Synthesis G. Bassolino et al. Paper

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

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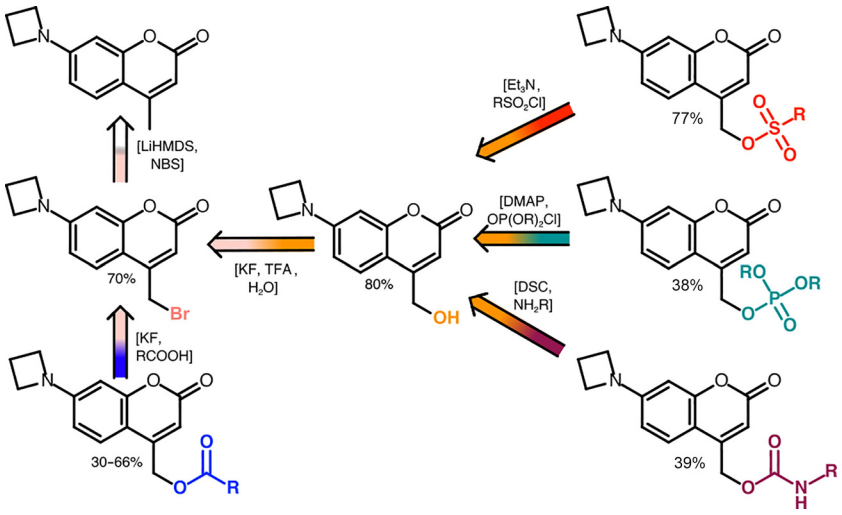

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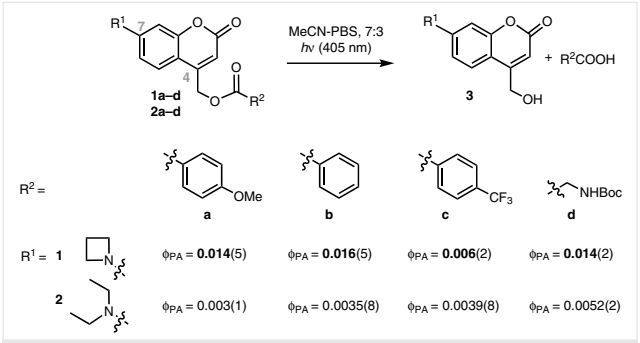
Abstract 7-Substituted 4-methylcoumarin derivatives are widely employed as photoprotecting groups in chemistry and biology. We have recently shown that the 7-azetidinylated version of this photocage releases carboxylic acids in aqueous solution more efficiently than the traditionally used 7-diethylamino 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 its use in the preparation of 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-Methylcoumarin derivatives substituted at the 7-position (for the numbering, see Scheme 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-workers[17] and Xu and co-workers[18] 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 proved[19] 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 increases when an azetidin-1-yl ring (**1a–d**) is used in place of a diethylamino group (**2a–d**, Scheme 1).



Scheme 1 Quantum yield of photoactivation (φ_{PA}) of 7-azetidin-1-yl (**1a–d**) and 7-diethylamino (**2a–d**) coumarin esters[19] (PBS = phosphate-buffered saline, pH 7.4)

Although these esters could be prepared from coumarinyl alcohol **3**, in our original report they were prepared by direct nucleophilic substitution, employing carboxylates and coumarinyl bromide **4** (Scheme 2).[19] Compound **3**, however, would be a versatile intermediate for the preparation of other biologically relevant compounds, including sulfonates, phosphates, and carbamates. Such compounds have been used to produce photoinduced pH jumps,[20] to achieve control of biologically relevant processes such as DNA transcription,[13] or to release bioactive molecules.[16,21]

