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# A New Colorimetric Protecting Group Allowing Deprotection Under Neutral Conditions

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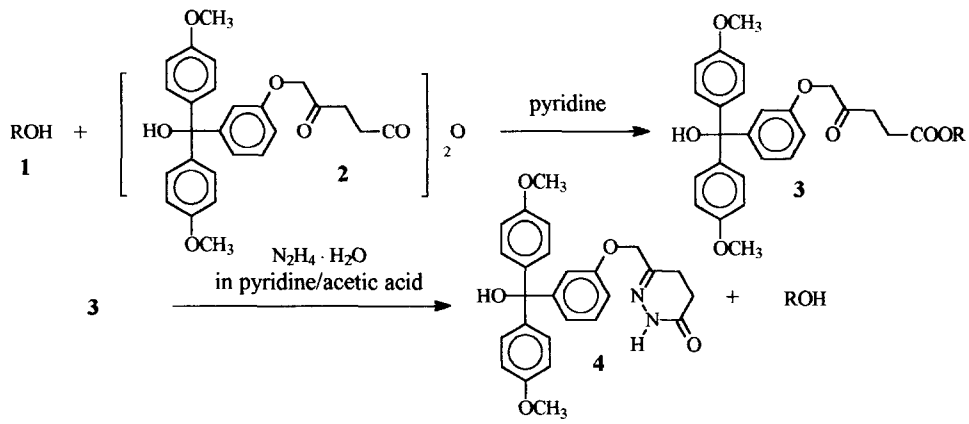
**Abstract:** A 4,4'-dimethoxytrityl derivative of the levulinyl group has been developed for protection of nucleophilic functionalities such as hydroxyl groups by reaction of its symmetrical anhydride. It can rapidly be removed under mild conditions using a hydrazine-pyridinium acetate based buffer at near neutral pH. This protecting group can be detected with high sensitivity at 513 nm ($\varepsilon$ = 78,600).

Key element for synthetic strategies in natural product chemistry is the availability of a variety of protecting groups which can be introduced and removed under different selectivities and chemical conditions; an orthogonal protecting group strategy is highly desired. Since many natural products are sensitive compounds mild conditions are needed for post-synthetic removal of protecting groups. For the detection of small amounts of protected material, sensitive monitoring of introduction and removal of the protecting group as well as for a better differentiation amongst other protecting groups for other functionalities within the same molecule it is desirable to have a protecting group which can be detected through a colorimetric reporter function with high molar extinction coefficient. In this paper we wish to introduce a protecting group which could become an important addition to the collection of protecting groups available to the natural product chemist.

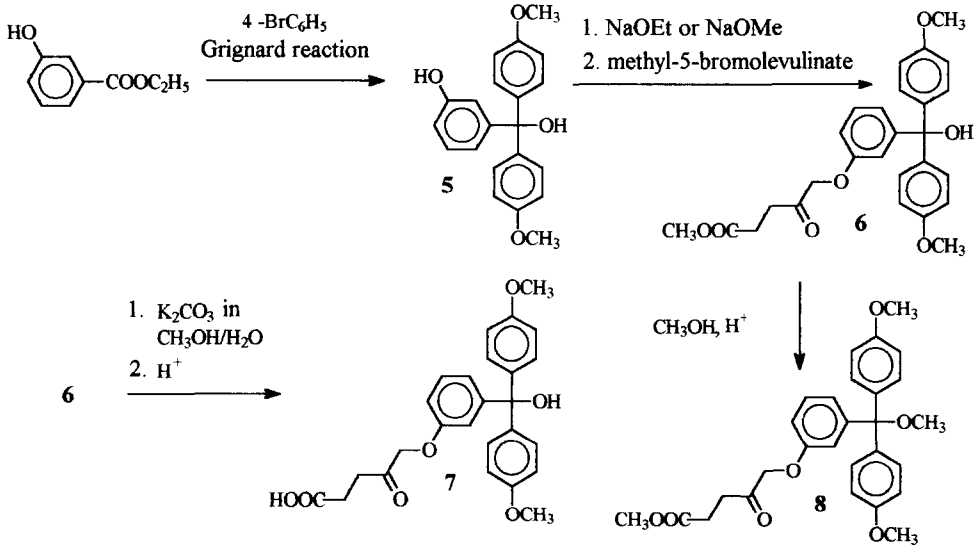
The new protecting group, 5-[3-(bis-4-methoxyphenyl)-hydroxymethyl]-phenoxy-levulinic acid (7) (scheme 2), combines the favorable features of the levulinyl group,[1-5] which can be removed from levulinyl




