1,2-Ethylene-3,3-bis(4′,4″-dimethoxytrityl Chloride) (E-DMT): Synthesis and Applications of a Novel Protecting Reagent

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Abstract: 1,2-Ethylene-3,3-bis(4′,4″-dimethoxytrityl chloride), (E-DMT) was developed as a novel, bifunctional protecting reagent. This new compound was found to have a potential as a multipurpose acid-labile protecting reagent which can afford a 5′,5′-tritylthymidine dimer and a unique 5′,3′-cyclic protected thymidine derivative in modest to good yields.

Key words: bis(trityl chloride), bifunctional protecting reagent, acid-labile protecting group, solution-phase DNA synthesis, nucleoside, thymidine

Triphenylmethyl (trityl) group and its derivatives have been widely used for the protection of hydroxyl, amino, and carboxyl groups. The usefulness and importance of the trityl group are clearly demonstrated by (i) highly selective protection of less-hindered functional groups, (ii) stability under neutral or alkaline conditions, (iii) cleavage under mild acidic conditions, (iv) remarkable hydrophobicity, and (v) sensitive detection of the corresponding trityl cation by visual or spectrophotometric methods.[1] Due to these advantages, the trityl groups and in particular its 4′,4″-dimethoxy trityl (DMT) analogue are widely used for the protection of the 5′-hydroxyl group during DNA and RNA synthesis.[2]

In addition to these conventional monofunctional trityl groups, some bis(trityl chloride)s having two trityl chlorides connected with a bifunctional ester linkage have been reported by Köster et al. as a scaffold for solution-phase DNA synthesis (**1**, Figure 1).[3] These bifunctional trityl chlorides[4] can react with 2 equivalents of a thymidine derivative at the 5′-OH group allowing synthesis of two strands of DNA via the 3′-ends. This method significantly differentiates the trityl bearing two oligonucleotide chains from the monomers in molecular size to enable a facile purification of the trityl bearing oligonucleotide by size exclusion gel permeation chromatography. Recently, a solution-phase approach has been evaluated as a scalable route for the large-scale synthesis of therapeutic oligonucleotides.[5] The growing demand for synthetic DNA and RNA and its analogues as therapeutics[6] and reagents for diagnostic[7] applications has triggered a need for improved synthetic protocols[5,8] and the development of new protecting reagents.[9]

Despite the mentioned advantages, application of compound **1** in nucleic acid chemistry is limited by its susceptibility to base treatment. This limitation is particularly significant for large-scale solution-phase synthesis of oligonucleotides where extensive treatment with ammonium hydroxide is required. We envisioned that this issue could be addressed if the two-trityl groups could be linked together via an all carbon bridge. This modification would lead to a chemically stable bis(trityl) group amenable for oligonucleotide synthesis. Moreover such a protecting group could be recovered and recycled to make the process environmentally friendly.[10] Our continued interest in the development of novel protecting groups[11] prompted us to explore the synthesis of 1,2-ethylene-3,3-bis(4′,4″-dimethoxytrityl chloride) (E-DMT, **2**; Figure 1). Herein, we wish to report our results on the expeditious synthesis of E-DMT and show the preliminary results of potential applications of **2** as a protecting reagent.

A general route for the synthesis of trityl derivatives is based on the Grignard reaction between an aryl halide and a carboxylic ester. This method could be implemented for

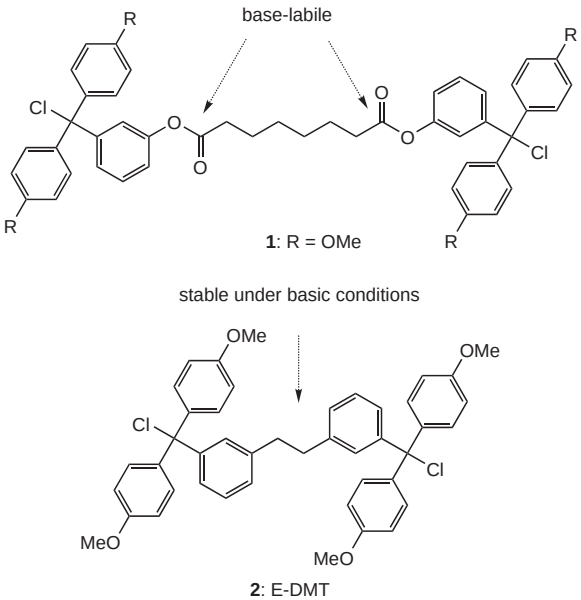

Figure 1 Chemical structures of bis(trityl chloride)s **1** and **2**.

