Supporting information for Chemical Communications

S(O)-pixyl protecting groups as efficient mass-tags

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General

Unless otherwise noted, starting materials were purchased from commercial sources and used without further purification. Anhydrous solvents were obtained from commercial sources (Romil Ltd) and used without any further treatment. All reactions with moisture sensitive reagents were performed in oven-dried glassware and under protective argon atmosphere. Whenever possible, reactions were monitored by thin-layer chromatography (TLC) using TLC silica gel coated aluminium plates 60F254 (MN).

Instrumentation

$^1$H NMR spectra were recorded on Bruker DPX200 instrument. Chemical shifts are quoted in parts per million with reference to residual protons of the deuterated solvent. UV spectra were recorded on a Shimadzu UV-2401 PC spectrometer, equipped with a cell temperature controller.

The synthesis of oligonucleotides was carried out in an Applied Biosystems 394 DNA/RNA four column synthesiser using, unless otherwise noted, standard phosphoramidite chemistry.

HPLC analyses and separations were carried out on a Waters system coupled to a photodiode array detector analyser and the data was processed using Waters Millenium software. Separations were achieved using a Chromolith Performance column (RP-18e 100-4.6mm). All separations were achieved by gradient elution of water and acetonitrile.

MALDI- and LDI-TOF mass spectra were recorded on a PE-ABI Voyager Elite Reflection Delayed Extraction Instrument. Spectra were acquired with an accelerating voltage of 20KV and 200 ns delay in the positive ion mode.

High resolution mass spectra were recorded on a AutoSpec-oaTof spectrometer.
INITIAL APPROACHES USING MODEL TRITYL COMPOUNDS

Initially, we investigated model trityls based on structures 18 and 19 (Figure 1) as candidates for trityl tags with adjustable stability. The formation of the corresponding cations was controlled by modifying the oxidation state of heteroatoms N, S or Se to the corresponding N-oxides, sulphoxides and selenoxides. Model compounds 18a, 18b, 19a and 19c were prepared according to literature procedures. Compound 19b was prepared using an adaptation of these procedures. Compounds based on structures 18a,b and 19a,b were found to be inadequate for our applications.

Candidates to trityl tags with adjustable stability.

S-PIXYL DERIVATIVES 4

S-pixyl derivatives 4a and 4b were prepared according to literature procedures.3 S-pixyl derivatives 4c and 4d were prepared by Grignard reaction of the appropriate commercial (Aldrich) arylmagnesium bromide solution (10mmol) and the appropriate thioxanthenone (5mmol) in anhydrous THF at room temperature. In a typical procedure, arylmagnesium bromide solution is dropwise added to a 0ºC cooled THF solution of the starting, thioxanthenone under argon atmosphere. The reaction mixture is then stirred for 4 h at room temperature. Aqueous workup followed by purification with silica gel chromatography using hexane/ethyl acetate (5:1) as eluent gave the pure S-pixyl derivatives 4.

3-methoxy-9-(p-methylphenyl)thioxanthen-9-ol (4c):

Yield: 70%

1H NMR (CDCl3):  δ 7.97 (dd, J = 1.1 and 7.8 Hz, 1H), 7.86 (d, J = 8.7 Hz, 1H), 7.39 (d, J = 7.7 Hz, 1H), 7.35 (dt, J = 7.6 and 0.9 Hz, 1H), 7.25 (dt, J = 7.50 and 1.2 Hz, 1H), 6.97 (d, J = 8.2 Hz, 2H), 6.94 (d, J = 2.54 Hz, 1 H), 6.90 (dd, J = 8.6 and 2.7 Hz, 1H), 6.88 (d, J = 8.2 Hz, 2H), 3.81 (s, 3H), 2.24 (s, 3H).

Exact mass (TOF MS ES+, (M-OH)+): Calcd for C21 H17 O S 317.1000, found 317.0992.

1 R. Berslow, L. Kaplan, D. LaFollette, J. Am. Chem. Soc. 1968, 90, 4051.
3,6-dimethoxy-9-(p-methylphenyl)thioxanthen-9-ol (4d):

Yield: 91%

$^1$H NMR (CDCl$_3$): δ 7.96 (d, $J = 8.60$ Hz, 2H), 7.10-6.90 (m, 8H), 3.85 (s, 6H), 2.33 (s, 3H).

Exact mass (TOF MS ES+, (M-OH)$^+$): Calcd for C$_{22}$H$_{19}$O$_2$S 347.1106, found 347.1101.

