Concise Synthesis of Photocleavable Molecular Tag for Laser Desorption Ionization Mass spectrometry via Fries Reaction

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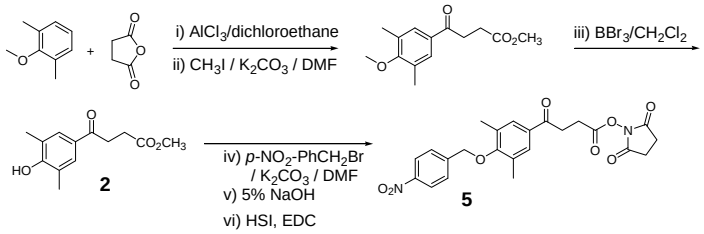
A new synthetic route for the photocleavable molecular tag for laser desorption ionization mass spectrometry (LDI-MS) was achieved using Fries reaction of 2,6-dimethylphenyl ester as its key reaction. Zirconium chloride was found as uniquely efficient adjuvant to promote the reaction. The molecular tag was obtained in 5 steps without chromatographic purification.

Introduction

Exploitation of new functional molecules is one of the most important missions imposed on organic chemistry. High synthetic accessibility strategies as an integral part of the preparation of such molecules are

evidently fundamental. Recently, we reported a new photocleavable molecule for laser desorption ionization mass spectrometry (LDI-MS) which facilitates ionization of tagged molecule[1]. Using this unique functional molecule, one can observe various tagged molecules in low mass range by simple operation as well as matrix assisted laser desorption ionization mass spectrometry (MALDI-MS) without matrix which frequently disturb observation of low mass region. The previous synthetic access route, which is depicted in Scheme 1, seemed acceptably direct and standard. However, the de-methylation step (iii) with BBr_3 contained unavoidable by-products requiring severe silica gel column chromatographic purification. As molecule for utility, synthetic accessibility must be significant part of its function. Improved synthetic access may open further utility of the molecular tag **5** as a new tool for screening in low mass region with highly sensitive LDI-MS. In this paper, we report a new efficient access to the photocleavable molecular tag **5** for LDI-MS.



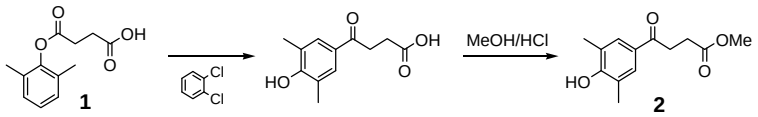
Scheme 1: Standard synthetic route for the photocleavable molecule

Discussion

Application of Fries reaction seemed promising to avoid the Friedel Crafts acylation of 2,6-dimethylanisole and following troublesome de-methylation step with BBr_3. Fries reaction is an important rearrangement in aromatic chemistry[2], and is a basic and efficient route to access acyl substituted phenols which

are frequently found in photo-reactive functional molecules as their key sub-structure[3]. The reaction also plays important roles in the synthesis of biomolecules[4]. Aiming for exploitation of a new route for molecular tag **5**, Fries reaction of 4-(2,6-dimethylphenoxy)-4-oxobutanoic acid (**1**) was examined under various conditions. The Fries product was *in situ* converted to corresponding methyl ester **2** by methanol/HCl treatment. The results are summarized in Table 1.

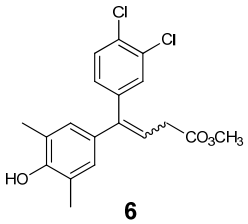
Table 1. Fries reaction of 4-(4-hydroxy-3,5-dimethylphenyl)-4- oxobutanoic acid (**1**)



run	Lewis acid	equi.	temp (°C)	Yield[a] (%)
1	$BF_3{\cdot}OEt_2$	3	170	<5
2	$FeCl_3$	3	170	<5
3	$ZnCl_2$	3	170	<5
4	$SnCl_4$	3	170	<5
5	$SbCl_3$	3	170	<5
6	$TiCl_4$	3	170	<5
7	$BiCl_3$	3	170	<5
8	$AlCl_3$	3	170	23
9	$ZrCl_4$	3	170	56
10[b]	$ZrCl_4$	3	170	78
11	$ZrCl_4$	3	160	43
12	$ZrCl_4$	1	170	<5
13	$ZrCl_4$	2	170	25
14	$ZrCl_4$	4	170	49

