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The S-pixyl group: an efficient photocleavable protecting group for the 5′ hydroxy function of deoxyribonucleosides

Michael P. Coleman and Mary K. Boyd *

Department of Chemistry, Loyola University Chicago, 6525 N. Sheridan Rd, Chicago, IL 60626, USA

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Abstract

The 9-phenylthioxanthyl (S-pixyl or S-Px) group has been investigated as a photocleavable protecting group for primary alcohols, and specifically as a 5′ hydroxy protecting group for deoxyribonucleosides. Several alcohols, including the four nucleosides with protected exocyclic amino functions, were protected in very good to excellent yield by treatment of 9-chloro-9-phenylthioxanthene **3** in dry pyridine to reveal the derivatized compounds. Irradiation of the protected substrates in neutral, aqueous solution regenerated the starting alcohols in excellent yield.

Keywords: protecting groups; photochemistry; nucleosides; alcohols.

The extensive utility of oligonucleotides in applications ranging from DNA sequencing to antisense drugs has led to progressive advances in techniques used in their manufacture.[1,2] The efficient production of oligonucleotides is now possible using synthetic protocols that are nearly as well established as those used in solid-phase peptide synthesis. This simplicity in handling combined with the undeniable utility of oligonucleotides has led to their very creative use in combinatorial chemistry.[3,4] Such advances have produced a high demand for new protective schemes for the 5′ hydroxy function of nucleic acids, a key step in the synthesis of oligonucleotides via the phosphoramidite approach.[2]

The regioselective protection of the primary hydroxyl function in the presence of other secondary hydroxyl groups in nucleosides is a high priority when choosing a protective scheme.[2] Further, a 5′ protective scheme must be orthogonal with the current protocols of solid-phase oligonucleotide synthesis,[5] such as protection of the exocyclic amino function of the DNA bases. The use of low energy UV light as a reactive medium to selectively deprotect a 5′ protecting group in the presence of other protective schemes is very advantageous in that one can avoid the acid-catalyzed depurination of a nucleic acid that has been observed with the use of several acid-labile 5′ protecting groups.[6] In addition, one very exciting advantage from a synthetic perspective is the ability to use light to spatially control reactions within well-defined regions.

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* Corresponding author. Tel: 773-508-3174; fax: 773-508-3086; e-mail: mboyd@luc.edu

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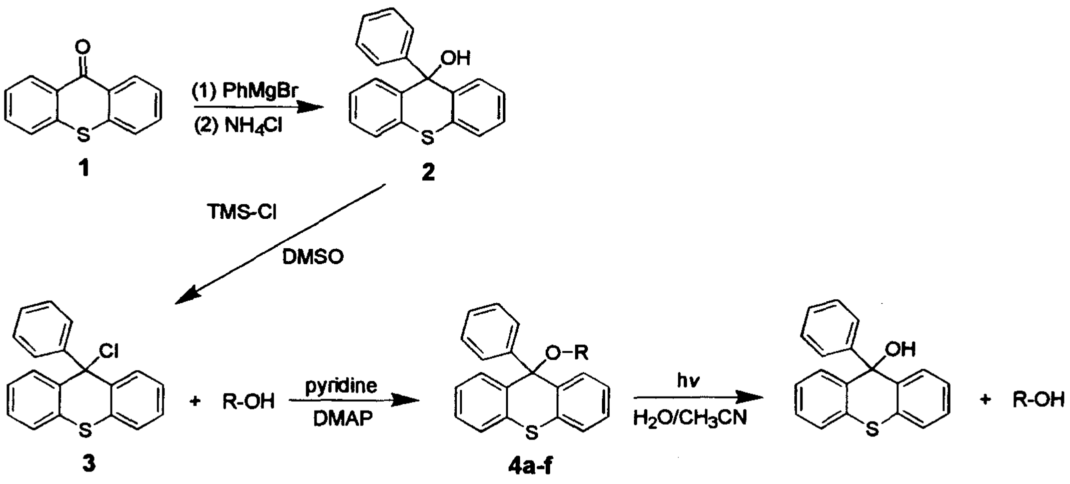
Fodor and co-workers have reported the efficient manufacture of a light directed, spatially-addressable synthesis of oligonucleotide arrays using the (α-methyl-2-nitropiperonyl)oxycarbonyl (MeNPOC) moiety as a 5′ photocleavable protecting group.[7] However, reactive byproducts produced upon irradiation of the nitrobenzyl and related protecting groups, as well as those produced by the dimethoxybenzoin moiety resulted in the loss of fidelity of the growing oligonucleotide, suggesting that alternative approaches are worthy of investigation.[8,9] We herein report that the S-pixyl group is an efficient photocleavable protective group for primary alcohols, including the 5′ hydroxy function of nucleic acids, and that the deprotection conditions are orthogonal with other protective schemes currently used in solid-phase oligonucleotide synthesis.