**Preparation of S-pixyl oxides 5**

S-pixyl oxides 5 were prepared by the dropwise addition of 1 eq of MCPBA in dichloromethane to a dichloromethane solution (0.1M) of the appropriate starting S-pixyls, at 0°C. The reaction mixture was stirred for 1 h and then quenched with a saturated solution of sodium bicarbonate. Aqueous workup followed by purification with silica gel chromatography using hexane/ethyl acetate (3:1) as eluent gave the pure S-pixyl oxide derivatives 5.

9-(p-methylphenyl)-10-oxothioxanthen-9-ol (5a):

Yield: 88%

$^1$H NMR (CDCl$_3$): δ 8.10 (d, $J = 7.6$ Hz, 2H), 7.87 (dd, $J = 7.6$ and 0.8 Hz, 2H), 7.57 (dt, $J = 7.6$ and 1.2 Hz, 2H), 7.50 (dt, $J = 7.6$ and 1.0 Hz, 2H), 6.97 (d, $J = 8.2$ Hz, 2H), 6.80 (d, $J = 8.2$ Hz, 2H), 2.24 (s, 3H).

Exact mass (TOF MS ES+, M + Na$^+$): Calcd for C$_{20}$H$_{16}$O$_2$NaS 343.0769, found 343.0768.

9-(p-methoxyphenyl)-10-oxothioxanthen-9-ol (5b):

Yield: 82%

$^1$H NMR (CDCl$_3$): δ 8.17 (d, $J = 7.7$ Hz, 2H), 7.82 (d, $J = 7.6$ and 0.8 Hz, 2H), 7.74 (dt, $J = 7.6$ and 1.1 Hz, 2H), 7.64 (dt, $J = 7.6$ and 0.6 Hz, 2H), 6.81-7.75 (m, 4H), 3.70 (s, 3H).

Exact mass (TOF MS ES+, M + Na$^+$): Calcd for C$_{20}$H$_{16}$O$_3$NaS 359.0718, found 359.0716.

3-methoxy-9-(p-methylphenyl)-10-oxothioxanthen-9-ol (5c):

Yield: 75%

$^1$H NMR (CDCl$_3$): δ 8.07 (d, $J = 7.7$ Hz, 1H), 7.89 (d, $J = 7.9$ Hz, 1H), 7.76 (d, $J = 8.8$ Hz, 1H), 7.60 (t, $J = 7.6$ Hz, 1H), 7.55-7.49 (m, 2H), 7.15 (d, $J = 8.2$ Hz, 2H), 7.10 (dd, $J = 8.8$ and 2.6 Hz, 1H), 7.05 (d, $J = 8.2$ Hz, 2H), 3.88 (s, 3H), 2.26 (s, 3H).

Exact mass (TOF MS ES+, M + Na$^+$): Calcd for C$_{21}$H$_{18}$O$_3$NaS 373.0874, found 373.0869.
3,6-dimethoxy-9-(p-methylphenyl)-10-oxothioxanthen-9-ol (5d):

Yield: 80%
1H NMR (CDCl3): δ 7.98 (d, J = 8.5 Hz, 2H), 7.44 (d, J = 2.6 Hz, 2H), 7.09 (dd, J = 8.5 and 2.6 Hz, 2H), 6.99 (d, J = 8.2 Hz, 2H), 6.85 (d, J = 8.2 Hz, 2H), 3.90 (s, 6H), 2.26 (s, 3H).

Exact mass (TOF MS ES+, M + Na+): Calcd for C22 H20 O4 Na S 403.0980, found 403.0960.

SYNTHESIS OF PHOSPHORAMIDITES 11a AND 11b

S-pixylols 6a and 6b

1.7M tert-BuLi in pentane (22mmol) was dropwise added to a stirred and cooled to -80°C solution of 1-[2-(4-bromophenoxy)ethyl]-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane 7 (20mmol) in THF (25ml). The temperature was allowed to rise to -40°C for 1 h and then lowered again to -80°C. A solution of ketone 8a or 8b (10mmol) in 10 ml of THF was then dropwise added and the reaction mixture was stirred overnight, allowing the temperature to reach the room temperature. The solution was evaporated to dryness, the residue was dissolved in ethyl acetate (150ml) with a few drops of triethylamine, washed with saturated sodium bicarbonate and brine, dried over anhydrous sodium sulphate and solvents rotavaporated. The residue was purified by column chromatography. Silica gel was previously treated for 30min with 1% triethylamine solution in hexane/ethyl acetate 5:1. This eluent was used to separate the starting materials. The product was then obtained using 1% ethylamine in hexane/ethyl acetate 2:1 as eluent.