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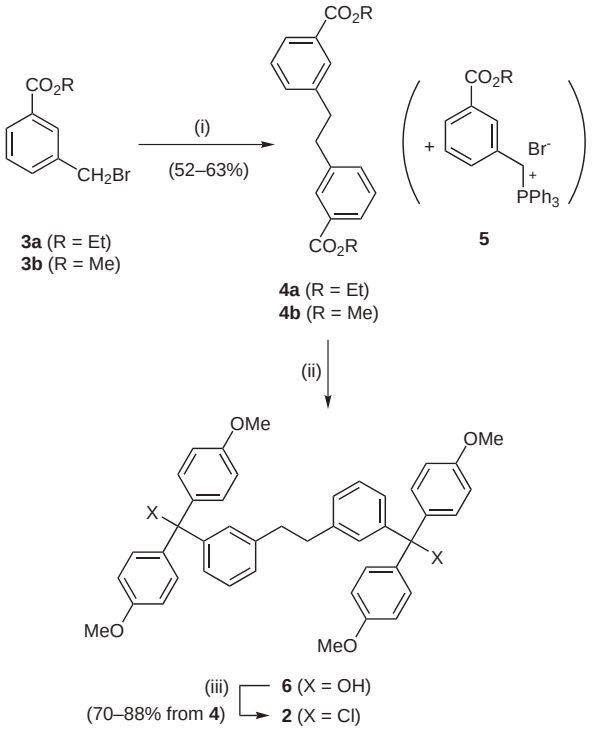
the synthesis of bis(trityl chloride)s using the appropriate bis(benzoate ester)s (Scheme 1). We decided to link the two benzoate esters at the *m*-position since substituents at *o*- or *p*-positions are known to alter significantly the stability of the resulting trityl ether. For example, under acidic conditions *p*-alkyl substituted DMT ethers are reported to be twice less stable than the parent DMT ether analogs.[12]

The above considerations led us to explore esters of the 1,2-ethylene-3,3-bis benzoic acid (**4a** or **4b**) as synthetic precursors for a new DMT dimer. Such a motif was expected to meet the above criteria and could also be easily accessible from coupling of the corresponding benzyl bromide monomers (**3a** or **3b**).[13]



Scheme 1 Synthesis of E-DMT **2**. *Reagents and conditions*: (i) $CoCl(PPh_3)_3$ (1.2 equiv), degassed benzene, 5–10 °C, 1 h, 52–63%; (ii) *p*-anisylmagnesium bromide (5 equiv), THF, reflux, 30 min; (iii) AcCl, reflux, 1.5 h then precipitated from C_6H_{12}, 70–88% from **4**.

The synthesis of E-DMT (**2**) is shown in Scheme 1. 3-Bromomethylbenzoic acid ethyl ester (**3a**) has been reported for the synthesis of **4a**.[13] However, **3a** is not commercially available and its reported synthesis is based on a carefully controlled bromination of ethyl *m*-toluate.[14] To circumvent this issue, we also used bromomethyl *m*-toluate (**3b**) which is commercially available.[15] Both **3a** and **3b** gave almost identical results in the step i and ii (Scheme 1).

The yield of step i was dependent on the purity of $CoCl(PPh_3)_3$.[16] We found that reproducible results were obtained by using freshly prepared $CoCl(PPh_3)_3$.[17] The competing by-product of this reaction was [(3-alkoxycarbonylphenyl)methyl]triphenylphosphonium bromide (**5**). The latter compound **5b** was generated in variable amounts (ca. 20–30%) when the reaction was carried out at room temperature. This side reaction could be suppressed when the reaction temperature was kept at 5–10 °C.

Next, we chose *p*-anisylmagnesium bromide for the Grignard reaction, which would give an analogue of 4′,4″-dimethoxytrityl chloride (DMTCl) as the final product. DMTCl is a well-studied and established protecting reagent for oligonucleotide synthesis, and we assumed that the stability of the resultant ether E-DMT (**2**) could probably be similar to the DMT protected nucleosides. The addition of a solution of *p*-anisylmagnesium bromide in THF to a solution of **4** in THF under a variety of conditions (–78 °C, 0 °C, r.t., or inverse addition) resulted in the formation of some unidentified by-products (stained orange with H_2SO_4–MeOH on TLC). Gratifyingly, the desired product **6** was obtained in nearly quantitative yield when a solution of *p*-anisylmagnesium bromide in THF was added drop wise to a solution of **4** in THF under reflux to ensure that the Grignard reagent was consumed immediately. The crude diol **6** was used for next step (iii) without further purification.

Treatment of **6** with AcCl under reflux for 1.5 h, followed by precipitation from cyclohexane at 4 °C afforded E-DMT (**2**) as an orange powder.[18] E-DMT (**2**) was stored at –20 °C for at least six months without noticeable degradation.