We have previously demonstrated the 9-phenylxanthyl (pixyl or Px) group to be an effective photocleavable protecting group for primary alcohols.[10] The pixyl group displays excellent crystalline properties as well as efficient deprotection yields under acceptable photolysis conditions. We then selected the 9-phenylthioxanthyl (S-pixyl or S-Px) group[11] as a potential photocleavable protecting group for primary alcohols, including nucleosides, due to a significant bathochromic shift in its absorption spectrum relative to the pixyl group, suggesting efficient deprotection yields with lower energy irradiations. A comparison of the UV–vis spectra of Px and S-Px protected phenethyl alcohol suggests that irradiations of subsequent S-pixyl derivatives at 300 nm would be suitable to deprotect the S-pixyl group and efficiently regenerate substrate alcohols. This is particularly important for nucleoside substrates which must be irradiated at wavelengths greater than 280 nm to avoid potentially damaging effects such as purine decomposition and the photoinduced dimerization of thymidine bases.[12] With such promising characteristics evident, we are particularly interested in developing the S-pixyl group as a photocleavable protecting group for the 5′ hydroxy function in the pentose sugar moiety of nucleosides.

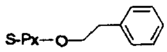
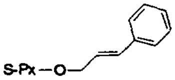
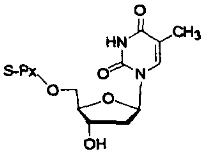
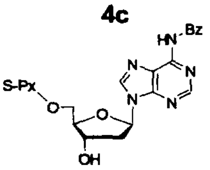
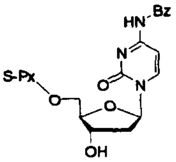
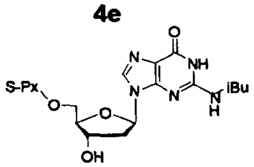
Thioxanthone **1** was reacted with the Grignard reagent phenylmagnesium bromide followed by weak acid hydrolysis to generate 9-phenylthioxanthenol **2** (Scheme 1). Compound **2** was then reacted with trimethylsilyl chloride and a catalytic amount of dimethylsulfoxide[13] to generate 9-chloro-9-phenylthioxanthene **3**. Protection of a series of substrate alcohols with **3** under anhydrous, basic conditions produced the derivatized alcohols **4a–f** as pure, off-white solids in good yields (Table 1).[14]



Scheme 1.

Compounds **4a–f** were irradiated in thoroughly degassed neutral-aqueous acetonitrile. The irradiations gave the recovered substrate alcohol and 9-phenylthioxanthenol as major photoproducts. Water concentrations and irradiation times were varied in order to achieve an optimum deprotection efficiency for each

Table 1

Derivatives 4a-f	Protection[a] %yield	Deprotection[b] %yield
 **4a**	80	93
 **4b**	94	95
 **4c**	79	97
 **4d**	86	96
 **4e**	82	75
 **4f**	92	89

