Synthesis of S-Pixyl Derivatives for Mass Spectrometric Applications

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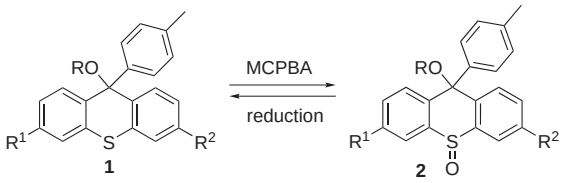
Abstract: Synthesis of novel S-pixyls based on the thioxanthyl skeleton is described. Thioxanthone derivatives were prepared by a regioselective intramolecular Friedel–Crafts acylation reaction and converted into S-pixyl derivatives by Grignard synthesis. S-Pixyl carbocations stabilised by electron donating groups on the thioxanthyl backbone produced exceptional mass spectra under (MA)LDI conditions (laser desorption ionisation, both with and without matrix), and can be detected down to femtomol levels.

Key words: S-pixyls, regioselective Friedel–Crafts acylation, (MA)LDI analysis, functionalised thioxanthen-9-ones, 2-aroylpyrene

We have been pursuing the synthesis of novel trityl labels as new molecular tools for mass spectrometric (MS) applications in genomics[1] and proteomics.[2] Our results have shown that trityl-based mass tags fly extremely well in the positive mode of laser desorption ionisation (LDI) MS with and without matrix, due to the formation of a highly stabilised carbocation.[3] The presence of *ortho*- or *para*-alkyl or -alkoxy groups on the phenyl ring of the trityl improve the LDI process by further stabilising the positive charge.

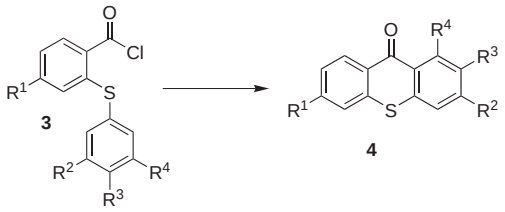
We were particularly interested in S-pixyl[4] derivatives due to their ability to stabilise carbocations and the fact that the sulfur atom can be oxidised to the corresponding sulfoxide. The resulting S(O)-pixyl derivatives were then found to be resistant to acid treatment and were not ionised even in 50% H_2SO_4. The reverse deoxygenation of the sulfoxide function in S(O)-pixyl derivatives can be satisfactorily carried out according to literature procedures[5] (Scheme 1). S-Pixyls are used currently as protecting groups in organic chemistry and can be cleaved from organic molecules by acid or UV irradiation.[6]



Scheme 1

For our applications the S-pixyl mass tag released as a cation can then be analysed by (MA)LDI analysis.

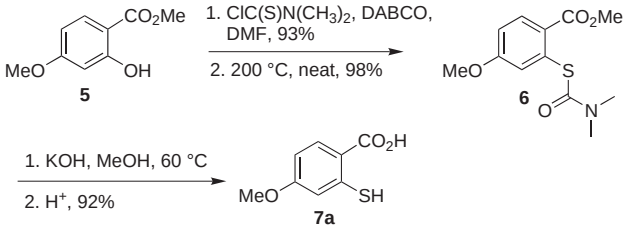
We have identified the thioxanthone ketone **4** as a key intermediate. Derivatised forms of this ketone are not commercially available. Attempts to prepare substituted ketones via reported literatures routes proved to be difficult.[7] A series of functionalised thioxanthen-9-ones were prepared via regioselective intramolecular Friedel–Crafts acylation reaction (Scheme 2).