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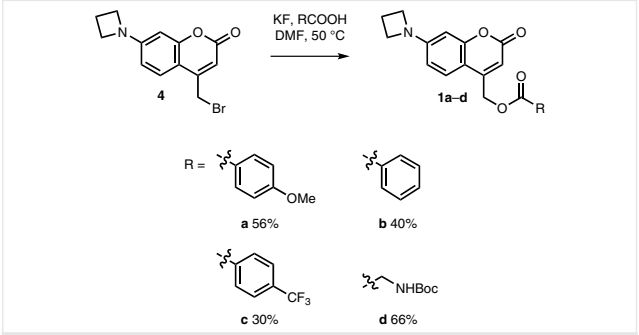
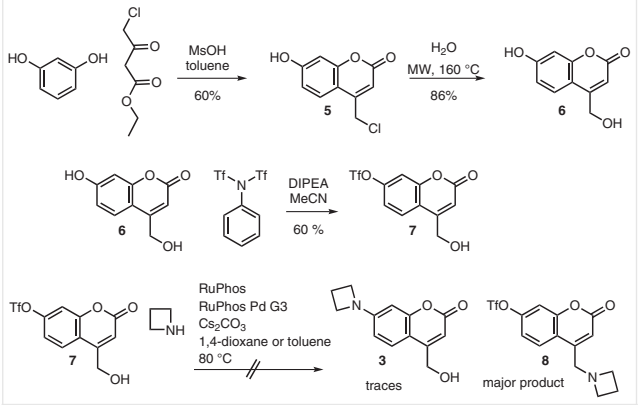


Scheme 2 Previous preparation of 7-azetidin-1-yl-4-methylcoumarin esters **1a–d**[19]

Oftentimes, 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 azetidinylcoumarins 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, compound **3** was used to prepare model sulfonate, phosphate, and carbamate derivatives, highlighting the versatility of this intermediate. Finally, we demonstrate that photolysis of these compounds proceeds smoothly, releasing only the photoprotected leaving group and alcohol **3**.

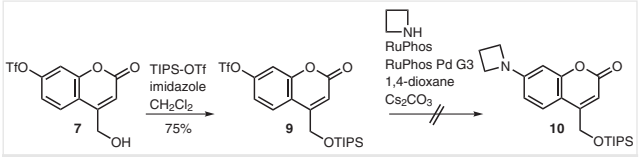
At the outset, we tried to install the azetidine substituent after introducing the 4-hydroxymethyl group on the coumarin skeleton (Scheme 3). After optimization of reported protocols, compound **6** was obtained from resorcinol upon hydrolysis of the intermediate chloride **5**.[8,22,23] Selective triflation of the 7-hydroxy substituent to yield intermediate **7** was also carried out according to a reported procedure.[24] To install the azetidine ring, we aimed to optimize the conditions reported by Lavis and co-workers:



Scheme 3 Route to compound **3** installing the azetidine in the last step

RuPhos/RuPhos Pd G3, with Cs_2CO_3 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 azetidinyl ring substituting the allylic hydroxy group of the 4-methyl, instead of the aromatic triflate. This product was identified by LC/MS analysis of the reaction mixture (m/z = 364; see Figure S1, Supporting Information). The desired alcohol **3** was formed only in trace amounts.

To disfavor coordination of palladium at the 4-hydroxymethyl group, a triisopropylsilyl group was installed before the Buchwald–Hartwig cross-coupling (Scheme 4). With this new substrate **9**, 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. ^{1}H NMR analysis revealed that the small amounts of product **10** that formed under these conditions underwent further nucleophilic ring opening of the azetidine ring (see Figure S2, Supporting Information).



Scheme 4 Buchwald–Hartwig coupling of **9**

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

The first routes tested were the thermal hydrolysis and photolysis of bromide **4** (Scheme 5), an intermediate which had already been prepared in the synthesis of esters **1a–d**. Briefly, compound **4** was obtained from the corresponding triflate by Buchwald–Hartwig cross-coupling to provide **11**,[17] followed by bromination at the 4-methyl substituent by sequential deprotonation with lithium hexamethyldisilazide 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 ^{1}H NMR analyses (see Figure S3, Supporting Information) of the reaction mixtures or the isolated products revealed the attack of a bromide ion at 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.

For an alternative halogenated intermediate, *m*-azetidin-1-ylphenol (**13**) was used in an attempt to prepare 7-azetidin-1-yl-4-(chloromethyl)coumarin (**14**) by Pechmann condensation with ethyl 4-chloroacetoacetate promoted by Ti(O*i*Pr)$_3$Cl at 95 °C (Scheme 6). This reaction has been successfully applied to the synthesis of julolidine-fused ana-

