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Practical and Scalable Synthesis of 7-Azetidin-1-yl-4-(hydroxymethyl)coumarin: An Improved Photoremovable Group

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Abstract 7-Substituted-4-methylcoumarin derivatives are widely employed as photoprotecting groups in chemistry and biology. We have recently shown that the azetidinylated version of this photocage releases carboxylic acids in aqueous solution more efficiently than the traditionally used 7-diethylamino variant. Here we present a robust and scalable route to prepare the 7-azetidinylated alcohol, a useful precursor for the photoprotection of a variety of leaving groups and use it to prepare model phosphate, sulfonate and carbamate derivatives.

Keywords azetidine; bioorganic chemistry; chromophores; photochemistry; protecting groups; uncaging

Coumarin derivatives are widely used as fluorophores^{1,2} and photocleavable (also known as “caging”) groups.^{3,4} 4-Methylene coumarin derivatives substituted in the 7 position (for the numbering, see Table 1) with an electron rich group have been used extensively as photoremovable protecting groups in biology,^{5,6} synthetic chemistry,⁷ and materials science,^{8,9} even though their quantum yields of photoactivation (ϕ_{PA}) are generally low.^{4,10–15} Photoactivation of poor leaving groups such as alcohols and amines usually relies on their conversion into carbonates or carbamates to drive their release through the irreversible formation of carbon dioxide.^{3,11,16}

Lavis and co-workers¹⁷ and Xu and co-workers¹⁸ reported that azetidin-1-yl and aziridin-1-yl rings can improve the fluorescence quantum yield of a wide variety of fluorophores when used in place of dialkylamino or larger ring substituents. We have recently proved¹⁹ that the same substitution approach can be used to improve the efficiency of other photochemical processes, showing that the ϕ_{PA} of a series of 7-substituted 4-

methylcoumarin esters (**1a–d**) increases when an azetidin-1-yl ring is used in place of a diethylamino group (**2a–d**, Figure 1).

Table 1 Quantum yield of photoactivation (ϕ_{PA}) of 7-azetidin-1-yl (**1a–d**) and 7-diethylamino (**2a–d**) coumarin esters.

$\phi_{PA} = 0.014(5)$	$\phi_{PA} = 0.016(5)$
$\phi_{PA} = 0.006(2)$	$\phi_{PA} = 0.0039(8)$
$\phi_{PA} = 0.014(2)$	$\phi_{PA} = 0.0052(2)$
$\phi_{PA} = 0.003(1)$	$\phi_{PA} = 0.0035(8)$

Data from ref. 19 PBS= Phosphate-buffered saline (pH = 7.4).

Scheme 1 Preparation of 7-azetidin-1-yl-4-methylcoumarin esters **1a–d** from ref. 19.

Although these esters could have been prepared from coumarinyl alcohol **3**, in our original report we prepared them by direct nucleophilic substitution, employing carboxylates and coumarinyl bromide **4** (Scheme 1). Compound **3**, however,

would be a versatile intermediate for the preparation of other biologically relevant compounds, including sulfonates, phosphates and carbamates. These compounds have been used to produce photoinduced pH jumps,²⁰ to achieve control of biologically relevant processes such as DNA transcription¹³ or to release bioactive molecules.^{16,21}

Often times, new tools in chemical biology are not widely adopted because their syntheses represent a considerable effort. We considered that a simple and reliable synthesis of building block **3** would facilitate the application of azetidyl coumarins as protecting groups, even in laboratories in which synthetic chemistry is not the main area of expertise. Here we report the results of exploring several potential synthetic routes and identify a reproducible and scalable protocol for the production of intermediate **3**. Furthermore, we used compound **3** to prepare model sulfonate, phosphate and carbamate derivatives, highlighting the versatility of this intermediate. Finally, we show that photolysis of these compounds proceeds smoothly releasing only the photoprotected leaving group and alcohol **3**.

Scheme 2 Route to compound **3** installing the azetidine in the last step. DIEA: *N,N*-diisopropylethylamine; MW = microwave irradiation; Tf= trifluoromethanesulfonate.