[a] Yields reported are based on isolated yields after column chromatography
[b] All deprotections were conducted in a Rayonet reactor with 16, 300nm mercury arc lamps. The concentration of each reaction was approximately 5E-4M in varying concentrations of water and acetonitrile. Yields reported are based on the recovery of substrate as analyzed by reverse-phase HPLC. Optimum deprotection yields were obtained in 10-25min. Deprotections could also be carried out using a Hanovia lamp with Pyrex glassware giving similar deprotection yields.

derivative. Complete removal of the S-pixyl group was achieved in the maximum percentage of water that solubility would allow (40–60% water), as determined by HPLC analysis. Lower percentages of water resulted in decreased recovery of substrate alcohols and an increase in thioxanthone bi-product formation. Parallel 'dark' reactions were conducted for all experiments and demonstrated that there was no thermal degradation under the photolysis conditions. The protected alcohols exhibit good thermal stability, for example **4c** exhibited no decomposition after several hours of refluxing in aqueous acetonitrile. Deprotection yields (Table 1) were based on recovery of substrate alcohols. It was determined that the photoproduct 9-phenylthioxanthenol **2** after long irradiation times underwent photo-oxidative secondary photochemistry to produce thioxanthone **1** but this did not effect the recovery of the substrate alcohol.

Two common protecting groups for the exocyclic amino functions within a nucleic acid, the isobutyryl group used to protect guanosine and the benzoyl group used to protect adenosine and cytidine were not affected under these photolysis conditions. Control experiments compared irradiations of the S-Px protected nucleosides to irradiation of the nucleosides with no $5'$ hydroxyl protecting group in the presence and absence of equimolar amounts of **2**. These experiments demonstrated that the photolysis conditions had little or no effect on each of the respective nucleosides. Photolysis of compound **4e** was problematic since the pyrimidine base of cytidine absorbs a significant amount of light at 300 nm, which made subsequent photolysis reactions sluggish and required longer irradiation times.

In summary, the S-pixyl group has proven to be a viable photocleavable protecting group for primary alcohols including the four nucleosides. It has also been proven to be orthogonal with other commonly used protective schemes currently used in solid-phase oligonucleotide synthetic protocols.

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14. The following synthesis is representative: $5'$-*O*-(9-phenylthioxanthyl)-$2'$-deoxythymidine **4c**: trimethyl silyl chloride (6 mL) was added dropwise to 9-phenylthioxanthenol **2** (1.480 mmol, 0.429 g) dissolved in 12 mL of methylene chloride and 0.2 mL of dimethylsulfoxide. After 12 h the solvent was removed and 9-chloro-9-phenylthioxanthene **3** was obtained in nearly quantitative yield. **3** was dissolved in 7 mL pyridine and added dropwise to thymidine (1.345 mmol, 0.328 g) and DMAP (0.041 mmol, 0.005 g) in 10 mL pyridine. TLC indicated that the reaction was complete within 7 h. The reaction was quenched with 10 mL of saturated aqueous Na_2CO_3, extracted with methylene chloride and dried with magnesium sulfate. Evaporation of the organic layer gave a faintly red glassy crystal. Column chromatography yielded