Scheme 1

esters in a few minutes at room temperature at near neutral pH using a hydrazinium-pyridinium acetate buffer and the highly sensitive 4,4'-dimethoxytrityl group[6] which can be detected under acidic conditions around 500 nm with enormously high molar extinction coefficient (around 70,000). The application of this new protecting group is demonstrated for the protection of hydroxyl groups in scheme 1. The hydroxyl group containing compound **1** such as 5'-O-(4,4'-dimethoxytrityl)-2'-deoxythymidine is being treated in pyridine with the symmetrical anhydride **2** which is prepared *in situ* from the corresponding acid **7** (see scheme 2) in presence of 4-dimethylaminopyridine. Removal of the 4,4'-dimethoxytrityl group was accomplished with 80% aqueous acetic acid under standard conditions[6] and the 3'-O-levulinyl ester **3** of deoxythymidine obtained in 69% overall yield. The ester bond in **3** can be cleaved quantitatively in less than 10 minutes at room temperature using a hydrazinium-pyridinium acetate buffer at near neutral pH to furnish the alcohol **1** or deoxythymidine respectively. Different reagent compositions have been tested without any change on the rate of cleavage (see experimental section). The reactions can easily be monitored e.g. by thin layer chromatography using an acidic sugar spray reagent such as anisaldehyde/sulfuric acid (see experimental section). Treatment with acids generates the trityl kation which in case of the heterocyclic compound **4** strongly absorbes in the visible spectral region with $\lambda_{max}$ at 513 nm and molar extinction coefficient of $\varepsilon_{513}$=78,600 (5% dichloroacetic acid in methylene chloride, v/v); the differences to the spectral properties of the parent 4,4'-dimethoxytrityl kation ($\lambda_{max}$= 498 nm; $\lambda_{498}$= 68,700) are remarkable.

The synthesis of **7** is summarized in scheme 2. The first step employs a Grignard reaction;[7] compound **5** could be obtained in crystalline form. For the introduction of the levulinic acid moiety 5-bromo levulinic




Scheme 2

acid methyl ester was synthesized;[8] coupling to **5** via a Williamson type etherification reaction furnished **6** as crystalline compound in good overall yield. The title compound **7** is being prepared from **6** by mild alkaline hydrolysis and transformed to the symmetrical anhydride **2** by treatment with N,N'-dicyclohexylcarbodiimide.

Although the tertiary hydroxyl group in **2**, **3** and **7** does not react under most conditions it can be transformed with a large excess of alcohol to methyl or ethyl trityl ethers. If **5**, **7** or with **7** protected compounds such as **3** are dissolved in either methanol or ethanol in the presence of traces of acids such as acetic acid the corresponding trityl methyl or ethyl ethers are obtained in quantitative yields. As an example a methanolic solution of **6** can be transformed into **8** by addition of small amounts of acetic acid; after evaporation of solvent **8** is obtained in pure form. The trityl ethers can be reverted to the corresponding trityl carbinol derivatives by a short treatment with 80% aqueous acetic acid. If alcoholic solutions of **7** containing compounds are used the formation of the corresponding trityl ethers can completely be avoided by the presence of traces of triethylamine.

The new protecting group **7** is being successfully used in our laboratory for the solid phase synthesis of oligodeoxynucleotides.[9] It could also be of great value in other areas of natural product chemistry to facilitate monitoring of various reaction steps in solution phase syntheses employing starting materials such as amino acids/peptides or sugars/oligosaccharides which have in contrast to nucleosides/nucleotides no uv-absorbing properties. Here the new group **7** does not only serve as a protecting group but at the same time provides for a reporter functionality.

