4,4′,4″-Tris(levulinoyloxy)trityl as a New Type of Primary Hydroxyl Protecting Group

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The 4,4′,4″-tris(levulinoyloxy)trityl (TLTr) group was introduced selectively on the 5′-oxygen of thymidine by use of *in situ* generated 4,4′,4″-tris(levulinoyloxy)trityl bromide. The TLTr group was found to be sufficiently stable to acids and readily removed by hydrazinolysis followed by warming to 50 °C in pyridine–acetic acid without damage of other protecting groups such as the *O*-acetyl and 4,4′-dimethoxytrityl groups. The utility of this new protecting group was demonstrated by the successful synthesis of thymidylyl(3′—5′)-thymidine where the TLTr group was employed as the 5′-hydroxyl-protecting group.

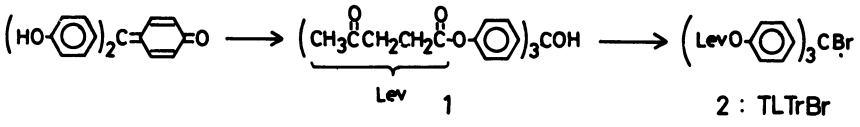
For synthetic work on polyfunctional natural products, a protecting group, which is removed under as mild conditions as possible, has often been required. Among a wide variety of unblocking agents reported, hydrazine has proved to be accessible in nucleotide chemistry.[1-4] Letsinger[1a] first reported β-benzoylpropionyl as a hydrazine-lable hydroxyl blocking group. Later, van Boom[2a] described levulinoyl as a more easily removable group. Very recently, Ogilvie[3] reported the use of the latter for protection of the exo amino groups of nucleosides. Matteucci[4] and we[5] also communicated dialkylamidines and phthaloyl, respectively, as hydrazine-labile blockers for the deoxyadenosine N^6-amino function.

We have found that the 4,4′,4″-tris(benzoyloxy)trityl (TBTr) group was rather stable to acids but yet susceptible to cleavage under alkaline conditions.[6] This hydrolytic cleavage was explained as the result of alkali-mediated elimination of rosolic acid from a 4,4′,4″-trihydroxytrityl ether initially formed. If the 4,4′,4″-trihydroxytrityl group could be removed under certain nonbasic conditions, we could choose various substituents capable of hydrolysis under mild conditions to design new "protected" protecting groups which satisfied the above mentioned stipulation.

Therefore, we have studied levulinoyloxy-substituted trityl as hydrazine-labile protecting groups. First, 4,4′,4″-tris(levulinoyloxy)tritanol (**1**) was prepared in 43% yield by levulination of rosolic acid. The tritanol was then converted to the trityl bromide (**2**) by treatment with acetyl bromide. This new reagent underwent facile 5′-*O*-tritylation with thymidine (**3**) in pyridine giving rise to the product (**4**) in 81% yield. Treatment of **4** with 80% acetic acid showed that the 4,4′,4″-tris(levulinoyloxy)trityl (TLTr) group had the same stability as the TBTr group ($T_{1/2}$=1 d, T_{com}=5 d).[6] On the other hand, hydrazinolysis of **4** with 1 M† H_2NNH_2 in hydrazine–acetic acid (3:2, v/v) at room temperature for 3 min gave quantitatively 5′-*O*-(4,4′,4″-trihydroxytrityl)thymidine (**5**). The latter was rapidly converted to **3** and rosolic acid by treatment with 80% acetic acid. Since our ultimate aim was to convert **5** to **3** under essentially neutral conditions, several experiments have been conducted. Some attempts to cleave the $(HO)_3Tr$–O ether bond of **5** oxidatively by using various metal salts[7] involving $FeCl_3$ and $CuCl_2$ have failed. Although **5** was expected to be decomposed to thymidine by passing it slowly through a silica-gel column, elution with CH_2Cl_2–MeOH gave a 80% recovery of **5** and a small amount (10%) of **3**. The final goal to conversion of **5** to **3** was achieved in pyridine–acetic acid (1:2, v/v) at 50 °C for 30 min. The hydrazine reaction of **4** followed by extraction and then warming in pyridine–acetic acid resulted in quantitative formation of **3**.

In order to see if the TLTr group can be used in the presence of other acid- or alkali-labile protecting groups, the syntheses of 3′-*O*-acetylthymidine (**6**) and 3′-*O*-(4,4′-dimethoxytrityl)thymidine (**7**) were conducted. Acetylation of **4** followed by unblocking of the TLTr group gave an 87% overall yield of **6**. Similarly, **7**, was obtained in 94% yield from **4**. The conditions for removal of the TLTr group described here proved to be very mild because the DMTr and acetyl groups were virtually stable.