At the outset, we tried to install the azetidine substituent after introducing the 4-hydroxymethyl group on the coumarin skeleton (Scheme 2). After optimization of reported protocols, we obtained compound **6** from resorcinol upon hydrolysis of the intermediate chloride **5**.^{8,22,23} Selective triflation of the 7-hydroxyl substituent to yield intermediate **7** was also carried out according to a reported procedure.²⁴ To install the azetidine ring, we tried to optimize the conditions reported by Lavis and co-workers: RuPhos/RuPhos Pd G3, with Cs₂CO₃ as a base, in 1,4-dioxane or toluene.¹⁷ In either solvent, the major product **8** was formed presumably by a Tsuji-Trost-type pathway, with the azetidyl ring substituting the allylic hydroxy group of the 4-methylene, instead of the aromatic triflate. This product was identified by LC/MS analysis of the reaction mixture (*m/z* = 364, Figure S1). The desired alcohol **3** was formed only in trace amounts.

Scheme 3 Buchwald-Hartwig coupling of **9**.

To disfavor coordination of palladium at the 4-hydroxymethylene group, a triisopropylsilyl (TIPS) group was installed before the Buchwald-Hartwig cross coupling (Scheme 3). With this new substrate, no Tsuji-Trost product **8** was observed during the cross-coupling reaction, but compound **10** was obtained in low yields (30%) when the reaction was performed on a small scale (15 mg). Upon scaling up to 220 mg, compound **10** was obtained only as a minor product. ¹H NMR analysis revealed that the small amounts of product **10** that formed under these conditions underwent further nucleophilic ring opening of the azetidine ring (Figure S2).

Because installing the azetidine at this late stage was not practical, we decided to explore alternative routes employing the azetidyl coumarin **11** reported by Lavis and co-workers.¹⁷

Scheme 4 Thermal hydrolysis and photolysis of bromide **4**. NBS= *N*-bromosuccinimide.

The first routes we tested were the thermal hydrolysis or photolysis of bromide **4** (Scheme 4), an intermediate we had already prepared in the synthesis of esters **1a-d**. Briefly, compound **4** was obtained from the corresponding triflate by Buchwald-Hartwig cross-coupling,¹⁷ followed by bromination on the 4-methylene by sequential deprotonation with lithium hexamethyldisilazane (LiHMDS) and treatment of the anion with *N*-bromosuccinimide.^{25–30} Neither the thermal nor the photochemical method yielded the alcohol **3** in satisfactory yields and purities. In all cases, LC/MS and ¹H NMR analyses (Figure S3) of the reaction mixtures or the isolated products revealed the attack of a bromide ion on the azetidine ring, yielding 7-(3-bromopropylamino)coumarin **12**. Attempts to intercept the formed bromide anion with silver salts did not change the outcome of the reaction.

Scheme 5 Tested route to compound **14**. LiHMDS: Hexamethyldisilazane lithium salt.

As an alternative halogenated intermediate, we also tried to use *m*-azetidin-1-yl-phenol (**13**) to prepare 7-azetidin-1-yl-4-chloromethylcoumarin (**14**) by Pechmann condensation with ethyl 4-chloroacetoacetate promoted by Ti(O*i*Pr)₃Cl at 95 °C (Scheme 5). This reaction has been successfully applied to the synthesis of julolidine-fused analogues.^{31–33} Unfortunately, this route was unsuccessful because at these high temperatures even the chloride ion, a poor nucleophile, attacked the azetidine

to open the ring, as determined by ^1H NMR and LC/MS analyses (see SI).

Finally, as we were able to form esters by nucleophilic displacement of the bromide in compound **4** at lower temperatures, we tried to obtain alcohol **3** from hydrolysis of an ester intermediate (Scheme 6). Anticipating the need to use mild hydrolysis conditions to avoid nucleophilic opening of the azetidine ring, we decided to use trifluoroacetic acid (TFA) to form the intermediate ester. Derivative **15** was so prone to hydrolysis that even adventitious H_2O present in an open TFA bottle was enough to hydrolyze the intermediate ester. In fact, we did not isolate or observe trifluoroacetate **15**; LC/MS analysis of the reaction mixture only detected alcohol **3** or bromide **4**. Under optimized reaction conditions, no significant attack on the azetidine ring was observed by any of the nucleophiles present in the reaction mixture. However, when the reaction was carried out with more equivalents of TFA- H_2O than KF, LC/MS analysis of the reaction mixture revealed the presence of a product arising from nucleophilic attack of a bromide ion on the azetidiny ring.