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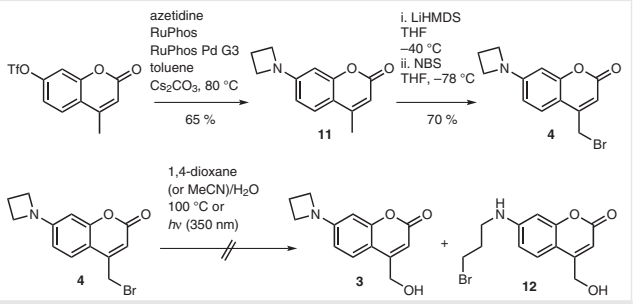
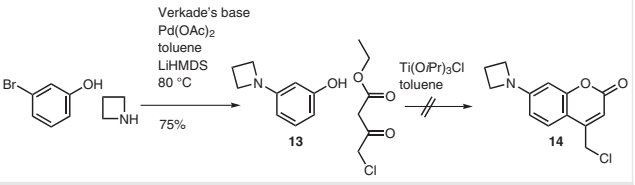


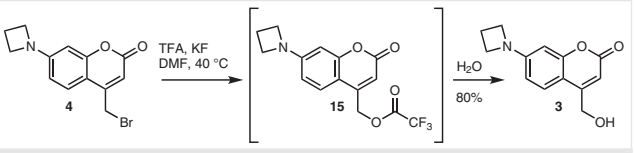
Scheme 5 Thermal hydrolysis and photolysis of bromide **4**

logues.[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 ^{1}H NMR and LC/MS analyses (see Supporting Information).



Scheme 6 Tested route to compound **14**

Finally, as we were able to form esters by nucleophilic displacement of the bromide in compound **4** at lower temperatures (Scheme 2), we aimed to obtain alcohol **3** by hydrolysis of an ester intermediate (Scheme 7). 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**; only alcohol **3** and bromide **4** were detected by LC/MS analysis of the reaction mixture. Under optimized reaction conditions, no significant attack at the azetidine ring by any of the nucleophiles present in the reaction mixture was observed. 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 at the azetidinyl ring.



Scheme 7 One-pot esterification and hydrolysis of bromide **4**

The involvement of the trifluoroacetate **15** as an intermediate is supported by a comparison of LC/MS analyses of the reaction of **4** in the presence of KF and the 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). A significant amount of alcohol **3** was detected only in the first case. In the absence of TFA, only unreacted starting material was observed, whereas without H_2O only traces of alcohol were 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 **3**.

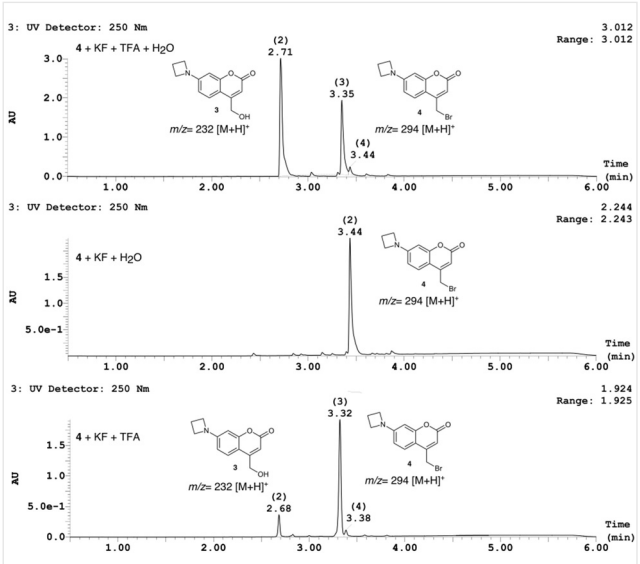


Figure 1 LC/MS analyses of the reaction of bromide **4** with both TFA and H_2O (top), only H_2O (middle), or only TFA (bottom). Analyses were undertaken between 2.3 and 3 hours after addition of the reactants.

Taking these results into account, the route depicted in Scheme 8 is recommended for the preparation of alcohol **3**. Whereas it is possible to purify bromide **4** by column chromatography, it is not necessary for the subsequent 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 alcohol **3** (R_f = 0.25, silica gel, CH_2Cl_2–Et_2O, 7:3) and bromide **4** (R_f = 0.55, silica gel, CH_2Cl_2–EtOAc, 95:5) is much larger than the difference between the latter and the parent compound **11** (R_f = 0.42, silica gel, 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 hand, it was used to prepare the model photoprotected sulfonate, phosphate, and carbamate derivatives depicted in Scheme 9. These compounds were synthesized to demonstrate the