[a] Determined by HPLC analysis, [b] Scaled up reaction (see text)

Most of standard Lewis acids did not promote the reaction (runs 1 to 7) under the applied conditions. Although $AlCl_3$ afforded desired product, the yield was not to a satisfactory level (run 8). In fact, reactions with $AlCl_3$ generated significant amount of side-product **6** at above 150 ^oC (Figure 1). We managed to find that $ZrCl_4$ was an effective Lewis acid for the reaction (runs 9 to 14). It was found that higher reaction temperature was essential to complete the reaction (runs 9 and 11). When nitrobenzene was applied instead of dichlorobenzene, resulting reaction mixture was seriously contaminated with unknown dark materials maybe derived from solvent. This contamination seriously disturbed recrystallization. Use of 3 equivalent of $ZrCl_4$ afforded the best yield (runs 9 and 12-14). Typically heavy and dark red precipitation was observed during the reaction with $ZrCl_4$. The heavy precipitation resulted to habitual sticking of the magnetic stirrer bar and it seemed to prevent completion of the reaction. Delightfully, however, scaled-up reaction with mechanical stirrer showed significantly improved isolated yield of 78% (run 10).



6

Figure 1 Structure of side product **6** formed by overreaction with $AlCl_3$.

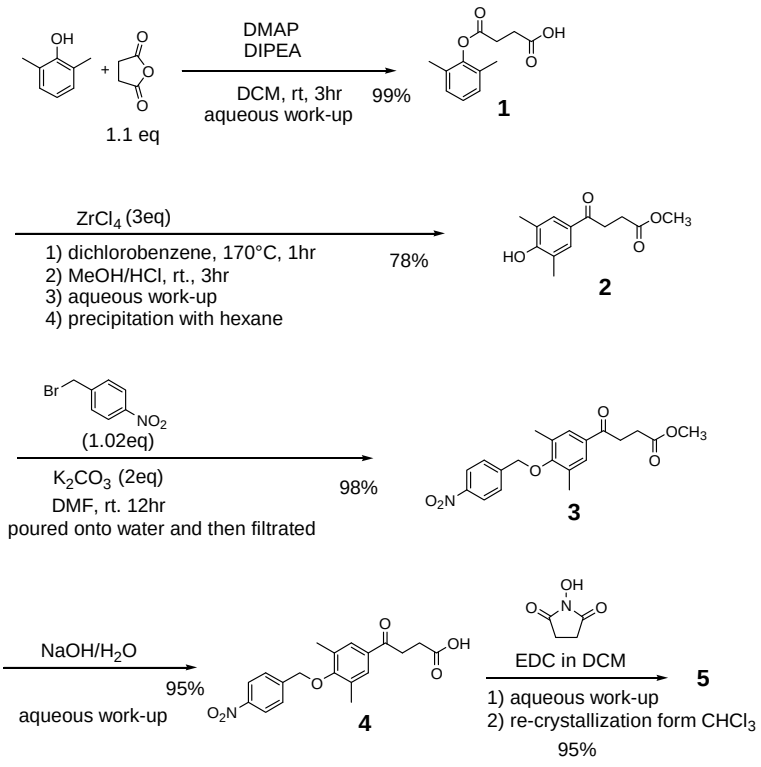
To the reaction mixture, methanol/HCl was added for esterification. No significant by-product was observed except a small amount of esterified starting material **1**. The product **2** was thus readily obtained in satisfactory purity after aqueous HCl work up and recrystallization with *n*-hexane/EtOAc.