## EXPERIMENTAL SECTION

$^1H$ (400 and 250 MHz) and $^{13}C$ (100 and 63 MHz) NMR spectra were recordered on a Bruker AMX 400 and a AC 250-P instrument. Samples were dissolved in the presence of tetramethylsilane as internal standard, unless otherwise stated. Chemical shifts are given in ppm. Mass spectra were obtained on a Finnigan MAT 311A mass spectrometer under EI conditions and a VG Analytical 70-250S mass spectrometer under FAB conditions (matrix: 3-nitro-benzyl alcohol, Xenon bombardment). Melting points are uncorrected. Elementary analyses were performed by the analytical Department of the Institute of Organic Chemistry, University of Hamburg. Thin layer chromatography (tlc) was carried out on 60 $PF_{254}$ silica gel coated aluminia sheets (Merck, Darmstadt, No 5562). Trityl and sugar containing compounds were visualized with the sugar spray reagent (0.5ml 4-methoxybenzaldehyde, 9 ml ethanol, 0.5 ml concentrated sulfuric acid and 0.1 ml glacial acetic acid) by heating with a fan or on a hot plate. Column chromatography was performed using silica gel from Merck.

**3-Hydroxy-4',4''-dimethoxytritylcarbinol (5)**

Compound **5** was obtained in a Grignard reaction.[7] In a typical reaction 11.10 g (457 mmol) magnesium, 85.4 g (457 mmol) 4-bromoanisole were treated with 19.0 g (114 mmol) 3-hydroxy-benzoic acid ethyl ester. The reaction product was acidified with 2 M hydrochloric acid. **5** could be obtained in crystalline form from toluene; the crystals were washed with toluene and dichloromethane until the filtrate was colorless; yield: 38.5 g (46%); m.p. 124-26 °C.

*$^1H$ NMR* (400 MHz, [$D_6$] DMSO): δ = 3.31 (s, 1H,$R_3C$-O<u>H</u>), 3.73 (s, 6H, -OC<u>$H_3$</u>), 6.1 (s,1H, aryl-O<u>H</u>), 6.55-7.08 (m, 12H, aryl-<u>H</u>). - *$^{13}C$ NMR* (63 MHz, [$D_6$] DMSO, internal standard [$D_6$] DMSO at 39.7 ppm): δ = 55.16 (q, -O<u>C</u>$H_3$), 80.01 (s, $R_3$<u>C</u>-OH), 112.85, 113.52, 115.22, 118.76, 128.39, 129.06 (d, <u>C</u>-H, aryl),

140.48, 150.07 (s, $R_2\underline{C}$-$CR_2$-OH, aryl,quarternary), 156.73, 157.94 (s, $R_2\underline{C}$-$OCH_3$ and $R_2\underline{C}$-OH,aryl, position not defined). - *MS* (EI): m/z (rel. intensity) : m/z calculated for $C_{21}H_{20}O_4$ ($M^+$): 336; found : 336 (57), 319 (13, M-$OH^+$), 243 (100), 135 (49).

**5-[3-(Bis-4-methoxyphenyl)-hydroxymethyl]-phenoxy-levulinic acid methyl ester (6)**

For the synthesis of 5-bromo-levulinic acid methyl ester diazomethane was prepared[10] and not distilled. Note: on page 561 is a typo; instead of 5 mol methylamine hydrochloride take 1.5 mol. CAUTION: diazomethane is explosive and poisonous. For the synthesis of **6** sodium ethoxide in ethanol (from 3.74 g/163 mmol sodium in 180 ml ethanol ) was added to compound **5** (54.1 g, 161 mmol) and the mixture refluxed for 30 minutes. Alternatively sodium methoxide dissolved in methanol can be used. Solvents were evaporated, the remaining sodium phenolate of **5** dissolved in 93 ml dry N,N-dimethyl-formamide (DMF) and 34.7 g (166 mmol) 5-bromo-levulinic acid methyl ester added in a dropwise manner. The reaction was allowed to stir overnight and its completition checked by tlc (dichloromethane/methanol 97/3, v/v). After the addition of 360 ml water the solution was extracted with toluene (2x210 ml), the organic layer successively extracted with water (1x670 ml), 0.2 M sodium hydroxide (4x500ml) and water (3x200 ml). A first crop of crystalline **6** could be directly obtained from methanol (containing a trace of triethylamine) ; yield: 21.5 g (29%); m.p. 78-79 °C. More product **6** could be purified from the mother liquor by silica gel column chromatography (100 g silica gel, Merck, Darmstadt; No 7734 for 33 g raw product, solvent dichloromethane) and subsequent crystallization from methanol (containing a trace of triethylamine).