Figure 1 LC/MS analyses of the reaction of bromide **4** with both TFA and H_2O (top), only H_2O (middle) or only TFA (bottom). LC/MS analyses were taken between 2.3 and 3 hours after addition of the reactants.

The involvement of the trifluoroacetate **15** as an intermediate is supported by comparison of LC/MS analyses of the reaction of **4** in presence of KF and addition of both TFA and H_2O (Figure 1, top), only H_2O (Figure 1, middle) and only TFA from a newly opened bottle (Figure 1, bottom). Only in the first case a significant amount of alcohol is detected. In the absence of TFA, only unreacted starting material is observed, whereas without

H_2O only traces of alcohol are formed. These experiments suggest that perhaps bromide **4** and trifluoroacetate **15** exist in an equilibrium that is strongly shifted towards the bromide, and water-driven, irreversible hydrolysis of **15** opens a pathway to the alcohol.

Taking these results into account, we recommend the route depicted in Scheme 7 to prepare the alcohol **3**. Whereas it is possible to purify the bromide **4** by column chromatography, it is not necessary for the following step to proceed with good yields. Indeed, we found it more practical to use the crude of the bromination reaction for the hydrolysis step because the difference in retention factors between the alcohol **3** ($R_f = 0.25$, SiO_2 , CH_2Cl_2 - Et_2O , 7:3) and the bromide **4** ($R_f = 0.55$, SiO_2 , CH_2Cl_2 - EtOAc , 95:5) is much larger than the difference between the latter and the parent compound **11** ($R_f = 0.42$, SiO_2 , CH_2Cl_2 - EtOAc , 95:5). This route has been scaled up to 0.4 g of starting material **11** without loss in isolated yield or purity of alcohol **3**.

With multi-milligram quantities of alcohol **3** in hands, we used it to prepare the model photoprotected sulfonate, phosphate and carbamate derivatives depicted in Scheme 8. We synthesized these compounds to demonstrate the versatility of building block **3** and to test the stability of the azetidine ring under the reaction conditions of these transformations. In all cases, the yields of these reactions were comparable to the ones reported when the same procedures were performed employing 7-diethylamino-4-hydroxymethylcoumarin as starting material.^{16,20} These results confirm that the azetidinylated coumarin can be used to prepare photoprotected versions of a variety of biologically relevant compounds.

Finally, we prepared ~ 0.3 mM solutions of compounds **16-18** in mixtures of MeCN-phosphate-buffered saline (PBS, pH 7.4), 7:3 and irradiated them using 405 nm LEDs to monitor the photolysis reactions (Figure 2) as described before.¹⁹ In all cases the photoreactions proceeded cleanly to give the photodeprotected products and alcohol **4** or chloride **14**. This halogenated coumarin is likely generated by a chloride ion trapping the intermediate ion pair of the photolysis¹² when the

reaction is carried out in PBS, which has a high concentration (155 mM) of NaCl.³⁴

Figure 2 LC/MS analyses of the mixtures of photolyzed compounds **16–18**. All indicated m/z values corresponds to the $[M + H]^+$ ions.

In summary, we presented a reproducible and scalable route to prepare alcohol **3**, a fundamental building block to obtain compounds for the photocontrolled release of different classes of substances such as phosphates, sulfonates, and amines. Whereas we do not exclude that with a thorough optimization of the reaction parameters other routes might also successfully lead to alcohol **3**, the one we propose here proved to be the most robust and scalable in our experience. We also prepared three photoprotected model compounds and showed that the photolysis reactions proceed cleanly. We envision that the availability of a short and reliable synthesis will encourage other scientists to adopt this photoremovable group, which is much more efficient than its diethylamino-substituted counterpart.