1-{2-[4-(9-Hydroxy-3-methoxythioxanthen-9-yl)phenyl]ethyl}-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane (6a):

Yield: 48%
1H NMR (CDCl3): δ 8.00 (dd, J = 7.8 and 1.2 Hz, 1H), 7.89 (d, J = 8.6 Hz, 1H), 7.42-7.36 (m, 4H), 7.10-6.90 (m, 5H), 3.90 (s, 6H), 3.83 (s, 3H), 2.75-2.60 (m, 2H), 1.92-1.81 (m, 2H), 0.80 (s, 3H).

1-{2-[4-(9-Hydroxy-3,6-dimethoxythioxanthen-9-yl)phenyl]ethyl}-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane (6b):

Yield: 56%
1H NMR (CDCl3): δ 7.84 (dd, J = 8.4 and 0.6 Hz, 2H), 7.13-6.85 (m, 8H), 3.90 (s, 6H), 3.82 (s, 6H), 2.70-2.60 (m, 2H), 1.90-1.80 (m, 2H), 0.81 (s, 3H).
**S-pixylols 9a and 9b**

Starting S-pixyl 6a or 6b (10mmol) was dissolved in w THF/water 9:1 mixture (100ml) and trifluoroacetic acid (0.25eq) was dropwise added. The mixture was stirred for 1h and then solvents were rotavaporated. Then the coloured residue was dissolved in dichloromethane and neutralised with a saturated solution of sodium bisulphate. Once neutralised, the organic phase was separated, washed with brine (x2) and solvents rotavaporated.

The residue was dissolved in 95% EtOH. Then LiOH (10eq) was added and the mixture was stirred overnight at room temperature. Solvents were then rotavaporated. To ensure the complete elimination of EtOH, water (100ml) was added to the residue and the solvent evaporated at reduced pressure. The residue was then dissolved in water (300ml) and an excess of ammonium chloride was added to the mixture until a fine solid starts to precipitate. At this point, dichloromethane was added to the mixture (300ml). The organic layer was separated, washed with brine, dried over anhydrous sodium sulphate and rotavaporated.

The residue (3mmol) was then dissolved in dry acetonitrile/DMF 9:1, under argon atmosphere at room temperature. N,N'-disuccinimidyl carbonate (DCC, 4mmol) and triethylamine (12mmol) were added to the solution and the mixture was stirred for 2h at room temperature. Solvents were then rotavaporated and then, upon the addition of water, a white solid was generated. The solid was filtered and dried under high vacuum.

3-[4-(9-Hydroxy-3-methoxythioxanthen-9-yl)phenyl]propionic acid, N-oxysuccinimide ester (9a):

Yield: 33% overall.

1H NMR (CDCl3): δ 8.00 (dd, J = 7.7 and 1.1 Hz, 1H), 7.89 (d, J = 8.5 hz, 1H), 7.45-7.15 (m, 4H), 7.07-6.89 (m, 5H), 3.84 (s, 3H), 3.20-2.80 (m, 8H).

3-[4-(9-Hydroxy-3,6-dimethoxythioxanthen-9-yl)phenyl]propionic acid, N-oxysuccinimide ester (9b):

Yield: 41% overall.

1H NMR (CDCl3): δ 7.84 (d, J = 8.4 Hz, 2H), 7.10-6.80 (m, 8H), 3.83 (s, sH), 3.00-2.80 (m, 8H).

**S(O)-pixyl ethers 10a and 10b**

9a or 9b was dissolved in the minimum necessary amount of dry acetonitrile. Then, a 54% solution of tetrafluoroboric acid in diethyl ether (1.5 eq,) was dropwise added. Upon addition, the solution acquired a strong orange/red colour. After stirring for 10 min, the tetrafluoroborate salt of the starting S-pixyl compounds was precipitated by the careful addition of anhydrous diethyl ether. The coloured solid was collected by filtration and used without any further purification.
The coloured solids were then suspended in dry acetonitrile under argon atmosphere. To the suspension, 10eq of anhydrous 1,3-butanediol and 1.1 eq of DIEA were added. After 1/2h of stirring at room temperature, the solution lost the vivid colouration. MCPBA was then portionwise added to the mixture, monitoring the conversion of the starting material by TLC after each addition (approx 1.5eq were required). Once the conversion is completed, solvents were rotavaporated and the residue is dissolved in dichloromethane, washed with 1M ammonium chloride, dried over sodium sulphate and solvents rotavaporated. The residue was purified by column chromatography, using chloroform/acetone 2:1 as eluent.