*$^1H$ NMR* (250 MHz, $CDCl_3$): δ = 2.63 (t, 2H, -$C\underline{H}_2$-$CH_2$-), 2.86 (t, 2H, -$CH_2$-$C\underline{H}_2$-), 2.91(s, 1H,$R_3C$-O$\underline{H}$), 3.65 (s, 3H, -$COOC\underline{H}_3$), 3.8 (s, 6H, -$OC\underline{H}_3$), 4.58 (s, 2H,-CO-$C\underline{H}_2$-O-), 6.78-7.26 (m, 12H, aryl-$\underline{H}$). - *$^{13}C$ NMR* (63 MHz, $CDCl_3$, internal standard $CDCl_3$ at 77.00 ppm): δ = 27.20 (t, -$\underline{C}H_2$-$CH_2$-), 33.66 (t, -$CH_2$-$\underline{C}H_2$-), 51.90 (q, -$COO\underline{C}H_3$), 55.23 (q, -$O\underline{C}H_3$), 72.68 (t, -CO-$\underline{C}H_2$-O-), 81.27 (s, $R_3\underline{C}$-OH), 113.14, 113.19, 113.98, 121.47, 128.95, 129.09, (d, $\underline{C}$-H, aryl), 139.21, 149.35 (s, $R_2\underline{C}$-$CR_2$-OH, aryl, quarternary), 157.43, 158.68 (s, $R_2\underline{C}$-$OCH_3$, $R_2\underline{C}$-$OCH_2$-CO-, aryl, position not defined), 173.05 (s, -$\underline{C}OOCH_3$), 205.88 (s, -$\underline{C}$O-). - *MS* (EI): m/z (rel. intensity) : m/z calculated for $C_{27}H_{28}O_7$ ($M^+$) : 464 ; found : 464 (30), 447 (10, M-$OH^+$), 243 (100), 135 (55). - *Elementary Analysis* (%): Found : C, 69.79/70.26; H, 6.06/6.07; $C_{27}H_{28}O_7$ requires C, 69.81; H, 6.08;

**5-[3-(Bis-4-methoxyphenyl)-methoxymethyl]-phenoxy-levulinic acid methyl ester (8)**

1.00 g of compound **6** is dissolved in 100 ml methanol containing 0.5 ml glacial acetic acid. Monitoring by tlc indicates completion of the reaction after less than 18 h. The solvent is evaporated under reduced pressure, followed by coevaporation with toluene (2-3 times).

*$^1H$ NMR* (400 MHz, $CDCl_3$): δ = 2.64 (t, 2H, -$C\underline{H}_2$-$CH_2$-), 2.89 (t, 2H, -$CH_2$-$C\underline{H}_2$-), 3.04 (s, 3H, $R_3C$-$OC\underline{H}_3$), 3.67 (s, 3H, -$COOC\underline{H}_3$), 3.79 (s, 6H,aryl -$OC\underline{H}_3$), 4.55 (s,2H,-CO-$C\underline{H}_2$-O-), 6.71-7.32 (m, 12H, aryl-$\underline{H}$). - *$^{13}C$ NMR* (63 MHz, $CDCl_3$, internal standard $CDCl_3$ at 77.00 ppm): δ = 27.20 (t, -$\underline{C}H_2$-$CH_2$-), 33.84 (t, -$CH_2$-$\underline{C}H_2$-), 51.85, 51.88 (q, -$COO\underline{C}H_3$, q, $R_3C$-$O\underline{C}H_3$, position not defined), 55.19 (q, aryl-$O\underline{C}H_3$), 72.76 (t, -CO-$\underline{C}H_2$-O-), 86.26 (s, $R_3\underline{C}$-$OCH_3$), 112.32, 113.05, 114.56, 121.70, 128.89, 130.15 (d, $\underline{C}$-H, aryl), 135.62, 147.43 (s, $R_2\underline{C}$-$CR_2$-$OCH_3$, aryl, quarternary), 157.32, 158.46 (s, $R_2\underline{C}$-$OCH_3$, $R_2\underline{C}$-$OCH_2$-CO-, aryl, position not defined), 172.94 (s, -$\underline{C}OOCH_3$), 206.11 (s, -$\underline{C}$O-). - *MS* (EI): m/z (rel. intensity) : m/z calculated for $C_{28}H_{30}O_7$ ($M^+$):478; found : 478 (14), 447 (100, M-$OCH_3^+$), 257 (55), 135 (50 ).

