroxy function in solid-phase oligonucleotide synthesis. A comparative study of the LMM*o*NBz, LMM*p*NBz and 2-(levulinyloxymethyl)-5-nitrobenzoyl (LMNBz) protecting groups for oligonucleotide synthesis proved strong feasibility for the LMM*o*NBz group.

Key Words: Base-labile protecting group; 5′-Hydroxy protection; Oligonucleotide synthesis.

Although reliable methods of solid-phase DNA synthesis using the 4,4′-dimethoxytrityl (DMTr) group to protect the 5′-hydroxy function of nucleotide units have been established, the DMTr group is not ideal for acidic deprotection at successive cycles.[1,2] The levulinyl (Lev) group has been demonstrated by Ohtsuka et al. to offer excellent protection for the 5′-hydroxy function of nucleotide units in RNA

*Correspondence: Kazuo Kamaike, Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji 192-0392 Tokyo, Japan; Fax: +81 42676 3073; E-mail: kamaikek@ps.toyaku.ac.jp.

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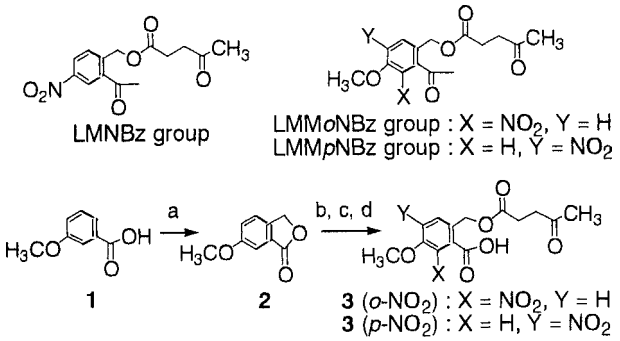
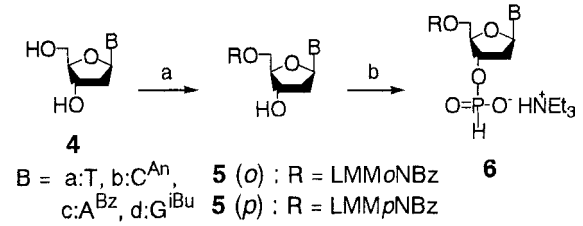
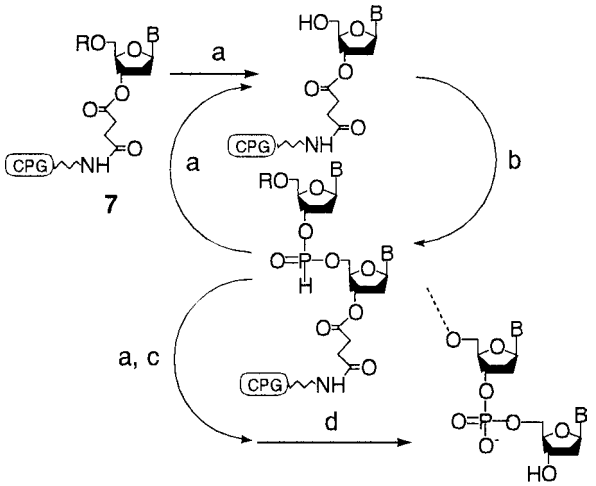


Scheme 1. a) HCHO, conc. HCl/1,4-dioxane, 50–55°C, 3 days; b) i. KOH, 85% MeOH-H_2O, 55°C, 30 min, ii. conc. HCl; c) levulinic anhydride, l-methylimidazole, 1,4-dioxane, r.t., 1 h; d) HNO_3, H_2SO_4, in an ice-salt bath, 20 min.



Scheme 2. a) 3 (*o*- or *p*-NO_2), TPSCl, pyridine, r.t., 2–3 h; b) i. Tris(1,2,3-triazolyl)phosphine, *N*-methylmorpholine, CH_2Cl_2, 0°C, 30 min, ii. 1 M TEAB aq.



Scheme 3. a) i. 0.5 M $NH_2NH_2.H_2O$, 2 : 3 : 5 CH_3COOH-pyridine-CH_3CN, 15 min, ii. 0.5 M imidazole, CH_3CN, 5 min; b) 0.05 M **6**, 0.25 M pivaloyl chloride, 1 : 1 pyridine-CH_3CN, r.t., 10 min; c) 3% I_2, H_2O-19% pyridine-76% THF, r. t., 10 min; d) conc. NH_4OH, r.t., 3 h-50°C, 6 h.

