Supplementary Material (ESI) for Chemical Communications

Supporting information for Chemical Communications

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

Pablo L. Bernad Jr,[a] Safraz Khan,[a] Vladimir A. Korshun,[b] Edwin M. Southern[c] and Mikhail S. Shchepinov*[a]

[a] *Tridend Technologies (a Division of OGT), Hirsch Building, Begbroke Business & Science Park, Sandy Lane, Yarnton, Oxford, OX5 1PF, UK. E-mail:misha@tridend.com; Fax: +44(0)186 5842 116*
[b] *Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Miklukho-Maklaya 16/10, Moscow 117 997, Russia*
[c] *Oxford Gene Technology (IP) Ltd, Hirsch Building, Begbroke Business & Science Park, Sandy Lane, Yarnton, Oxford, OX5 1PF, UK.*

Supplementary Material (ESI) for Chemical Communications

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 $60F_{254}$ (MN).

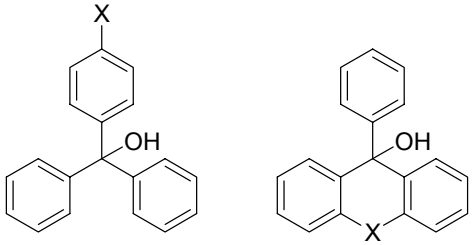
Instrumentation

^{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.

Supplementary Material (ESI) for Chemical Communications

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**,[1] **18b**,[1] **19a**[2] and **19c**[3] 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.



18 **19**

compound	X
18a	NMe_2
18b	SMe
19a	NMe
19b	Se
19c	S

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%
^{1}H NMR ($CDCl_3$): 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+, $(M\text{-}OH)^+$): 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.
2 A. R. Katritzky, W. H. Ramer, *J. Org. Chem.* 1985, **50,** 852.
3 a) N. Balgobin, J. Chattopadhyaya, *Chim. Scripta* 1982, **19,** 143; b) J. M. Bedlek, M. R. Valentino, M. K Boyd, *J. Photochem. Photobiol. A: Chem.* 1996, **94,** 7; c) M. P. Coleman, M. K. Boyd, *J.Org. Chem.* 2002, **67,** 7641.

Supplementary Material (ESI) for Chemical Communications

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+, $(M\text{-}OH)^+$): Calcd for C22 H19 O2 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 0ºC. 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 C20 H16 O2 Na S 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 C20 H16 O3 Na S 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 C21 H18 O3 Na S 373.0874, found 373.0869.

Supplementary Material (ESI) for Chemical Communications

3,6-dimethoxy-9-(*p*-methylphenyl)-10-oxothioxanthen-9-ol (5d):

Yield: 80%
^{1}H NMR ($CDCl_3$): 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 -80ºC 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 -40ºC for 1 h and then lowered again to -80ºC. 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%
^{1}H NMR ($CDCl_3$): 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%
^{1}H NMR ($CDCl_3$): 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), 1.90-1.8o (m, 2H), 0.81 (s, 3H).

Supplementary Material (ESI) for Chemical Communications

***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 and 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 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.
^{1}H NMR ($CDCl_3$): 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.
^{1}H NMR ($CDCl_3$): 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 0ºC. 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.

Supplementary Material (ESI) for Chemical Communications

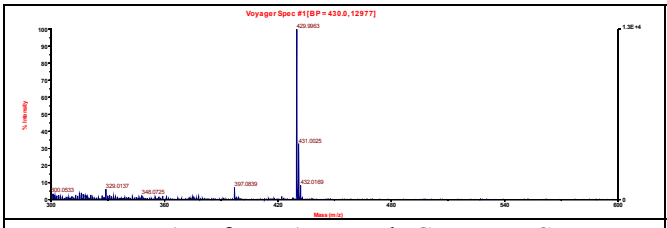
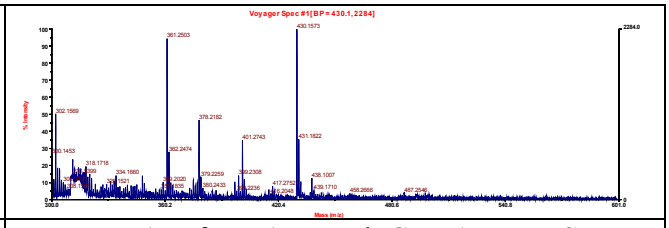
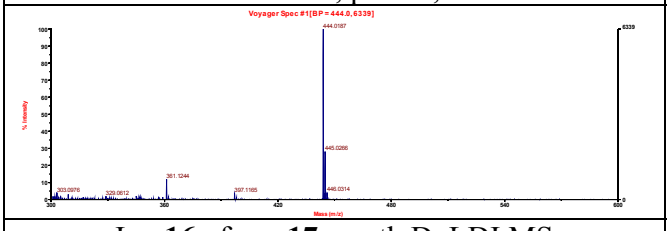
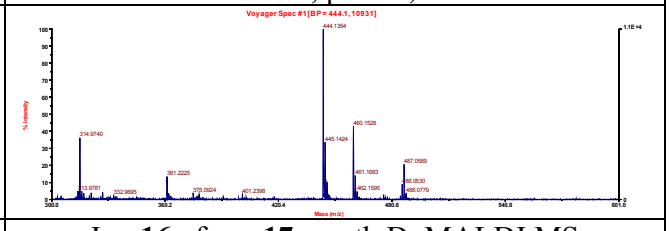
SYNTHESIS OF OLIGONUCLEOTIDES