Scheme 2

Similar approaches using acid-mediated cyclisations of *o*-(phenylthio)benzoic acids are reported for the synthesis of simple thioxanthen-9-one ketones. The regioselectivity is poor giving a mixture of regioisomers that require separation.[8]

Intermediate **7a** was prepared via a Newman–Kwart reaction, O→S transposition starting from commercially available methyl 2-hydroxy-4-methoxybenzoate **5** (Scheme 3).[9,10]



Scheme 3

Compound **5** was treated with dimethylthiocarbamoyl chloride in the presence of DABCO in DMF. The desired *O*-aryl *N*,*N*-dimethylthiocarbamoyl derivative was then thermally rearranged into the *S*-aryl *N*,*N*-dimethylthiocarbamate. Alkaline hydrolysis in methanol gave 78 g of **7a** in 92% yield. The Ullmann reaction was used to prepare methoxyphenylthiobenzoic acid **9**[11,12] as described in the

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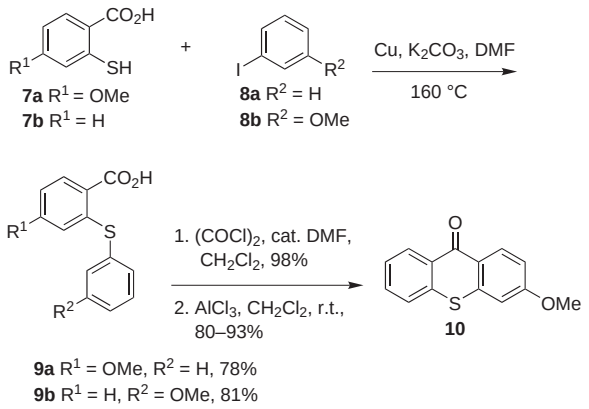
literature (Scheme 4). Friedel–Crafts acylation gave the desired ketone in 80–93% yields, excellent regioselectivity was observed for the synthesis of **10** (R^1 = H, R^2 = OMe), 1-methoxy-9*H*-thioxanthen-9-one (**11**) was not isolated (Table 1).[13]



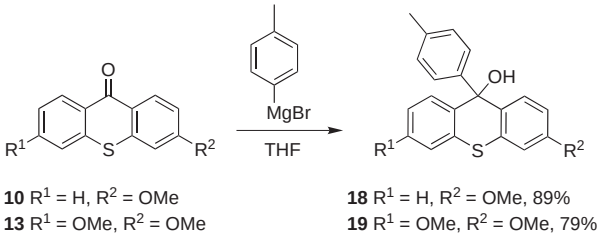
Scheme 4

Interestingly, Boyd et al. described the one-pot Friedel–Crafts acylation of anisole with thiosalicyclic acid in the presence of sulfuric acid and sulfonyl chloride, generating 3-methoxy-9*H*-thioxanthen-9-one in 44% yield.[6a] However, their ^{1}H NMR and ^{13}C NMR spectroscopic data were not in full agreement with our data for **10**. We suspected that they had in fact prepared the wrong regioisomer 2-methoxy-9*H*-thioxanthen-9-one (**12**, Table 1). We prepared **12** via reaction of **7b** with 4-iodoanisole, followed by Friedel–Crafts acylation in 68% yield. Compound **12** gave ^{1}H NMR and ^{13}C NMR data in full agreement with their reported data.[13]

Thiosalicyclic acids **7a** and **7b** both yield an identical ketone **10**, the high regioselectivity observed for **10** can be explained by the strong *para*-directing effect of the 3-methoxy group of **9b**. The versatility of this approach was further extended towards the synthesis of functionalised ketones **14**–**17**, which were prepared in good overall yields from the starting aryl acyl chlorides (Table 1).

Compounds **16** and **17** are of particular importance since they give rise to side chain derivatised mass tags for genomics and proteomics applications.

To evaluate the properties of S-pixyls as mass-tags for analysis by (MA)LDI, S-pixyls **18** and **19** were prepared by the addition of 4-methylphenylmagnesium bromide to thioxanthen-9-ones **10** and **13**, respectively (Scheme 5).