**5-[3-(Bis-4-methoxyphenyl)-hydroxymethyl]-phenoxy-levulinic acid (7)**

Compound **6** (20.0 g, 43.1 mmol) was stirred with a mixture of $K_2CO_3$/methanol/water (6.95 g/87.4 ml/69.5 ml) at room temperature; reaction is complete after less than 72 h. The reaction mixture was filtered, methanol evaporated, 300 ml water added and the mixture brought to pH 3 by addition of 2% aqueous $KHSO_4$. After addition of dichloromethane the mixture was extracted with water, dried with $Na_2SO_4$. After evaporation of $CH_2Cl_2$ **7** was obtained as brittle foam which could easily be handled. Yield: 18.7 g (41.5 mmol, 96 %).

*$^1H$ NMR* (250 MHz, $CDCl_3$): δ = 2.65 (t, 2H, -C<u>H</u>$_2$-CH$_2$-), 2.83 (t, 2H, -CH$_2$-C<u>H</u>$_2$-), 3.78 (s, 6H, -OC<u>H</u>$_3$), 4.58 (s, 2H,-CO-C<u>H</u>$_2$-O-), 6.76-7.25 (m, 12H, aryl-<u>H</u>). - *$^{13}C$ NMR* (63 MHz, $CDCl_3$, internal standard $CDCl_3$ at 77.00 ppm): δ = 27.08 (t, -<u>C</u>H$_2$-CH$_2$-), 33.41 (t, -CH$_2$-<u>C</u>H$_2$-), 55.24 (q, -O<u>C</u>H$_3$), 72.62 (t, -CO-<u>C</u>H$_2$-O-), 81.45 (s, R$_3$<u>C</u>-OH), 113.07, 113.20, 113.88, 121.51, 128.94, 129.11 (d, <u>C</u>-H, aryl), 139.08, 149.27 (s, R$_2$<u>C</u>-CR$_2$-OH, aryl, quarternary), 157.41, 158.68 (s, R$_2$<u>C</u>-OCH$_3$, s, R$_2$<u>C</u>-OCH$_2$-CO-, aryl, position not defined), 176.83 (s,-<u>C</u>OOH), 205.74 (s, -<u>C</u>O-). - *MS* (FAB, pos. mode) : m/z (rel. intensity): m/z calculated for $C_{26}H_{26}O_7$ ($M^+$): 450; found : 450 (11), 433 (64, M-$OH^+$).

**Alcoholysis of 2 with 2'-deoxythymidine (1) to 3**

Compound **2** is prepared *in situ* by reacting 10.0 g (22.2 mmol) of levulinic acid derivative **7** (scheme 2) with 4.76 g (23.1 mmol) N,N'-dicyclohexylcarbodiimide in 65 ml dioxane. N,N'-Dicyclohexylurea is removed by filtration and washed with dioxane. The solvent was evaporated *in vacuo* and to the residue is added a solution of 6.00 g (11.0 mmol) 5'-O-4,4'-dimethoxytrityl-2'-deoxythymidine and 0.108 g (0.884 mmol) 4-dimethylaminopyridine in 18 ml dry pyridine. Completion of reaction was checked by tlc. 30 minutes after the addition of a mixture of 1.43 ml of acetic acid and 2.7 ml of pyridine, 0.5 ml water were added, 60 minutes later an excess of ethyl acetate was added, N,N'-dicyclohexylurea removed by filtration and washed with ethyl acetate. The mixture was extracted with water, 5% aqueous sodiumhydrogen carbonate and water. After drying, the solvent was evaporated, then coevaporated with toluene to obtain a foam. The residue was directly detritylated with 80% aqueous acetic acid. Overall yield of **3** (R= 5'-O-DMT-2'-deoxythymidine) after silica gel column chromatography is 69% (silica gel 60 H, Merck, Darmstadt, No 7736, using a step gradient from dichloromethane to dichloromethane/methanol : 96/4, v/v, in presence of 0.04% pyridine).