Thymidine tetramers were synthesised on the ABI synthesiser, using standard protocols starting from 0.2µmole CPG standard dT columns (Cruachem). Phosphoramidites **11a** and **11b** were added at the final step of the synthesis. The final capping step was skiped 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 75µl of a 50% ethanolamine solution in methanol, for 45min at 55ºC. Oligonucleotides **17a** and **17b** were then precipitated by the addition of 75µl 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.

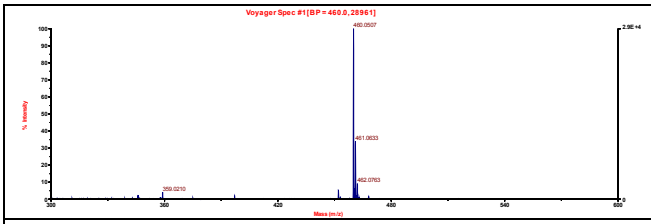
MASS SPECTROMETRY

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).

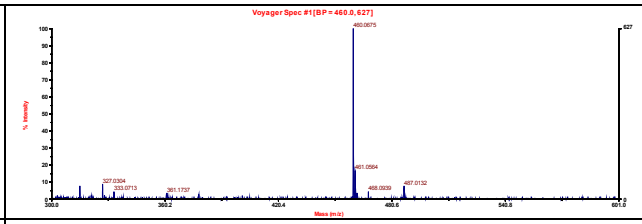
(MA)LDI spectra of oligonucleotides **17a** and **17b** are included below, indicating the path in Scheme 4 through they were synthesised and the

<table>
<tr><td>  </td><td>  </td></tr>
<tr><td>Ion 15a from 17a, path C, LDI MS</td><td>Ion 15a from 17a, path C, MALDI MS</td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td>Ion 16a from 17a, path D, LDI MS</td><td>Ion 16a from 17a, path D, MALDI MS</td></tr>
</table>

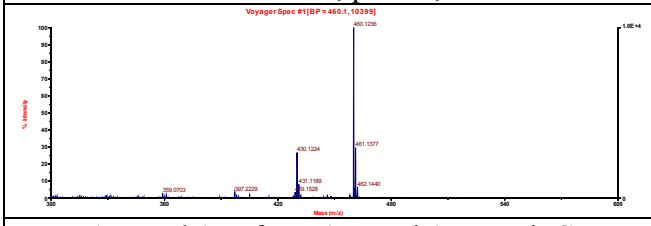
Supplementary Material (ESI) for Chemical Communications



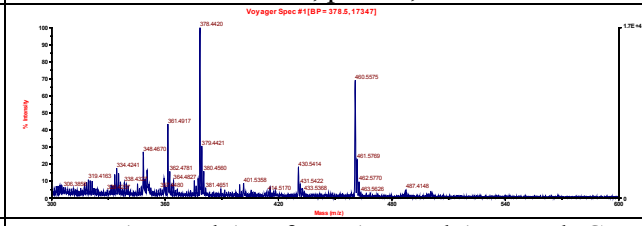
Ion **15b** from **17b**, path C, LDI MS



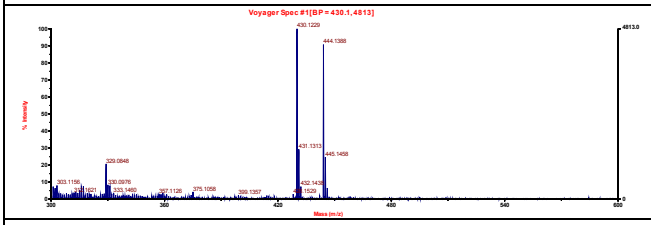
Ion **15b** from **17b**, path C, MALDI MS



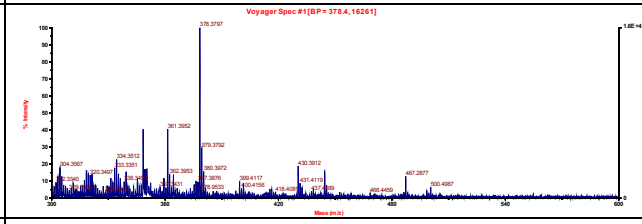
Ions **15a** and **15b** from **17a** and **17b**, path C, LDI MS



Ions **15a** and **15b** from **17a** and **17b**, path C, MALDI MS



Ions **15a** and **16a** from **17a**, paths C and D, LDI MS



Ions **15a** and **16a** from **17a**, paths C and D, MALDI MS