Only few reports on Fries reaction of 2,6-disubstitued phenyl esters exist probably due to the esters' inactivity [5]. In fact, unsuccessful result of acylation of 2,6-dimethylphenol was reported in a recently emerged microwave assisted catalytic direct acylation of *p*-cresol and 1-naphthol[6] which may include *in situ* Fries reaction process. The property of zirconium chloride to promote Fries reaction at room temperature was also reported[7a]. However, the reaction of **1** did not proceed at room temperature probably due to 2,6-dimethyl groups. Thus, 2,6-dimethylphenyl esters seemed to be less reactive for Fries reaction and therefore it was challenging.

Our earlier comparison between $ZrCl_4$ and $AlCl_3$ did not show significant difference in their apparent yields in Fries reaction of simple 2,6-dimethylphenyl acetate and *i*-butyrate, and propionate (see supporting information Figure 1S). However, Fries reaction of **1** showed clear superiority of $ZrCl_4$ over $AlCl_3$ at various temperatures. In addition, compound **1** essentially required higher temperature as described in Table 1. Three conceivable reasons for these observations could be drawn as follows; i) bulkiness of ester moiety, ii) electron withdrawing inductive effect of carboxylic acid functionality, and iii) coordination of carboxylic acid of **1** on Lewis acid may suppress Lewis acidity of adjuvant. Importantly, sometime small impurity may be fatal for purification by recrystallization.

Superiority of $ZrCl_4$ for Fries reaction was also reported for electronically inactivated aromatic esters[7b]. Our result may add a new agreement with an efficient character of $ZrCl_4$ for Fries reaction. Since presence of multi functionality in one molecule is basic requirement for many practical syntheses, the observed fact may add fruitful information for application of Fries reaction for multi functionalized systems.

Improved key Fries reaction conditions in hand, we have established a new synthetic route of photocleavable molecular tag **5** for LDI-MS (Scheme 2). Alkylation of **2** was carried out with *p*-nitrobenzyl bromide in the presence of potassium carbonate in DMF. The product **3** was obtained as white solid when the reaction mixture was simply poured onto water after the reaction. After standard alkaline hydrolysis and following condensation reaction with 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDAC), the molecular tag **5** was obtained with satisfactory purity. Complete purification for analytical grade was achieved by recrystallization from dichloromethane. Thus preparation of the molecular tag **5** for LDI-MS was achieved in 68% overall yield from economical 2,6-xylenol as a stating material without the need for chromatographic purification.



Scheme 2: New synthetic route of photocleavable molecular tag for LDI-MS

In conclusion, we have developed a new concise and practical route for photocleavable molecular tag **5**. Xylenol is far economical than originally reported dimethylanisol as starting material[1]. The route contains Fries reaction of 2,6-dimethylphenol ester as a key reaction which was found to be efficiently promoted by $ZrCl_4$. The exploited new simple access to acylphenol scaffold may prompt exploitation of a variety of photo-reactive functional molecules for many purposes.

Experimental Section

4-(2,6-dimethylphenoxy)-4-oxobutanoic acid (1). To the solution of 2,6-dimethylphenol (20 mmol) and DMAP (4 mmol) in dichloromethane, twenty two millimoles of succinic anhydride was added. The solution was stirred for 12 hrs at room temperature then extracted in three potions of EtOAc. Combined organic layer was washed with 1M aqueous HCl and brine successively, dried over magnesium sulphate and then the solvent was evaporated in vacuo to yield a cream colored powder (97%). **1**; m.p. 114-116°C. ^{1}H-NMR(300MHz, $CDCl_3$, TMS,r.t.) δ: 2.14(6H, s), 2.82(2H, t, *J*=6.84Hz), 2.93(2H, t, *J*=6.36Hz), 7.04(3H, s); ^{13}C-NMR(400MHz, $CDCL_3$, TMS, r.t.) δ: 16.19, 28.47, 28.86, 125.91, 128.56, 130.09, 148.02, 170.00, 178.15. HRMS *m/z* Calcd. for $C_{12}H_{13}O_4$ 222.0892, Found 222.0883.