Scheme 5

(MA)LDI analysis of an equimolar solution of S-pixyls **18** and **19** (5 μL of 2.5×10^{-6} M solution in THF) in the absence (Figure 2) and in the presence of matrix (Figure 1), 2,5-dihydroxybenzoic acid was performed. These results confirm that the presence of alkyl or alkoxy groups on the phenyl ring improves the sensitivity in the positive mode of laser desorption ionisation at femtomol concentrations, thus suggesting the way to modulate the desorption properties of the mass-tags by changing the number of electron donating substituents on the ring system.

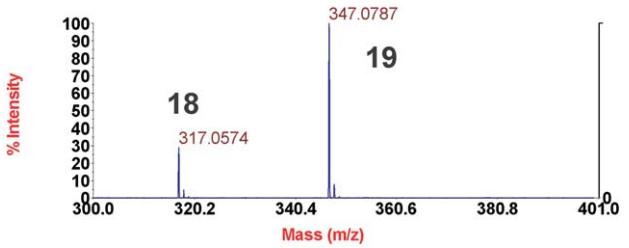


Figure 1 Equimolar mix of S-pixyls **18** and **19** in the presence of matrix

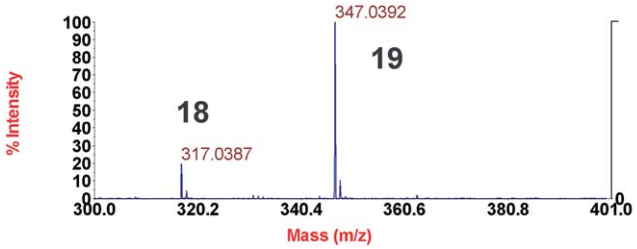
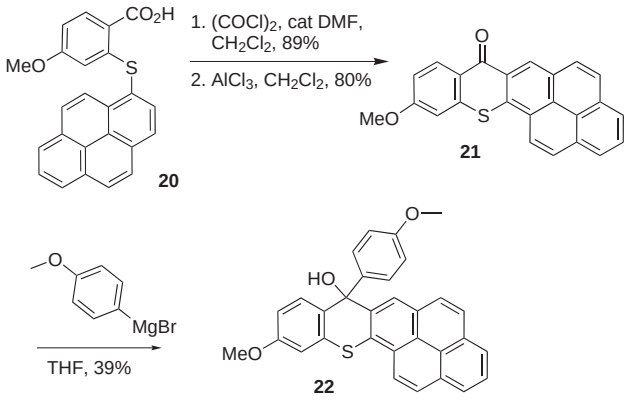


Figure 2 Equimolar mix of S-pixyls **18** and **19** in the absence of matrix

We also describe the synthesis of a novel pyrene ketone **21**, prepared from the reaction of 2-mercapto-4-methoxybenzoic acid with 1-bromopyrene, followed by a Friedel–Crafts acylation reaction to give **20** in 80% yield. The pyrene ketone was treated with 4-methoxyphenylmagnesium bromide to give the desired pyrene S-pixyl **22** in 39% yield (Scheme 6). This combination of a fluorophore



Scheme 6

and a mass tag in one molecule can potentially be used for orthogonal detection by mass spectrometry and optical detection methods.[3c]

Initial MS analysis of S-pixyl **22** gave the desired molecular ion (Figure 3). Further work on the fluorescent and MS properties of **22** is currently ongoing and will be reported in due course.

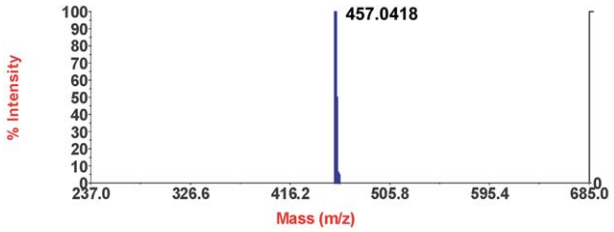
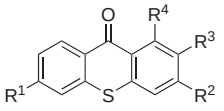


Figure 3

In conclusion, we have synthesised several substituted thioxanthone derivatives via a regioselective Friedel–Crafts acylation reaction in gram quantities. We have also reported the preparation of a new pyrene S-pixyl **22** for MS applications. (MA)LDI analysis of S-pixyls **18** and **19** confirm that the presence of electron donating groups on the thioxanthyl backbone improves the sensitivity in the positive mode of laser desorption ionisation. Further work is ongoing and the synthesis of bifunctional trityl derivatives will be reported in due course.