To evaluate the reactivity of **2** toward nucleosides, compound **2** was treated with 2 molar equivalents of thymidine in pyridine at room temperature (Scheme 2, reaction A). To ensure its quality prior to use, **2** was treated with $(COCl)_2$ for 1 hour to rechlorinate any trace amounts of trityl carbinol **6**, that may be formed due to adverse conditions. After removal of $(COCl)_2$ under vacuum, **2** was suspended in dry pyridine, and to this dry thymidine (co-evaporated with pyridine) was added in one portion. The symmetric dimer **7**,[19] having two thymidines attached to the E-DMT motif, was obtained in 53% yield. Under these conditions we also isolated the monosubstituted adduct in 19% yield. Incomplete conversion of the latter compound to dimer **7** may be due to the residual moisture and a slower reaction rate for the incorporation of the second thymidine.

In addition to the above products, a less-polar by-product was detected (TLC). Interestingly, the structure of this product was confirmed as 5′,3′-cyclic thymidine **8** (^{1}H NMR, ^{13}C NMR and MS).[20] This unexpected by-product is very attractive since selective protecting reagents for 5′,3′-hydroxyl groups of ribonucleosides are significantly important for the synthesis of base- and/or 2′-modified ribonucleosides derivatives.[11,21] Regardless of the importance, there are only a few bifunctional protecting reagents that are selective for the 5′,3′-hydroxyl groups of ribonucleosides. To the best of our knowledge, there is no cyclic protecting group, which is labile under mild, anhydrous acidic conditions, for 5′,3′-hydroxyl groups of

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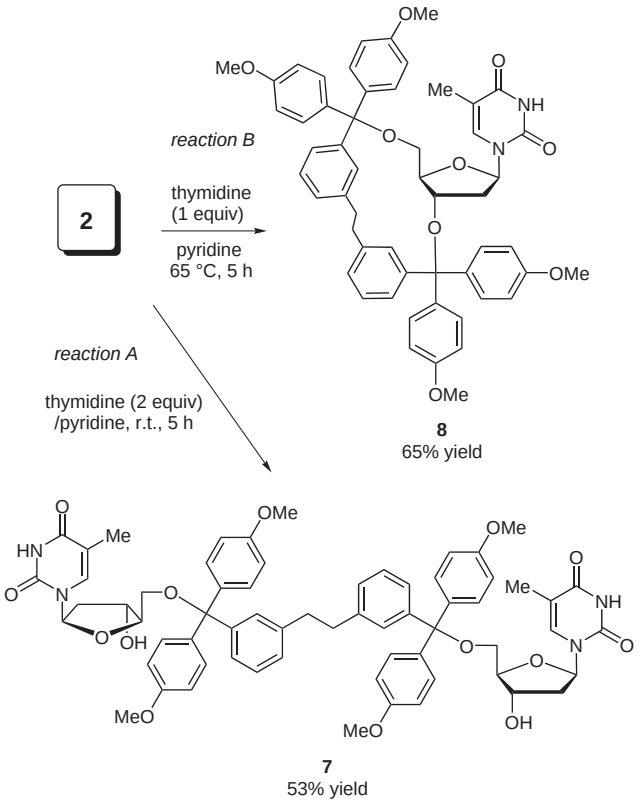
ribonucleosides. To improve the yield of **8** we tested a variety of conditions. Best results were obtained by drop wise addition of a pyridine solution of thymidine to a solution of **2** in pyridine at 65 °C. Using these conditions **8** was obtained in 65% yield. The optimized conditions furnished the best results (Scheme 2, reaction B). We postulate that this 'bending' property of **2** is due to the flexible ethylene bridge that may undergo free rotation to react with the neighboring 3′-OH group under appropriate conditions.

To evaluate the lability of this new protecting group, **7** and **8** were treated with 3% dichloroacetic acid–CH_2Cl_2, a standard detritylating reagent for DMT groups, at room temperature.[2] In both cases, the protecting group was completely removed within 3 minutes to give thymidine quantitatively as in the case of DMT group.

In conclusion, a novel 1,2-ethylene-3,3-bis(DMTCl) (E-DMT, **2**) has been synthesized as a multipurpose, flexible bifunctional protecting reagent. Under optimized conditions, compound **2** reacted with 2 equivalents of thymidine to afford the di(thymidine-5′-yl)trityl ether **7**. This property of **2** may be useful as a purification handle during solution-phase oligonucleotide syntheses and is currently under investigation. We also demonstrated that **2** reacted with 1 equivalent of thymidine to afford the 5′,3′cyclic thymidine **8**. We believe that the unusual ability of E-DMT group to 'stretch' or 'bend' is unique and could be further exploited for more complex synthetic manipulations.[22]



Scheme 2 Reactions of E-DMT (**2**) with thymidine.