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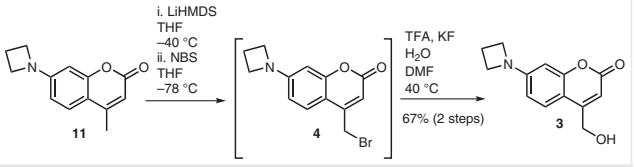
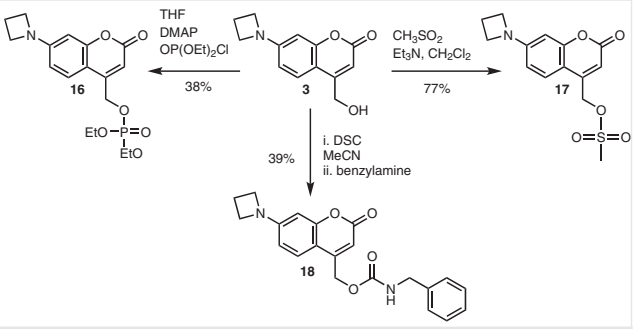


Scheme 8 Optimized synthesis of alcohol **3**

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 those reported when the same procedures were performed employing 7-(diethylamino)-4-(hydroxymethyl)coumarin 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.



Scheme 9 Synthesis of compounds **16**–**18** starting from building block **3** (DSC = *N*,*N*′-disuccinimidyl carbonate)

Finally, ~0.3 mM solutions of compounds **16**–**18** in 7:3 mixtures of MeCN–phosphate-buffered saline (PBS, pH 7.4) were prepared and then irradiated using 405 nm LEDs to monitor the photolysis reactions (Figure 2), as described previously.[19] In all cases, the photoreactions proceeded cleanly to give the photodeprotected products and alcohol **3** or chloride **14**. This halogenated coumarin is likely generated by a chloride ion trapping the intermediate ion pair of the photolysis[12] when the reaction is carried out in PBS, which has a high concentration (155 mM) of NaCl.[34]

In summary, we have 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 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

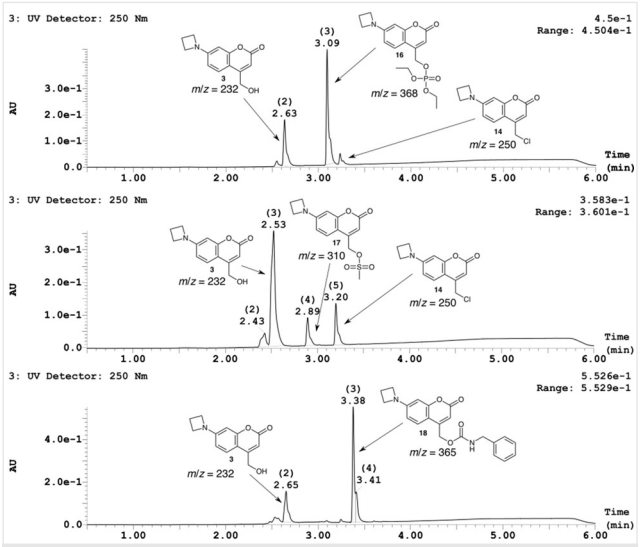


Figure 2 LC/MS analyses of the mixtures of photolyzed compounds **16**–**18**. All indicated *m*/*z* values corresponds to the $[M + H]^+$ ions.

reliable synthesis will encourage other scientists to adopt this photoremovable group, which is much more efficient than its diethylamino-substituted counterpart.

All reagents were purchased from commercial sources and used as received. Anhydrous solvents were procured from Acros Organics and used as received. Preparative HPLC was carried out using an Hitachi instrument equipped with an autosampler and a diode array detector and employing an ACE Excel 5 C18-Amide column (25 cm length, 10 mm internal diameter). 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 photodiode array detector, and an SQ Detector 2 ZSpray (ESI). The samples were eluted with a MeCN–H_2O gradient running from 2% to 98% MeCN, with a constant 0.1% HCOOH. NMR spectra were acquired on Bruker AV300 or Bruker 400 instruments. ^{1}H NMR chemical shifts are reported in ppm relative to $SiMe_4$ ($\delta = 0$) and were referenced internally with respect to residual protons in the solvent ($\delta = 2.50$ for DMSO-d_6, $\delta = 7.26$ for $CDCl_3$). 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). ^{13}C NMR chemical shifts are reported in ppm relative to $SiMe_4$ ($\delta = 0$) and were referenced internally with respect to the solvent signal ($\delta = 39.5$ for DMSO-d_6, $\delta = 77.2$ for $CDCl_3$). Peak assignments are based on calculated chemical shift, multiplicity, and 2D experiments. Atom-numbering is indicated in the Supporting Information. ^{31}P NMR (with ^{1}H decoupling) chemical shifts were referenced upon addition of triphenylphosphine as internal standard ($\delta = -4.9$ for $CDCl_3$).[35] IR spectra were measured on a Perkin Elmer Spectrum Two FTIR spectrophotometer. High-resolution mass spectrometry was conducted by staff of the Molecular and Biomolecular Analysis Service (MoBiAS, ETH Zürich) employing a Bruker maXis ESI/NanoSpray-Qq-TOF-MS or a Bruker solariX ESI/MALDI-FTICR-MS instrument. Elemental analysis was conducted by staff of MoBiAS (ETH Zürich) employing a LECO