Table 1 Synthesis of Substituted Thioxanthen-9-ones



Ketone	R^1	R^2	R^3	R^4	Yield (%)[a]
10	H	OMe	H	H	80–93
11	H	H	H	OMe	0[b]
12	H	H	OMe	H	68
13	OMe	OMe	H	H	83–95
14	H	Me	H	H	39[c]
15	H	H	H	Me	35[c]
16	H	$(CH_2)_2CO_2Me$	H	H	32
17	OMe	OMe	$O(CH_2)_2CO_2Et$	H	60

[a] Isolated yields.
[b] Synthesis of **10**, regioisomer **11** not obtained ($AlCl_3$, CH_2Cl_2, r.t.).
[c] Obtained as a mixture, purified via recrystallisation.

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(12) **General Procedure for the Copper-Mediated Ullmann Reaction, Selected Compounds 9a and 20.**
To a solution of thiosalicylic acid (0.2 mol) in 300 mL of DMF was added aryl halide (0.2 mol), K_2CO_3 (0.2 mol) and Cu powder (0.2 equiv), the mixture was warmed to reflux. After 16–24 h the mixture was cooled to r.t., filtered and poured into 1 N HCl and then extracted with EtOAc (4 ×). The combined organic phases were then washed with H_2O (4 ×), and dried over Na_2SO_4, filtered and concentrated in vacuo. The product was dried under high vacuum and used with out further purification (78–95%).
Analytic data of **9b**: ^{1}H NMR (200 MHz, DMSO-d_6): δ = 8.30–7.50 (d, *J* = 9.2 Hz, 1 H), 7.50–7.35 (dd, *J* = 9.6 Hz, 1 H), 7.30–7.18 (t, *J* = 7.5 Hz, 1 H), 7.15–7.00 (m, 3 H), 6.85–6.74 (d, *J* = 9.0 Hz, 1 H), 3.8 (s, 3 H). ^{13}C NMR (400 MHz, DMSO-d_6): δ = 168.00, 160.99, 142.35, 134.16, 133.26, 131.79, 131.68, 128.51, 127.88, 125.59, 120.77, 115.98, 56.15. HRMS (ESI): *m/z* calcd for $C_{14}H_{12}NaO_3S$ [M + Na^+]: 283.0405; found: 283.0416.

Analytic data of **20**: ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.50–8.30 (m, 8 H), 8.25–8.12 (t, *J* = 7.65 Hz, 1 H), 8.10–8.00 (d, *J* = 8.7 Hz, 1 H), 6.80–6.70 (d, *J* = 8.8 Hz, 1 H), 5.70–5.60 (d, *J* = 2.4 Hz, 1 H), 3.50–3.40 (br OH, 1 H), 3.35 (s, 3 H). ^{13}C NMR (400 MHz, DMSO-d_6): δ = 167.95, 162.98, 145.59, 135.73, 134.73, 134.04, 133.23, 131.49, 131.15, 130.22, 129.75, 128.12, 127.74, 127.12, 126.76, 126.44, 125.48, 125.18, 124.34, 119.95, 113.49, 109.61. HRMS (ESI): *m*/*z* calcd for $C_{24}H_{16}NaO_3S$ [M + Na^+]: 407.0718; found: 407.0717.