Acknowledgment

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(18) **Experimental for Synthesis of 2:** Ethyl ester **4a** (1.96 g, 6.0 mmol) was dried by repeated coevaporations with dry toluene and dissolved in dry THF (30 mL) under argon. Freshly prepared 1 M *p*-anisyl magnesium bromide solution in dry THF was added drop wise to the mixture under reflux and stirred for 30 min. The mixture was cooled to r.t., diluted with Et_2O (150 mL), and washed with sat. NH_4Cl aq solution (150 mL). The aqueous layer was back-extracted with Et_2O (2 × 150 mL). The combined organic layers were washed with sat. NaCl aq solution (150 mL), dried over $MgSO_4$, filtered, and concentrated to dryness. The residue was dried by repeated coevaporations with dry toluene and dissolved in AcCl (3 mL). The mixture was heated under reflux for 1.5 h, and then cooled to r.t. The mixture was added to cyclohexane (30 mL), stirred for 5 min, and kept at 4 °C for 20 h. The mixture was warmed to r.t. and the resultant precipitate was collected by suction filtration. The collected solid was washed with dry cyclohexane (5 × 3 mL) and dried under vacuum to afford **2** (2.97 g, 4.22 mmol, 70%) as an orange powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.16–7.11 (m, 4 H), 7.08 (d, *J* = 8.8 Hz, 8 H), 7.01 (d, *J* = 7.6 Hz, 2 H), 6.94 (d, *J* = 8.8 Hz, 2 H), 6.78 (d, *J* = 8.8 Hz, 8 H), 3.80 (s, 12 H), 2.86 (s, 4 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.7, 145.4, 140.6, 137.6, 130.9, 129.9, 127.9, 127.3, 127.1, 112.8, 82.5, 55.3, 37.5. ESI-MS: *m/z* for $C_{44}H_{41}Cl_2O_4$ (M + H^+) 703. Anal. Calcd. for $C_{44}H_{40}Cl_2O_4$: C, 75.10; H, 5.73; Cl, 10.08. Found: C, 74.68; H, 6.07; Cl, 9.51.

(19) Analytical data for **7**: ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.3 (brs, 2 H), 7.50 (s, 2 H), 7.19–7.14 (m, 14 H), 6.94–6.93 (m, 2 H), 6.85–6.82 (m, 8 H), 6.20 (dd, *J* = 6.7, 6.7 Hz, 2 H), 5.32 (d, *J* = 4.4 Hz, 2 H), 4.33 (m, 2 H), 3.84 (m, 2 H), 3.71 (s, 12 H), 3.12 (m, 4 H), 2.72 (s, 4 H), 2.24 (ddd, *J* = 13.5, 6.7, 6.7 Hz, 2 H), 2.15 (ddd, *J* = 13.5, 6.7, 3.2 Hz, 2 H), 1.38 (s, 6 H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 163.4, 157.8, 157.8, 150.1, 143.8, 140.4, 135.6, 135.4, 135.2, 129.5, 129.3, 127.8, 127.6, 126.7, 125.3, 113.0, 109.4, 85.7, 85.4, 83.6, 79.1, 70.5, 63.6, 55.0, 37.1, 11.7. HRMS: calcd for $C_{64}H_{65}N_4Na_2O_{14}^+$ (M – H^+ +2 Na^+) 1159.4287; found: 1159.4342.

(20) Analytical data for **8**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.04 (brs, 1 H), 7.54 (s, 1 H), 7.37–6.69 (m, 24 H), 6.56 (dd, *J* = 9.2, 5.2 Hz, 1 H), 4.34 (m, 1 H), 4.04 (m, 1 H), 3.80 (s, 3 H), 3.78 (s, 3 H), 3.76 (s, 3 H), 3.72 (s, 3 H), 3.15–2.57 (m, 6 H), 2.27 (dd, *J* = 10.8, 3.2 Hz, 1 H), 2.11 (m, 1 H), 1.41 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 163.3, 158.6, 158.2, 158.1, 157.8, 150.0, 141.6, 141.5, 140.5, 140.1, 139.8, 138.5, 136.9, 135.6, 133.9, 130.9, 130.8, 129.4, 129.3, 129.3, 128.2, 127.7, 127.7, 127.6, 127.4, 127.3, 127.1, 113.2, 113.1, 113.0, 112.9, 110.8, 87.6, 86.6, 86.2, 85.5, 75.5, 55.3, 55.3, 55.3, 55.2, 40.4, 37.6, 36.8, 11.8. HRMS: calcd for $C_{54}H_{53}N_2O_9$ (M + H^+) 873.3745; found: 873.3732.

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