$^1$H-NMR of compounds 10a and 10b resulted unexpectedly complicated. $^1$H signals appeared duplicated due to the presence of two asymmetric centres in the molecule. Giving the low resolution (200MHz) of the available NMR spectrometer, $^1$H spectra provided just with qualitative information about the expected products (i.e. the presence of the methoxy groups, the hydroxysuccinimide moieties, the presence of the 1,3-butanodiol molecule, etc). The identity of the products was confirmed by high resolution MS and by chemical test (not shown).

3-{4-[9-(3-Hydroxybutanoxy)-10-oxo-3-methoxythioxanthen-9-yl]phenyl}propionic acid, N-oxysuccinimide ester (10a):

Yield: 75% overall.

Exact mass (TOF MS ES+, M + Na$^+$): Calcd for C31 H31 N O8 Na S 600.1668, found 600.1676.

3-{4-[9-(3-Hydroxybutanoxy)-10-oxo-3,6-dimethoxythioxanthen-9-yl]phenyl}propionic acid, N-oxysuccinimide ester (10b):

Yield: 82% overall.

Exact mass (TOF MS ES+, M + Na$^+$): Calcd for C32 H33 N O9 Na S 630.1774, found 630.1775.

Phosphoramidites 11a and 11b

Phosphoramidites 11a and 11b were freshly prepared immediately prior to their utilization and were used without characterisation.

Starting compounds 10a and 10b (1mmol) dissolved in dry dichloromethane (10ml) under argon atmosphere at 0°C. 2-cyanoethyl diisopropylchlorophosphoramidite (1eq) and DIEA (1.1eq) were added to the mixture. The conversion of the starting material was monitored by TLC. After 2h the reaction mixture was diluted with 50ml of chloroform, washed with water (2x100ml) and dried over sodium sulphate. Solvents were evaporated and the residue was thoroughly dried under high vacuum. For their use in the ABI DNA synthesiser, 0.2mmol of the obtained residues were dissolved in 1.5ml of dry acetonitrile.
SYNTHESIS OF OLIGONUCLEOTIDES

Thymidine tetramers were synthesised on the ABI synthesiser, using standard protocols starting from 0.2\(\mu\)mole CPG standard dT columns (Cruachem). Phosphoramidites 11a and 11b were added at the final step of the synthesis. The final capping step was skipped to avoid possible side reactions of the capping reagent with the sulphoxide function. Protected oligonucleotides 13a and 13b were reacted with amines by the treatment of the CPG columns with 0.1M solutions of the corresponding amines in THF for 15 min. Then, they were deprotected by the treatment of the GPC resins with 75\(\mu\)l of a 50% ethanolamine solution in methanol, for 45 min at 55ºC. Oligonucleotides 17a and 17b were then precipitated by the addition of 75\(\mu\)l of a 2M lithium perchlorate solutions and 1.5ml of acetone at 0ºC. The obtained residues were then dissolved in 1ml of water. A fraction of these solutions were purified by HPLC.

MASS SPECTROMETRY

A comprehensive study to determine the most optimal conditions for the detection the \(S\)-pixyl mass tags attached to the oligonucleotides by (MA)LDI-TOF spectrometry was carried out. All the analysis were completed preparing the samples both with and without matrix (2,5-dihydroxybenzoic acid, DHB). The best spectra were obtained when the solutions of the oligonucleotides 17a and 17b were spotted directly on the spectrometer stainless steal plate, without any treatment. Some other the conditions gave similar results (i.e. treatment with HI, spectra not show).

(MA)LDI spectra of oligonucleotides 17a and 17b are included below, indicating the path in Scheme 4 through they were synthesised and the
<table>
<thead>
<tr>
<th>Ion 15b from 17b, path C, LDI MS</th>
<th>Ion 15b from 17b, path C, MALDI MS</th>
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<tbody>
<tr>
<td>Ions 15a and 15b from 17a and 17b, path C, LDI MS</td>
<td>Ions 15a and 15b from 17a and 17b, path C, MALDI MS</td>
</tr>
<tr>
<td>Ions 15a and 16a from 17a, paths C and D, LDI MS</td>
<td>Ions 15a and 16a from 17a, paths C and D, MALDI MS</td>
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