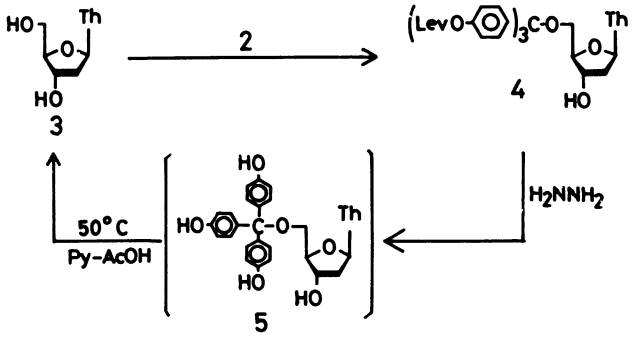
Furthermore, to demonstrate the synthetic utility of the TLTr group, we tried to synthesize thymidylyl-(3′—5′)-thymidine 3′-phosphate (TpTp) by using this new hydroxyl-protecting group. Phosphorylation of **4** with cyclohexylammonium *S,S*-diphenyl phosphorodithioate (**8**)[8] in the presence of mesitylenedisulfonyl chloride (MDS)[9] gave the 3′-phosphorylated product



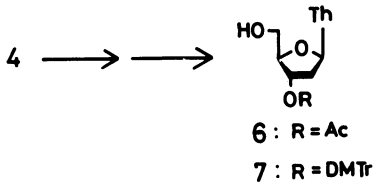
Scheme 1.

(**9**) in 86% yield. A similar hydrazinolysis of **9** and successive workup gave *S*,*S*-diphenyl thymidine 3′-phosphorodithioate (**10**) in 86% yield. The base-labile bis(phenylthio)phosphoryl group was found to be stable during these treatments. On the other hand, treatment of **9** with 3.3 M pyridinium phosphinate,[10,11] in pyridine resulted in quantitaive formation of Triethylammonium *S*-phenyl 5′-*O*-tris(levulinoyloxy)trityl]thymidine 3′-phosphorothioate (**11**). Upon this treatment, the TLTr group remained also intact. Condensation of **10** with **11** in the presence of MDS and 3-nitro-1,2,4-triazole (NT)[12] gave the dimer (**12**) in 82% yield. Treatment of **12** with AgOAc[5,13] in aqueous pyridine followed by ammonia gave TpTp in 91% yield.

Since the skeleton of the TLTr group is derived from a dye of rosolic acid, compounds containing the TLTr group can be readily detected as distinct orange spots upon heating TLC plates.



Scheme 2.



Scheme 3.

We have also examined the 9-[(4-levulinoyloxy)-phenyl]-9-xanthenyl and 4-dimethylamino-4′,4″-bis(levulinoyloxy)trityl groups as the possible hydrazine-labile protecting groups. However, the former was found to be too unstable during silica-gel column chromatography, and the latter was stable on silica gel but the corresponding hydroxyl-substituted xanthenyl group resulted from the hydrazine treatment was resistant to the pyridine–acetic acid treatment even at 60 °C.

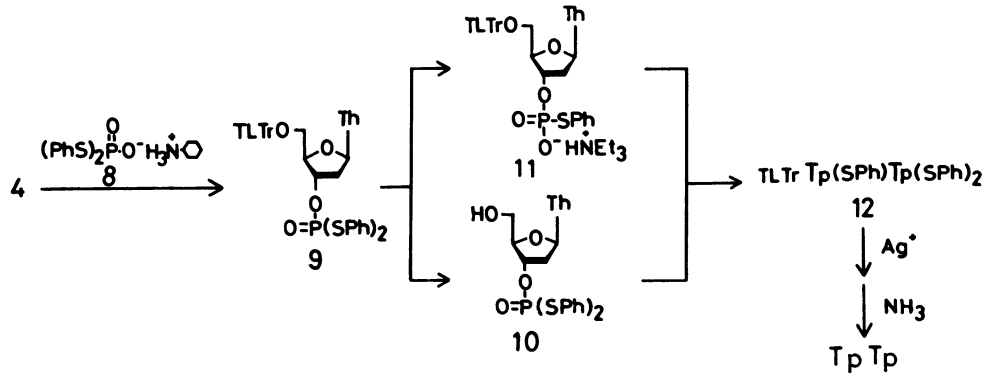
In conclusion, the TLTr group can be selectively introduced into a primary hydroxyl as an easily detectable group and used substantially as an equivalent of the levulinoyl group.