5′[9-phenylthioxanthyl]2′deoxythymidine ether **4c** as a white solid in 79% yield; mp (dec) 137–145°C. ^{1}H NMR (DMSO) δ: 1.45 (s, 3H); 2.35 (m, 2H); 3.24 (dd, 2H); 3.95 (d, 1H); 4.41 (br, 1H); 5.40 (t, 1H); 6.23 (t, 1H); 7.20–7.77 (m, 13H); 8.28 (s, 1H); 11.40 (s, 1H). ^{13}C NMR δ: 62.2; 70.4; 79.4; 79.9; 82.0; 82.8; 109.9; 124.8; 125.5; 126.7; 127.0; 128.9; 128.1; 128.2; 128; 129.1; 129.3; 130.0; 133.8; 133.9; 148.1; 150.5; 163.9. 9-Phenylthioxanthyl phenethyl ether **4a**: mp (dec) 129–132°C. ^{1}H NMR (CDCl$_3$) δ: 2.91 (t, 2H); 3.35 (t, 2H); 7.01–7.37 (Ar, 18H). ^{13}C NMR δ: 36.66; 64.70; 79.69; 124.98; 125.19; 126.07; 126.11; 126.52; 127.33; 127.91; 128.15; 129.11; 129.68; 130.11; 134.897; 139.266; 149.31. 9-Phenylthioxanthyl cinnamyl ether **4b**: mp (dec) 135–143°C. ^{1}H NMR (CDCl$_3$) δ: 3.88 (d, 2H); 6.31 (m, 1H); 6.63 (d, 1H); 7.00–7.61 (Ar, 18H). ^{13}C NMR δ: 65.08; 80.44; 125.427; 125.685; 126.52; 126.62; 126.77; 126.94; 127.69; 127.79; 128.23; 128.73; 129.88; 130.62; 131.12; 135.20; 137.2; 148.97. 5′-*O*-(9-Phenylthioxanthyl)-N6-benzoyl-2′-deoxyadenosine **4d**: mp (dec) 130–137°C. ^{1}H NMR (DMSO) δ: 2.51 (m, 1H); 2.84 (m, 1H); 3.59 (m, 2H); 3.93 (q, 1H); 4.47 (t, 1H); 5.43 (s, 1H); 6.51 (t, 1H); 6.90 (t, 1H); 7.09–7.68 (Ar, 16H); 8.03 (d, 2H); 8.58 (s, 1H); 8.61 (s, 1H); 11.21 (s, 1H). ^{13}C NMR δ: 64.21; 70.17; 79.20; 83.93; 85.80; 124.95; 125.02; 125.35; 126.03; 126.45; 126.82; 127.85; 128.05; 128.15; 128.51; 129.42; 129.51; 132.25; 133.23; 134.00; 143.44; 148.32; 150.17; 151.31; 151.89; 165.45. 5′-*O*-(9-Phenylthioxanthyl)-N4-benzoyl-2′-deoxycytidine **4e**: mp (dec) 150–152°C. ^{1}H NMR (DMSO) δ: 2.18–2.24 (m, 2H); 3.23–3.3.45 (m, 2H); 4.06 (q, 1H); 4.38 (q, 1H); 5.38 (br s, 1H); 6.20 (t, 1H); 7.09–7.65 (Ar, 17H); 8.07 (d, 2H); 8.22 (d, 1H); 11.32 (br s, 1H). ^{13}C NMR δ: 63.37; 69.43; 79.76; 85.45; 85.99; 96.02; 124.96; 125.32; 125.28; 126.58; 126.66; 126.93; 128.01; 128.12; 128.17; 128.38; 129.34; 129.40; 129.57; 129.81; 132.66; 133.65; 133.82; 144.44; 152.07; 162.89; 166.45. 5′-*O*-(9-Phenylthioxanthyl)-N2-isobutyryl-2′-deoxyguanosine **4f**: mp (dec) 159–163°C. ^{1}H NMR (DMSO) δ: 1.13 (d, 6H); 2.35 (m, 1H); 2.72 (m, 2H); 3.27 (q, 2H); 4.09 (m, 1H); 4.45 (m, 1H); 5.39 (s, 1H); 6.27 (t, 1H); 6.89 (t, 1H); 7.09–7.58 (Ar, 13H); 8.07 (s, 1H); 9.19 (br s, 1H); 11.45 (br s, 1H). ^{13}C NMR δ: 18.88; 64.49; 70.42; 79.29; 83.20; 85.82; 124.66; 125.03; 125.32; 126.00; 126.48; 126.76; 127.66; 127.92; 128.09; 129.09; 129.14; 129.30; 129.56; 133.99; 134.02; 137.62; 147.71; 148.22; 148.32; 154.77; 180.00.