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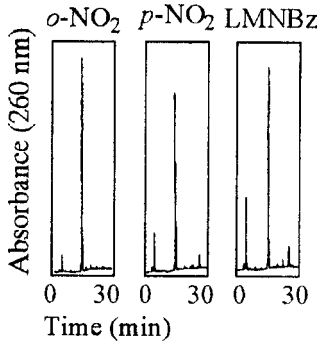

Figure 1. Reversed-phase HPLC profiles of crude products of TpT preparations using **6a** (DMTr) on CPGs [**7a** (*o*, *p*, and LMNBz)], respectively. Conditions of reversed-phase HPLCs: column μBONDASPHERE 5μ C18 (3.9 mm ID × 150 mm L); elution buffer 7.25-50% CH_3CN/0.1 M TEAA (pH 7); flow rate 1 mL/min.

synthesis on CPG support.[3a,b] The Lev group is easily removed by hydrazinolysis although the yield of 5′-*O*-levulinylation is unsatisfactory due to low regioselectivity.[3] We have previously reported developing the LMNBz group, which has higher regioselectivity, while maintaing easy removability of the Lev group.[4] In this paper, we report the development of *o*- and *p*-nitrobenzoyl derivatives, the LMM*o*NBz and LMM*p*NBz groups, as novel base-labile protection for the 5′-hydroxy function in solid-phase oligonucleotide synthesis.

The protecting reagents [**3** (*o*- and *p*-NO_2)] were easily prepared from *m*-anisic acid (**1**) as shown in Sch. 1. Introduction of the LMM*o*NBz and LMM*p*NBz groups to the 5′-position of 2′-deoxyribonucleosides (**4**) was accomplished by treatment with **3** (*o*- and *p*-NO_2), respectively, in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) in pyridine. The corresponding 5′-*O*-benzoyl derivatives **5** (*o* and *p*) were obtained in 55–76% yields (Sch. 2). We performed a comparative study of

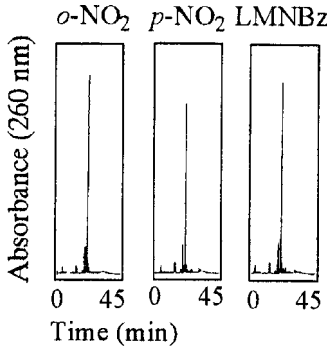

Figure 2. Reversed-phase HPLC profiles of crude products of TpTpTpT preparations using **6a** (*o*, *p*, and LMNBz) on CPG **7a** (DMTr), respectively. Conditions of reversed-phase HPLCs: column μBONDASPHERE 5μ C18 (3.9 mm ID × 150 mm L); elution buffer 7.25-50% CH_3CN/0.1 M TEAA (pH 7); flow rate 1 mL/min.

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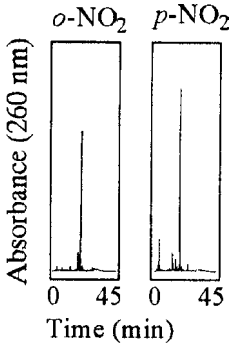

Figure 3. Reversed-phase HPLC profiles of crude products of TpTpTpT preparations using **6a** (*o* and *p*) on CPGs [**7a**(*o* and *p*)], respectively. Conditions of reversed-phase HPLCs: column μBONDASPHERE 5μ C18 (3.9 mm ID × 150 mm L); elution buffer 7.25-50% CH_3CN/0.1 M TEAA (pH 7); flow rate 1 mL/min.

the LMM*o*NBz, LMM*p*NBz and LMNBz protecting groups for oligonucleotide synthesis (Sch. 3), as exemplified by synthesis of TpT and TpTpTpT using *H*-phosphonates [**6a** (DMTr, *o*, *p*, and LMNBz)][5] on CPGs [**7a** (DMTr, *o*, *p*, and LMNBz)] by the manual synthesis, respectively. The excellence of the LMM*o*NBz group over the LMMN*p*Bz and LMNBz groups was confirmed as can be seen in Figs. 1–3.

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