Experimental

^{1}H NMR spectra were recorded at 100 MHz on a JEOL JNM PS-100 spectrometer using tetramethylsilane as an internal standard. Column chromatography was performed with silica gel C-200 purchased from Wako Co. Ltd., and a mini pump for a goldfish basin was conveniently used to gain a medium pressure for rapid chromatographic separation. Rosolic acid and the other regents were purchased from Wako Co. Ltd.

4,4′,4″-Tris(levlinoyloxy)tritanol (1). To a mixture of rosolic acid (29 g, 0.1 mol) and levulinic acid (23.2 g, 0.2 mol) in pyridine was added dicyclohexylcarbodiimide (41.2 g, 0.2 mol). After the solution was stirred for 2 h, water (1.8 ml) was added to hydrolyze the quinomethane initially formed to 4-hydroxy-4′,4″-bis(levulinoyloxy)tritanol. After being stirred at 55 °C for 50 min, the solution was dried over powdered molecular sieves 3A (23 g) for 20 min and treated with levulinic acid (23.2 g, 0.2 mol) and dicyclohexylcarbodiimide (41.2 g, 0.2 mol) for 3 h. The mixture was quenched with water, filtered, extracted with CH_2Cl_2, dried over Na_2SO_4, and chromatographed on a silica-gel column with CH_2Cl_2 to yield **1** (26 g, 43%) as an oily material which gradually solidified upon standing: ^{1}H NMR ($CDCl_3$) δ=2.20 (s, 9H, $CH_3C(O)$), 2.81 (s, 12H, CH_2), 3.58 (br, 1H, OH), 6.93 (d, 6H, J=8.6 Hz, ArH), 7.22 (d, 6H, J=8.0 Hz, ArH). Calcd for $C_{34}H_{34}O_{10}$: C, 67.77; H, 5.69. Found: C, 67.82; H, 5.81.

5′-O-[4,4′,4″-tris(levulinoyloxyl)trityl]thymidine (4). To a solution of **1** (5.0 g, 8.3 mmol) in benzene (28 ml) was added acetyl bromide (3.9 ml, 53 mmol). The mixture was



Scheme 4.

refluxed for 15 min, and then the benzene and excess reagent were removed by evaporation. The residue was coevaporated three times with dry benzene under reduced pressure and finally dissolved in pyridine (30 ml). This solution was added to **3** (1.34 g, 5.5 mmol) predried by repeated coevaporations with dry pyridine. The resulting solution was stirred for 10 h and then water was added. Extraction with CH_2Cl_2 followed by chromatography on silica gel gave **4** (3.7 g, 81%) as foam: ^{1}H NMR ($CDCl_3$) δ=1.61 (s, 3H, CH_3), 1.33 (m, 2H, 2'-H), 3.22 (m, 2H, 5'-H), 3.78 (s, 3H, CH-O), 4.01 (m, 1H, 4'-H), 4.40 (m, 1H, 3'-H), 5.03 (d, 1H, J=4 Hz, OH), 6.42 (t, 1H, J=7 Hz, 1'-H), 6.83 (d, 2H, J=5.2 Hz, ArH), 7.00—7.52 (m, 10H, ArH), 7.66 (s, 1H, 6-H), 10.71 (br, 1H, NH). Calcd for $C_{44}H_{46}O_{14}N_2$: C, 63.92; H, 5.61; N, 3.39. Found: C, 63.76; H, 5.48; N, 3.24.

3'-O-Acetylthymidine (6). To a solution of **4** (414 mg, 0.5 mmol) in dry pyridine (5 ml) was added acetic anhydride (1 ml). After being stirred for 3 h, the solution was quenched with water and extracted with CH_2Cl_2 (3×20 ml). The combined extracts were dried over Na_2SO_4, concentrated to dryness, and the residue was dissolved in 1 M H_2NNH_2 in pyridine–acetic acid (3:2, v/v, 7.5 ml). The same workup as described in the synthesis of **10** gave **6** (123 mg, 87%). This compound was identified with an authentic sample by comparison of their ^{1}H NMR spectra.

3'-O-(4,4'-Dimethoxytrityl)thymidine (7). To a solution of **4** (414 mg, 0.5 mmol) in dry pyridine (5 ml) was added 4,4'-dimethoxytrityl chloride (339 mg, 1 mmol). The mixture was kept at 70 °C for 4 h. Then, the same workup as described in the synthesis of **10** gave **7** (255 mg, 94%). This compound was identified with an authentic sample by comparison of their ^{1}H NMR spectra.