*$^1H$ NMR* (400 MHz, $CDCl_3$,): δ = 1.88 (s, 3H, -C<u>H</u>$_3$ of thymine), 2.38-2.34 (m, 2H, H2'$^a$/H2'$^b$), 2.64 (t, 2H, -C<u>H</u>$_2$-CH$_2$-), 2.81 (t, C(5')-O<u>H</u>), 2.88 (t, 2H, -CH$_2$-C<u>H</u>$_2$-), 3.30 (s, 1H, DMTr-O<u>H</u>), 3.78 (s, 6H, -OC<u>H</u>$_3$), 3.88-3.83 (m, 2H, H5'$^a$/H5'$^b$), 4.06 (m, 1H, H4'), 4.57 (s, 2H, -CO-C<u>H</u>$_2$-CO-), 5.32 (m, 1H, H3'), 6.2 (t, 1H, H1'), 6.78-7.23 (m, 12H, aryl-<u>H</u>), 7.49 (s, 1H, H6), 8.72 (s, 1H, N-<u>H</u> of thymine). - *$^{13}C$ NMR* (100 MHz, $CDCl_3$, internal standard $CDCl_3$ at 77.00 ppm): δ = 12.51 (q, -<u>C</u>H$_3$ of thymine), 27.43 (t, -<u>C</u>H$_2$-CH$_2$-), 33.62 (t, -CH$_2$-<u>C</u>H$_2$-), 37.06 (t, C2'), 55.23 (q, aryl-O<u>C</u>H$_3$), 62.44 (t, C5'), 72.60 (t, -CO-<u>C</u>H$_2$-O-), 74.98 (d, C3'), 81.27 (s, <u>C</u>(DMTr)-OH), 84.6 (d, C4'), 85.97 (d, C1'), 111.33 (s, C5 of thymine), 113.17, 113.26, 113.73, 121.68, 128.92, 129.09 (d, <u>C</u>-H, aryl), 136.26 (d, C6 of thymine), 139.18, 149.39 (s, R$_2$<u>C</u>-CR$_2$-OH, aryl, quarternary), 150.33 (s, C2 of thymine), 157.39, 158.64 (s, R$_2$<u>C</u>-OCH$_3$, s, R$_2$<u>C</u>-OCH$_2$-CO-, aryl, position not defined), 163.49 (s, C4 of thymine), 172.37 (s, -<u>C</u>OOR), 205.96 (s, -<u>C</u>O-). - *MS* (FAB, pos. mode): m/z (rel.

intensity): m/z calculated for $C_{36}H_{38}N_2O_{11}$ ($M^+$): 674; found: 674 (12), 657 (100, M-$OH^+$). - *Elementary Analysis* (%): Found: C, 63.84/63.83; H, 5.84/5.83; N, 4.04/4.02; $C_{36}H_{38}N_2O_{11}$ requires C, 64.15; H, 5.68; N, 4.16.

**Reagents tested for hydrazinolysis**

a) 0.5 M Hydrazinium hydrate in pyridine/glacial acetic acid (4:1, v/v) ; b) 0.5 M hydrazinium hydrate in pyridine/glacial acetic acid/water (4:1:0.25, v/v), pH 6.5; c) 1.0 M hydrazinium hydrate in pyridine/glacial acetic acid/water (4:3:0.35, v/v), pH 5.4. Addition of water avoids possible crystallization of the reagent in case of automated solid phase oligonucleotide syntheses with ß-cyanoethylphosphoamidites[11] using **7** as a temporary protecting group.

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