Methyl 4-(4-hydroxy-3,5-dimethylphenyl)-4- oxobutanoate (2). The compound **1** (18 mmol) was added into dichlorobenzene (36 mL) and pre-heated at 100^{o}C. Following the pre-heating for about 30 minutes, compound **1** dissolved completely resulting into a clear solution into which $ZrCl_4$ (54 mmol) was added. The resulting mixture was mechanically stirred for 2h at 170^{o}C. The resulting dark-red colored precipitate was

cooled to room temperature before addition of 72 mL HCL/MeOH. The resulting clear wine red solution was stirred for 12h at room temperature. The solution was concentrated *in vacuo* and then EtOAc (50 mL) was added. The solution was washed with three potion of 1M aqueous HCl and brine successively, dried over magnesium sulphate and then solvent was evaporated under reduced pressure. After recrystallization from EtOAc and n-hexane, **2** (78%) was obtained as a white solid. All the physical data matched with our previous publication[1].

4-[3,5-Dimethyl-4-(4-nitro-benzyloxy)-phenyl]-4-oxo-butyric acid methyl ester (3). To a solution of **2** (14 mmol) in DMF(20 mL), potassium carbonate (28 mmol) and *p*-nitro-benzyl bromide (14.7 mmol) were added. The suspension was stirred for 6hrs at room temperature. Then the mixture was poured onto water (100 mL) and resulting white solid was filtrated. After washing with H_2O and drying under air stream, the compound **3** was obtained in 98%. All the physical data matched with our previous publication[1].

4-[3,5-Dimethyl-4-(4-nitro-benzyloxy)-phenyl]-4-oxo-butyric acid 2,5-dioxo-pyrrolidin-1-yl ester (5). To a solution of **3**(13.7 mmol) in THF (30 mL), aqueous sodium hydroxide (5% 50 mL) was added. The solution was stirred for 3hrs at room temperature. Then the mixture was poured onto 1M HCl solution (100 mL) and resulting white solid was filtrated. After washing with H_2O and drying under air stream, corresponding carboxylic acid **4** was obtained in 95%. To the crude carboxylic acid, CH_2Cl_2(30 mL), *N*-hydroxysuccinimide (20 mmol) and

N-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDAC, 25 mmol) were added successively and the mixture stirred for 3hrs at room temperature to clear solution. The solution was poured onto water and extracted with CH_2Cl_2 (3x30 mL). The organic portion was then dried over magnesium sulfate. The filtrate was evaporated *in vacuo*. The molecular tag **5** was obtained as a white solid (95%). All the physical data matched with our previous publication[1]. The obtained compound was typically pure at this stage, recrystallization from CH_2Cl_2 worked efficiently for further purification.

Z and ***E*-methyl 4-(3,4-dichlorophenyl)-4-(4-hydroxy-3,5-dimethylphenyl)but-3-enoate**. Compound **6**; ^{1}H-NMR(500MHz, $CDCl_3$, TMS,r.t.) δ: 2.19(2.20H, s), 2.24(3.80H, s), 3.10(0.72H, d, *J*=7.5Hz), 3.19(1.28H, d, *J*=7.5Hz), 3.70(3H, s), 4.86(3H, t, *J*=7.0Hz), 6.13(0.36H, t, *J*=7.5Hz), 6.15(0.64H, t, *J*=7.5Hz), 6.73(1.29H, s), 6.82(0.71H, s), 7.03(0.5H, d, *J*=8.5Hz), 7.08(0.5H, d, *J*=8.5Hz), 7.28(0.5H, d, *J*=2Hz), 7.31(0.5H, d, *J*=8.5Hz), 7.33(0.5H, d, *J*=2Hz), 7.45(0.5H, d, *J*=8.5Hz). ^{13}C-NMR(500MHz, $CDCL_3$, TMS, r.t.) δ: 15.9, 15.9, 35.1, 35.2, 51.9, 51.9, 121.2, 126.8, 127.6, 129.2, 129.3, 129.8, 129.9, 130.3, 131.5, 142.6, 151.9, 152.2, 172.1, 172.2. HRMS *m/z* Calcd. for $C_{19}H_{18}C_{l2}O_3$ 364.0633, Found 365.0711.

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