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TruSpec Micro instrument. Melting points were measured on a Büchi Melting Point M-560 instrument and are uncorrected; unless otherwise given, the temperature gradient used was 20 °C·min^{-1}.

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

Compound **11** (400 mg, 1.86 mmol) was added to a flame-dried multi-neck flask and suspended in anhydrous THF (34 mL). The suspension was stirred for 10 min at r.t., then cooled to –40 °C in an acetone–dry ice bath, and LiHMDS (1 M in THF; 4.7 mL, 4.7 mmol) was added dropwise to the mixture through a dropping 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 anhydrous THF (6 mL) was added dropwise through a dropping funnel, ensuring 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 dibrominated byproduct (*m*/*z* = 374). The reaction was quenched with HCl (0.1 M) and the mixture repeatedly extracted with CH_2Cl_2. The combined organic phases were dried over $MgSO_4$, and the solvent was removed under reduced pressure until an orange solid was obtained. KF (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 anhydrous DMF (10 mL) and TFA–H_2O (4:1, 450 µL) was added to the flask (2.5 equiv of each). The flask was sealed, and the system was stirred at 40 °C for 12–15 h, until completion, as indicated by TLC (silica gel; CH_2Cl_2–EtOAc, 95:5) by the disappearance of the intermediate bromide **4**. CH_2Cl_2 was added and the organic phase was washed with H_2O. The aqueous phase was back-extracted once with CH_2Cl_2. The combined organic phases were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure until a solid was obtained. The crude was dissolved in CH_2Cl_2 and as little MeOH as possible, and was fixed on Celite. The product was purified by silica gel column chromatography (CH_2Cl_2–Et_2O, 95:5 → 8:2), and isolated as a yellow-orange powder; yield: 290 mg (67%).

Mp 198 °C; R_f = 0.25 (silica gel; CH_2Cl_2–Et_2O, 7:3).

IR (neat): 3386, 1690, 1600, 1530, 1415, 1090 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.45 (d, *J* = 8.7 Hz, 1 H, H-3), 6.36 (dd, *J* = 8.7, 2.3 Hz, 1 H, H-2), 6.26 (d, *J* = 2.3 Hz, 1 H, H-1), 6.11 (t, *J* = 1.5 Hz, 1 H, H-4), 5.51 (t, *J* = 5.6 Hz, 1 H, H-8), 4.67 (dd, *J* = 5.6, 1.5 Hz, 2 H, H-5), 3.93 (t, *J* = 7.4 Hz, 4 H, H-6), 2.35 (quint, *J* = 7.4 Hz, 2 H, H-7).

^{13}C NMR (100 MHz, DMSO-d_6): 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 $C_{13}H_{14}NO_3$: 232.0968; found: 232.0970.

Anal. Calcd for $C_{13}H_{13}NO_3$·0.33 H_2O: 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)-2*H*-chromen-2-one (4)

Compound **11** (500 mg, 2.32 mmol) was added to a flame-dried multi-neck flask and suspended in anhydrous THF (45 mL). The suspension was cooled to –40 °C in an acetone–dry ice bath, and LiHMDS (1 M in THF; 5.81 mL, 5.81 mmol) was added dropwise to the mixture through a dropping funnel. The dropping funnel was rinsed by adding THF (1 mL). The solution was warmed to –30 °C, followed by cooling to –78 °C. A solution of *N*-bromosuccinimide (455 mg, 2.55 mmol) in anhydrous THF (7 mL) was freshly prepared in a flame-dried flask and added dropwise, and the mixture was stirred at –78 °C. The reaction mixture was neutralized after 1 h, when LC/MS and TLC analyses revealed the presence of a dibrominated byproduct (*m*/*z* = 374). The mixture was treated with HCl (0.1 M) and extracted three times with CH_2Cl_2. The combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The product was fixed on Celite and purified by silica gel column chromatography (CH_2Cl_2–EtOAc, 99.5:0.5) to yield an orange solid; yield: 490 mg (70%).