(13) **General Procedure for the Intramolecular Friedel–Crafts Acylation Reaction, Selected Compounds 10, 12, 13 and 21.**

Preparation of Acid Chlorides.

o-(Phenylthio)benzoic acid derivatives (0.1 mol), placed in a dry 250 mL round-bottom flask under a positive pressure of argon. Then, 100 mL of dry CH_2Cl_2 were added and the reaction cooled to 0 °C. A few drops of DMF were added followed by the dropwise addition of oxalyl chloride (1.5 equiv) with stirring. The reaction was stirred for 1 h or longer until the suspension had completely dissolved. The product was concentrated in vacuo and dried under high vacuum to give the acid chloride, which was used without further purification (90–100%).

Intramolecular Friedel–Crafts Acylation.

The acid chloride (0.1 mol) was placed in a dry 250 mL round-bottom flask under a positive pressure of argon. Then, 100 mL of dry CH_2Cl_2 were added and the reaction was stirred at r.t., $AlCl_3$ (1.5 equiv) was added slowly and the reaction was stirred for 1–2 h. The reaction was quenched with H_2O and extracted with CH_2Cl_2 (2 ×). The combined organic phases were washed with dilute $NaHCO_3$ and dried over Na_2SO_4, filtered and concentrated in vacuo. The product was purified by flash chromatography on silica gel, hexane–EtOAc gradient elution to give the desired product (80–95%).

Analytic data of **10**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.65–8.58 (d, *J* = 8.1 Hz, 1 H), 8.55–8.50 (d, *J* = 9.0 Hz, 1 H), 7.60–7.40 (m, 3 H), 7.05–6.97 (d, *J* = 11.6 Hz, 1 H), 6.90 (d, *J* = 2.4 Hz, 1 H), 3.90 (s, 3 H). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 179.02, 162.48, 139.54, 136.89, 132.88, 131.92, 129.69, 129.27, 126.28, 125.71, 123.02, 115.08, 107.79, 55.66. HRMS (ESI): *m*/*z* calcd for $C_{14}H_{11}NaO_2S$ [M + H]: 243.0480; found: 243.0473.

Analytic data of **12**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.65–8.60 (d, *J* = 8.3 Hz, 1 H), 8.02 (d, *J* = 2.8 Hz, 1 H), 7.60–7.45 (m, 4 H), 7.30–7.20 (d, *J* = 8.9 Hz, 1 H), 3.95 (s, 3 H). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 179.65, 158.35, 137.49, 131.99, 130.19, 129.84, 129.14, 128.55, 127.27, 126.06, 125.97, 122.72, 110.29, 55.67. HRMS (ESI): *m*/*z* calcd for $C_{14}H_{11}NaO_2S$ [M + H]: 243.0480; found: 243.0469.

Analytic data of **13**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.60–8.50 (d, *J* = 4.5 Hz, 1 H), 7.05–6.98 (d, *J* = 5.1 Hz, 1 H), 6.90 (s, 1 H), 3.90 (s, 3 H). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 178.30, 162.21, 139.12, 131.75, 123.03, 114.81, 107.98, 55.64. HRMS (ESI): *m*/*z* calcd for $C_{15}H_{13}NaO_3S$ [M + H]: 273.0585; found: 273.0576.

Analytic data of **21**: ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.90 (s, 1 H), 8.70–8.60 (d, *J* = 8.9 Hz, 1 H), 8.59–8.48 (d, *J* = 9.3 Hz, 1 H), 8.35–7.90 (m, 6 H), 7.24–7.00 (m, 2 H), 4 (s, 3 H). ^{13}C NMR (500 MHz, $CDCl_3$): δ = 180.14, 162.71, 138.72, 132.18, 131.98, 131.73, 131.01, 129.15, 128.44, 128.37, 127.79, 127.56, 126.79, 126.50, 126.21, 125.87, 125.78, 125.49, 124.20, 122.58, 122, 115.33, 108.53, 55.84. HRMS (ESI): *m*/*z* calcd for $C_{24}H_{15}O_2S$ [M + H]: 267.0793; found: 267.0797.