The experimental section has no title; please leave this line here.

All reagents were purchased from commercial sources and used as received. Dry solvents were procured from Acros Organics and used as received. For preparative column chromatography, petroleum ether (Pet. Et.) 40–60 °C was used in place of hexane. Preparative HPLC was carried out using a Hitachi instrument equipped with an autosampler and a diode array detector (DAD) and employing an ACE Excel 5 C18-Amide column (25 cm length, 10 mm internal diameter). Solvent A: H₂O, Solvent B: MeCN (flow 4.7 mL min⁻¹, 70% A → 30% A). Automated flash chromatography was performed using a Büchi Reveleris PREP system. LC/MS chromatograms were recorded on a Waters Acquity UPLC system equipped with a Waters Acquity BEH C18 column (5 cm length, 2.1 mm internal diameter), a diode array detector PDA and an SQ Detector 2 ZSPRAY (ESI). The samples were eluted with a MeCN-H₂O gradient running from 2% to 98% of MeCN, with a constant 0.1% of HCOOH. NMR spectra were acquired on Bruker AV300 or Bruker 400 instruments. ¹H NMR chemical shifts are reported in ppm relative to SiMe₄ (δ = 0) and were referenced internally with respect to residual protons in the solvent (δ = 2.50 for (CD₃)₂SO, δ = 7.26 for CDCl₃). Coupling constants are reported in Hz and multiplicities of signals are described as singlet (s), doublet (d), triplet (t), quartet (q), quintuplet (quint), or multiplet (m). ¹³C NMR chemical shifts are reported in ppm relative to SiMe₄ (δ = 0) and were referenced internally with respect to solvent signal (δ = 39.5 for (CD₃)₂SO, δ = 77.2 for CDCl₃). Peak assignments are based on calculated chemical shift, multiplicity, and 2D experiments. ³¹P NMR with ¹H

decoupling were referenced upon addition of triphenylphosphine as internal standard (δ = -4.9 for CDCl₃).³⁵ IR spectra were measured on FTIR spectrometer Perkin Elmer Spectrum Two. High-resolution mass spectrometry was conducted by staff at the Molecular and Biomolecular Analysis (MoBiAs) center (ETH Zurich) employing a Bruker maXIS ESI/NanoSpray-Qq-TOF-MS or a Bruker solariX ESI/MALDI-FTICR-MS instrument. Elemental analysis was conducted by staff at the MoBiAs center (ETH Zurich) employing a LECO TruSpec Micro instrument. Melting points were measured on a Büchi Melting Point M-560 instrument and are uncorrected. The temperature gradient used was 20 °C min⁻¹.

Procedures

7-(Azetidin-1-yl)-4-(hydroxymethyl)-2H-chromen-2-one (**3**)

Compound **11** (400 mg, 1.86 mmol) was added to a flame-dried multi-neck flask and suspended in dry THF (34 mL). The suspension was stirred for 10 min at room temperature, then cooled to -40 °C in an acetone-dry ice bath and LiHMDS (4.7 mmol, 1 M in THF, 4.7 mL) was added dropwise to the mixture through a dripping funnel. The solution was warmed to -30 °C, followed by cooling to -78 °C. A freshly prepared solution of *N*-bromosuccinimide (364 mg, 2.05 mmol) in dry THF (6 mL) was added dropwise through a dripping funnel, checking that the temperature did not rise above -70 °C. The solution was kept stirring at -78 °C for about 1 h, when LC/MS and TLC analyses revealed the presence of a di-brominated byproduct (m/z = 374). The reaction was quenched with HCl (0.1 M) and repeatedly extracted with CH₂Cl₂. The combined organic phases were dried over MgSO₄, and the solvent was removed under reduced pressure until an orange solid was obtained. Potassium fluoride (118 mg, 2.04 mmol) was flame-dried in a separate two-neck flask. The crude of the bromination reaction was added as a solid, and the system was evacuated and backfilled with nitrogen three times. The solids were suspended in dry DMF (10 mL) and 450 μ L of a TFA-H₂O, 4:1 mixture were added to the flask (2.5 eq of each). The flask was sealed, and the system was stirred at 40 °C for 12–15 h, until completion was indicated by TLC (SiO₂, CH₂Cl₂-EtOAc, 95:5) by the disappearance of the intermediate bromide **4**. CH₂Cl₂ was added and the organic phase was washed with water. The extraction waters were back-extracted once with CH₂Cl₂. The combined organic phases were washed with brine, dried over MgSO₄ and evaporated under reduced pressure until a solid was obtained. The crude was dissolved in CH₂Cl₂ and as little MeOH as possible, and was fixed on Celite. The product was purified by column chromatography (SiO₂, CH₂Cl₂-Et₂O, 95:5 → 8:2) and isolated as a yellow/orange powder (290 mg, 67%). Mp 198 °C; R_f = 0.25 (SiO₂, CH₂Cl₂-Et₂O, 7:3).