Mp 200 °C (when measured with a slower gradient of 5 °C·min^{-1}, a color change was observed around 185 °C, yielding a solid that does not melt at 200 °C); R_f = 0.55 (silica gel; CH_2Cl_2–EtOAc, 95:5).

IR (neat): 1723, 1617, 1529, 1412, 1306, 1230, 1161 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.48 (d, *J* = 8.7 Hz, 1 H, H-3), 6.33 (dd, *J* = 8.7, 2.3 Hz, 1 H, H-2), 6.22 (d, *J* = 2.3 Hz, 1 H, H-1), 6.17 (s, 1 H, H-4), 4.40 (s, 2 H, H-5), 4.01 (t, *J* = 7.3 Hz, 4 H, H-6), 2.45 (quint, *J* = 7.4 Hz, 2 H, H-7).

^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{13}H_{13}NO_2Br$: 294.0124; found: 294.0118.

Anal. Calcd for $C_{13}H_{12}NO_2Br$: 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-2*H*-chromen-2-one (11)[17]

4-Methyl-2-oxo-2*H*-chromen-7-yl trifluoromethanesulfonate (800 mg, 2.6 mmol), RuPhos (182 mg, 0.4 mmol), RuPhos Pd G3 (334 mg, 0.4 mmol), and Cs_2CO_3 (1.27 g, 3.9 mmol) were transferred to a flame-dried Schlenk flask, which was evacuated and backfilled with nitrogen three times. Anhydrous toluene (21 mL) was added, followed by azetidine (0.2 mL, 162 mg, 2.8 mmol). The reaction mixture was stirred for 15 h at 80 °C, then filtered over Celite, fixed on Celite, purified by silica gel column chromatography (CH_2Cl_2–EtOAc, 99:1), and washed with pentane to yield compound **11** as a bright yellow solid; yield: 360 mg (65%).

R_f = 0.42 (silica gel; CH_2Cl_2–EtOAc, 95:5).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.38 (d, *J* = 8.6 Hz, 1 H, H-3), 6.30 (dd, *J* = 8.6, 2.3 Hz, 1 H, H-2), 6.21 (d, *J* = 2.3 Hz, 1 H, H-1), 5.97 (q, *J* = 1.2 Hz, 1 H, H-4), 3.99 (t, *J* = 7.3 Hz, 4 H, H-6), 2.43 (quint, *J* = 7.3 Hz, 2 H, H-7), 2.34 (d, *J* = 1.2 Hz, 3 H, H-5).

^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{13}H_{14}NO_2$: 216.1019; found: 216.1019.

(7-(Azetidin-1-yl)-2-oxo-2*H*-chromen-4-yl)methyl Diethyl Phosphate (16)

Compound **3** (50 mg, 0.21 mmol) and DMAP (3 mg, 0.02 mmol) were added to a flame-dried flask, and the system was evacuated and backfilled with nitrogen three times. Anhydrous THF (1 mL) and Et_3N (46 µL, 0.33 mmol) were added. A solution of diethyl chlorophosphate (35 µL, 0.24 mmol) in THF (1 mL) was added dropwise. The reaction mixture was stirred at r.t. for 12–15 h, until the starting material was consumed, as determined by TLC (silica gel; CH_2Cl_2–Et_2O, 7:3). Upon completion, CH_2Cl_2 was added, and the organic phase was washed with H_2O. The aqueous phase was back-extracted once with CH_2Cl_2. The combined organic phases were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The crude was dissolved in CH_2Cl_2 and fixed on Celite. The product was purified by silica gel column chromatography (CH_2Cl_2–Et_2O, 92:8 → 95:5), followed by HPLC [solvent A: H_2O, 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:

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10% A; 9–10.5 min: 10–50% A]. Compound **16** was isolated as an oil that became an off-white powder after cooling overnight at –20 °C; yield: 30 mg (38%).

Mp 100 °C; R_f = 0.1 (silica gel; CH_2Cl_2–Et_2O, 95:5).