S,S-*Diphenyl 5'-O-[4,4',4''-tris(levulinoyloxy)trityl]thymidine 3'-Phosphorodithioate (9).* A mixture of **4** (3.26 g, 3.94 mmol) and **8** (1.81 g, 4.73 mmol) were rendered anhydrous by repeated evaporations with dry pyridine (2×5 ml) and finally dissolved in dry pyridine (20 ml). MDS (1.88 g, 5.92 mmol) was added to the solution and the mixture was stirred for 30 min. The solution was diluted with CH_2Cl_2 and washed with water and 5% $NaHCO_3$. The usual workup followed by chromatography gave **9** (4.06 g, 94%): ^{1}H NMR ($CDCl_3$) δ=2.22 (s, 9H, $CH_3C(O)$), 2.30 (m, 2H, 2'-H), 2.82 (s, 12H, CH_2), 3.28 (m, 2H, 5'-H), 4.01 (m, 1H, 4'-H), 5.25 (m, 1H, 3'-H), 6.23 (t, 1H, J=7.5 Hz, 1'-H), 6.95 (d, 6H, J=8.5 Hz, ArH), 7.28 (d, 6H, J=8.5 Hz, ArH), 7.15—7.62 (m, 11H, ArH and 5-H), 8.47 (br, 1H, NH). Calcd for $C_{56}H_{55}O_{15}N_2PS_2$: C, 61.64; H, 5.08; N, 2.57. Found: C, 61.53; H, 5.21; N, 2.44.

S,S-*Diphenyl Thymidine 3'-Phosphorodithioate (10).* Compound **9** (546 mg, 1 mmol) was dissolved with stirring in 1 M H_2NNH_2 in pyridine–acetic acid (3:2, v/v, 15 ml). After being kept for 3 min, the solution was diluted with CH_2Cl_2 and washed with water and 5% $NaHCO_3$. The CH_2Cl_2 extract was dried over Na_2SO_4, filtered, and evaporated to dryness under reduced pressure. The residue was dissolved in pyridine–acetic acid (1:2, v/v, 15 ml) and the solution was warmed at 50 °C for 30 min. At this time the TLTr group was completely removed. The solution was extracted with CH_2Cl_2 and washed with water and 5% $NaHCO_3$. After filtration the solvent was removed under reduced pressure, and the residue was chromatographed on a silica gel to yield **10** (218 mg, 86%). This compound was identified with an authentic sample by comparison of their ^{1}H NMR spectra.

Synthesis of Dimer 12. Compound **9** (262 mg, 0.24 mmol) was treated with a mixture of 5 M phosphinic acid in pyridine (2.4 ml) and triethylamine (1.2 ml) at room temperature for 30 min. At this time one of the two phenylthio group was removed completely. The mixture was diluted with CH_2Cl_2 and washed with water and 0.2 M triethylammonium hydrogencarbonate (10 ml). The CH_2Cl_2 extract was dried over Na_2SO_4, filtered, and evaporated to dryness under reduced presure. The residue was mixed with **10** (253 mg, 0.2 mmol) and 3-nitro-1,2,4-triazole (82 mg, 0.72 mmol), and rendered anhydrous by evaporation with dry pyridine (3×5 ml). The mixture was dissolved in dry pyridine (2 ml) and MDS (228 mg, 0.72 mmol) was added. After being stirred for 30 min, the solution was worked up as descrived in the case of **9**. Chromatography yielded **12** (244 mg, 82%).

Full Deprotection of 12. Compound **12** (58 mg, 0.04 mmol) was dissolved in a solution of silver acetate (668 mg, 4 mmol) in pyridine–water (2:1, v/v, 4 ml) and the solution was stirred at 50 °C for 9 h. The resulting solution was diluted with pyridine-water (2:1, v/v, 40 ml). Then, hydrogen sulfide gas was bubbled into the solution at 0 °C until a clear supernatant had been obtained. The excess gas was removed under reduced pressure with stirring and the solution was centrifuged to remove the precipitate. The supernatant was evaporated to dryness under reduced pressure and dissolved in pyridine (5 ml). Concentrated aqueous ammonia (45 ml) was added and the solution was kept with stirring at 50 °C for 4 h. Then, the excess ammonia was evaporated with stirring by an aspirator and rosolic acid was removed by extraction with CH_2Cl_2. The aqueous layer was concentrated under reduced pressure and the residue was chromatographed on Whatman 3 MM papers developed with 2-propanol-concentrated ammonia–water (6:1:3, v/v/v). A band at R_f 0.2 was cut and eluted with water to give TpTp (630 OD, 91%). This product (20 OD) was incubated with spleen phosphodiesterase (10 μg, Boehringer) in a mixture of 0.05 M ammonium acetate (pH 6.5, 0.4 ml) and 0.01 M pyrophosphate buffer (pH 6.5, 0.2 ml) for 10 h gave only Tp (21 OD).

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