IR (neat) = 3386, 1690, 1600, 1530, 1415, 1090 cm⁻¹.

¹H NMR (400 MHz, (CD₃)₂SO): δ = 7.45 (d, J = 8.7 Hz, 1H, H-3), 6.36 (dd, J = 8.7, 2.3 Hz, 1H, H-2), 6.26 (d, J = 2.3 Hz, 1H, H-1), 6.11 (t, J = 1.5 Hz, 1H, H-4), 5.51 (t, J = 5.6 Hz, 1H, H-8), 4.67 (dd, J = 5.6, 1.5 Hz, 2H, H-5), 3.93 (t, J = 7.4 Hz, 4H, H-6), 2.35 (quint, J = 7.4 Hz, 2H, H-7).

¹³C NMR (100 MHz, (CD₃)₂SO): δ = 160.9, 157.0, 155.1, 153.7, 124.9, 107.8, 106.9, 104.6, 96.3, 59.1, 51.5, 15.9.

HRMS-ESI: m/z $[M + H]^+$ calcd for C₁₃H₁₄NO₃: 232.0968; found 232.0970.

Anal. Calcd for C₁₃H₁₂NO₃·0.33H₂O: C, 65.81; H, 5.81; N, 5.90. Found: C, 65.50; H, 5.68; N, 5.85.

7-(Azetidin-1-yl)-4-(bromomethyl)-2H-chromen-2-one (**4**)

Compound **11** (500 mg, 2.32 mmol) was added to a flame-dried multi-neck flask and suspended in dry THF (45 mL). The suspension was cooled to -40 °C in an acetone-dry ice bath and LiHMDS (5.81 mmol, 1 M in THF, 5.81 mL) was added to the mixture dropwise through a dripping funnel. The dripping funnel was rinsed by adding 1 mL of THF. The solution was warmed to -30 °C, followed by cooling to -78 °C. A solution of *N*-bromosuccinimide (455 mg, 2.55 mmol) in dry THF (7 mL) was prepared freshly in a flame-dried flask and added dropwise, and the solution was stirred at -78 °C. The reaction was neutralized after 1 h, when LC/MS and TLC analyses revealed the presence of a di-brominated byproduct (m/z = 374). The mixture was treated with HCl (0.1 M) and extracted three times with CH₂Cl₂. The combined organic phases were dried over MgSO₄ and the solvent was

removed under reduced pressure. The product was fixed on Celite and purified by column chromatography (SiO₂, CH₂Cl₂-EtOAc, 99.5:0.5) to yield an orange solid (490 mg, 70% yield).

Mp 200 °C. If measured with a slower gradient of 5 °C min⁻¹, a color change is observed around 185 °C, yielding a solid that does not melt at 200 °C; *R*_f = 0.55 (SiO₂, CH₂Cl₂-EtOAc, 95:5).

IR (neat) = 1723, 1617, 1529, 1412, 1306, 1230, 1161 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ = 7.48 (d, *J* = 8.7 Hz, 1H, H-3), 6.33 (dd, *J* = 8.7, 2.3 Hz, 1H, H-2), 6.22 (d, *J* = 2.3 Hz, 1H, H-1), 6.17 (s, 1H, H-4), 4.40 (s, 2H, H-5), 4.01 (t, *J* = 7.3 Hz, 4H, H-6), 2.45 (quint, *J* = 7.4 Hz, 2H, H-7).

¹³C NMR (100 MHz, CDCl₃): δ = 161.6, 156.4, 154.2, 150.6, 125.4, 110.1, 108.0, 107.4, 97.3, 51.8, 27.3, 16.6.

HRMS-ESI: *m/z* [M + H]⁺ calcd for C₁₃H₁₃NO₂Br: 294.0124; found: 294.0118.

Anal. Calcd for C₁₃H₁₂NO₂Br: C, 53.08; H, 4.11; N, 4.76. Found: C, 53.02; H, 4.18, N, 4.62.

7-(Azetidin-1-yl)-4-methyl-2H-chromen-2-one (11)¹⁷

4-Methyl-2-oxo-2H-chromen-7-yl trifluoromethanesulfonate (800 mg, 2.6 mmol), RuPhos (182 mg, 0.4 mmol), RuPhos Pd G3 (334 mg, 0.4 mmol) and Cs₂CO₃ (1.27 g, 3.9 mmol) were transferred to a flame-dried Schlenk flask, which was evacuated and backfilled with nitrogen three times. Dry toluene (21 mL) was added, followed by azetidine (162 mg, 2.8 mmol, 0.2 mL). The reaction was stirred for 15 h at 80 °C, then filtered over Celite, fixed on Celite, purified by column chromatography (SiO₂, CH₂Cl₂-EtOAc, 99:1), and washed with pentane to yield compound **1** as a bright yellow solid (360 mg, 65% yield).

*R*_f = 0.42, (SiO₂, CH₂Cl₂-EtOAc, 95:5).

¹H NMR (400 MHz, CDCl₃): δ = 7.38 (d, *J* = 8.6 Hz, 1H, H-3), 6.30 (dd, *J* = 8.6, 2.3 Hz, 1H, H-2), 6.21 (d, *J* = 2.3 Hz, 1H, H-1), 5.97 (q, *J* = 1.2 Hz, 1H, H-4), 3.99 (t, *J* = 7.3 Hz, 4H, H-6), 2.43 (quint, *J* = 7.3 Hz, 2H, H-7), 2.34 (d, *J* = 1.2 Hz, 3H, H-5).

¹³C NMR (100 MHz, CDCl₃): δ = 162.1, 155.7, 154.1, 153.1, 125.5, 110.4, 109.6, 107.8, 97.2, 51.9, 18.7, 16.6.

HRMS-ESI: *m/z* [M + H]⁺ calcd for C₁₃H₁₄NO₂: 216.1019; found 216.1019.

(7-(Azetidin-1-yl)-2-oxo-2H-chromen-4-yl)methyl diethyl phosphate (16)

Compound **3** (50 mg, 0.21 mmol) and DMAP were added to a flame-dried flask, and the system was evacuated and backfilled with nitrogen three times. Dry THF (1 mL) and triethylamine (46 μL, 0.33 mmol) were added. A solution of diethylchlorophosphate (35 μL, 0.24 mmol) in THF (1 mL) was added dropwise. The reaction was stirred at room temperature for 12–15 h, until the starting material was consumed as determined by TLC (SiO₂, CH₂Cl₂-Et₂O, 7:3). Upon completion, CH₂Cl₂ was added, and the organic phase was washed with H₂O. The water phase was back-extracted once with CH₂Cl₂. The combined organic phases were washed with brine, dried over MgSO₄ and evaporated under reduced pressure. The crude was dissolved in CH₂Cl₂ and fixed on Celite. The product was purified by column chromatography (SiO₂, CH₂Cl₂-Et₂O, 92:8 → 95:5), followed by HPLC. Method: Solvent A: H₂O, Solvent B: MeCN (flow 1 mL min⁻¹, 0–1 min: 50% A; 1–6.5 min: 50–20% A; 6.5–7.5 min: 20–10% A; 7.5–9 min: 10% A; 9–10.5 min: 10–50% A). The compound was isolated as an oil that became an off-white powder after cooling overnight at –20 °C (30 mg, 38%).

Mp 100 °C; *R*_f = 0.1 (SiO₂, CH₂Cl₂-Et₂O, 95:5).

IR (neat) = 2980, 1726, 1608, 1530, 1419, 1270, 1033 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ = 7.26 (d, *J* = 8.7 Hz, 1H, H-3), 6.28 (dd, *J* = 8.7, 2.3 Hz, 1H, H-2), 6.24 (t, *J* = 1.4 Hz, 1H, H-4), 6.22 (d, *J* = 2.3 Hz, 1H, H-1), 5.16 (dd, *J* = 6.7, 1.4 Hz, 2H, H-5), 4.16 (dq, *J* = 7.7, 7.1 Hz, 4H, H-8), 3.99 (t, *J* = 7.4 Hz, 4H, H-6), 2.44 (quint, *J* = 7.4 Hz, 2H, H-7), 1.35 (td, *J* = 7.1, 1.0 Hz, 4H, H-9).

¹³C NMR (100 MHz, CDCl₃): δ = 161.7, 155.9, 154.0, 149.8, 149.7, 124.2, 108.0, 107.0, 106.8, 97.2, 64.5 (d, *J* = 6.0 Hz), 64.4 (d, *J* = 4.4 Hz), 51.8, 16.6, 16.2 (d, *J* = 6.7 Hz).

³¹P NMR (162 MHz, CDCl₃): δ = –0.5.

HRMS-ESI: *m/z* [M + H]⁺ calcd for C₁₇H₂₃NO₆P: 368.1258; found 368.1256.

Anal. Calcd for C₁₇H₂₂NO₆P: C, 55.59; H, 6.04; N, 3.81. Found: C, 55.79; H, 6.26; N, 3.55.

(7-(Azetidin-1-yl)-2-oxo-2H-chromen-4-yl)methyl methanesulfonate (17)

Compound **3** (50 mg, 0.21 mmol) was flame-dried in a two-neck flask equipped with a dripping funnel, and the system was evacuated and backfilled with nitrogen three times. Dry CH₂Cl₂ (8 mL) and triethylamine (60 μL) were added, and the system was cooled in an ice bath. A solution of methanesulfonyl chloride (25 μL, 0.32 mmol) in CH₂Cl₂ (2 mL) was added dropwise. The reaction was stirred at 0 °C for 45 min, until completion as determined by TLC (SiO₂, CH₂Cl₂-Et₂O 7:3) by the disappearance of the starting material. CH₂Cl₂ was added, and the organic phase was washed with H₂O. The water phase was back-extracted once with CH₂Cl₂. The combined organic phases were washed with brine, dried over MgSO₄ and evaporated under reduced pressure until a solid was obtained. The crude was dissolved in CH₂Cl₂ and as little MeOH as possible, and was fixed on Celite. The product was purified by column chromatography (SiO₂, CH₂Cl₂-Et₂O 95:5), and isolated as an off-white solid (50 mg, 77%).

Mp 225 °C (possible phase changes at 153 °C, color change at 200 °C); *R*_f = 0.25 (SiO₂, CH₂Cl₂-Et₂O, 95:5).

IR (neat) = 2934, 1720, 1605, 1532, 1417, 1356, 1176, 1068, 963, 833 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ = 7.30 (d, *J* = 8.7 Hz, 1H, H-3), 6.30 (dd, *J* = 8.7, 2.3 Hz, 1H, H-2), 6.21 (d, *J* = 2.3 Hz, 1H, H-1), 6.20 (t, *J* = 1.1 Hz, 1H, H-4), 5.29 (d, *J* = 1.1 Hz, 2H, H-5), 4.01 (t, *J* = 7.4 Hz, 4H, H-6), 3.10 (s, 3H, H-8), 2.45 (quint, *J* = 7.4 Hz, 2H, H-7).

¹³C NMR (100 MHz, CDCl₃): δ = 161.2, 156.1, 154.1, 147.2, 124.4, 108.5, 108.2, 106.6, 97.2, 65.7, 51.7, 38.4, 16.5.

HRMS-ESI: *m/z* [M + H]⁺ calcd for C₁₄H₁₆NO₅S: 310.0744; found 310.0742.

Anal. Calcd for C₁₄H₁₆NO₅S: C, 54.36; H, 4.89; N, 4.53. Found: C, 54.26; H, 5.04; N, 4.53.

(7-(Azetidin-1-yl)-2-oxo-2H-chromen-4-yl)methyl benzylcarbamate (18)

N,N'-Disuccinimidyl carbonate (117 mg, 0.46 mmol) was transferred to a flame-dried, multi-neck flask equipped with a dripping funnel. Compound **3** (50 mg, 0.21 mmol) was transferred to the dripping funnel as a solid and the system was evacuated and backfilled with nitrogen three times. The flask was covered with aluminum foil. Dry MeCN was added to the carbonate (1 mL) and in the dripping funnel (2 mL). *N,N*-Diisopropylethylamine (150 mL, 0.84 mmol) was added to the suspension of compound **3**. The suspension was added in portions to the carbonate, and dry MeCN (6.5 mL) was added in portions to the dripping funnel to recover as much as possible of the solid **3**. The mixture was stirred at room temperature for 12 h, until TLC analysis (SiO₂, CH₂Cl₂-Et₂O 7:3) revealed no more compound **3**. Benzylamine (250 mL, 2.3 mmol) was added to the flask, and the reaction was stirred for 6 h, until LC/MS analysis revealed the desired carbamate to be the predominant product of the reaction mixture. CH₂Cl₂ was added and the organic phase was washed with H₂O. The water phase was back-extracted once with CH₂Cl₂. The combined organic phases were washed with brine, dried over MgSO₄ and evaporated under reduced pressure. The crude was dissolved in CH₂Cl₂ and as little MeOH as possible, and was fixed on Celite. The product was purified by automated flash column chromatography (SiO₂, CH₂Cl₂-EtOAc, 100:0 → 9:1), and isolated as a yellow solid (30 mg, 39% yield).

Mp 215 °C; *R*_f = 0.26 (SiO₂, CH₂Cl₂-EtOAc 95:5).

IR (neat) = 3332, 1718, 1605, 1527, 1419, 1238, 1145 cm⁻¹.

¹H NMR (400 MHz, (CD₃)₂SO): δ = 8.09 (t, *J* = 6.2 Hz, 1H, H-8), 7.47 (d, *J* = 8.7 Hz, 1H, H-3), 7.38 – 7.18 (m, 5H, H-10, H-11 and H-12), 6.38 (dd, *J* = 8.7, 2.3 Hz, 1H, H-2), 6.27 (d, *J* = 2.2 Hz, 1H, H-1), 6.03 (t, *J* = 1.3 Hz, 1H, H-

4), 5.25 (d, $J = 1.4$ Hz, 2H, H-5), 4.24 (d, $J = 6.1$ Hz, 2H, H-9), 3.95 (t, $J = 7.4$ Hz, 4H, H-6), 2.35 (p, $J = 7.4$ Hz, 2H, H-7).

^{13}C NMR (100 MHz, $(\text{CD}_3)_2\text{SO}$): $\delta = 160.5, 155.7, 155.2, 153.9, 152.1, 139.5, 128.3, 127.0, 126.9, 125.2, 108.0, 106.4, 105.0, 96.3, 61.1, 51.5, 43.9, 15.9$.

HRMS-ESI: m/z [$M + H$] $^+$ calcd for $\text{C}_{21}\text{H}_{21}\text{N}_2\text{O}_4$: 365.1496; found 365.1493.

Anal. Calcd for $\text{C}_{21}\text{H}_{20}\text{N}_2\text{O}_4$: C, 69.22; H, 5.53; N, 7.69. Found: C, 68.97%; H, 5.59; N, 7.58.

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Supporting Information

Supporting information for this article is available online.

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