IR (neat): 2980, 1726, 1608, 1530, 1419, 1270, 1033 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 7.26 (d, *J* = 8.7 Hz, 1 H, H-3), 6.28 (dd, *J* = 8.7, 2.3 Hz, 1 H, H-2), 6.24 (t, *J* = 1.4 Hz, 1 H, H-4), 6.22 (d, *J* = 2.3 Hz, 1 H, H-1), 5.16 (dd, *J* = 6.7, 1.4 Hz, 2 H, H-5), 4.16 (dq, *J* = 7.7, 7.1, Hz, 4 H, H-8), 3.99 (t, *J* = 7.4 Hz, 4 H, H-6), 2.44 (quint, *J* = 7.4 Hz, 2 H, H-7), 1.35 (td, *J* = 7.1, 1.0 Hz, 6 H, H-9).

^{13}C NMR (100 MHz, $CDCl_3$): 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).

^{31}P NMR (162 MHz, $CDCl_3$): δ = –0.5.

HRMS-ESI: *m*/*z* $[M + H]^+$ calcd for $C_{17}H_{23}NO_6P$: 368.1258; found: 368.1256.

Anal. Calcd for $C_{17}H_{22}NO_6P$: 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-2*H*-chromen-4-yl)methyl Methanesulfonate (17)

Compound **3** (50 mg, 0.21 mmol) was flame-dried in a two-neck flask equipped with a dropping funnel, and the system was evacuated and backfilled with nitrogen three times. Anhydrous CH_2Cl_2 (8 mL) and Et_3N (60 µL) were added, and the system was cooled in an ice bath. A solution of methanesulfonyl chloride (25 µL, 0.32 mmol) in CH_2Cl_2 (2 mL) was added dropwise. The reaction mixture was stirred at 0 °C for 45 min, until completion, as determined by TLC (silica gel; CH_2Cl_2–Et_2O, 7:3) by the disappearance of the starting material. CH_2Cl_2 was added, and the organic phase was washed with H_2O. The aqueous phase was back-extracted once with CH_2Cl_2. The combined organic phases were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure until a solid was obtained. The crude was dissolved in CH_2Cl_2 and as little MeOH as possible, and was fixed on Celite. The product was purified by silica gel column chromatography (CH_2Cl_2–Et_2O, 95:5), and isolated as an off-white solid; yield: 50 mg (77%).

Mp 225 °C (possible phase changes at 153 °C, color change at 200 °C); R_f = 0.25 (silica gel; CH_2Cl_2–Et_2O, 95:5).

IR (neat): 2934, 1720, 1605, 1532, 1417, 1356, 1176, 1068, 963, 833 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 7.30 (d, *J* = 8.7 Hz, 1 H, H-3), 6.30 (dd, *J* = 8.7, 2.3 Hz, 1 H, H-2), 6.21 (d, *J* = 2.3 Hz, 1 H, H-1), 6.20 (t, *J* = 1.1 Hz, 1 H, H-4), 5.29 (d, *J* = 1.1 Hz, 2 H, H-5), 4.01 (t, *J* = 7.4 Hz, 4 H, H-6), 3.10 (s, 3 H, H-8), 2.45 (quint, *J* = 7.4 Hz, 2 H, H-7).

^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{14}H_{16}NO_5S$: 310.0744; found: 310.0742.

Anal. Calcd for $C_{14}H_{15}NO_5S$: 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-2*H*-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 dropping funnel. Compound **3** (50 mg, 0.21 mmol) was transferred to the dropping funnel as a solid, and the system was evacuated and backfilled with nitrogen three times. The flask was covered with aluminum foil. Anhydrous MeCN was added to the carbonate (1 mL) and to the dropping funnel (2 mL). *N*,*N*-Diisopropylethylamine (150 µL, 0.84 mmol) was added to the suspension of compound **3**. The suspension was added in portions to the carbonate, and anhydrous MeCN (6.5 mL) was added in portions to the dropping funnel to recover as much of the solid **3** as possible. The mixture was stirred at r.t. for 12 h, until TLC analysis (silica gel; CH_2Cl_2–Et_2O, 7:3) revealed the absence of compound **3**. Benzylamine (250 µL, 2.3 mmol) was added to the flask, and the reaction mixture was stirred for 6 h, until LC/MS analysis revealed that the desired carbamate was the predominant product of the reaction. CH_2Cl_2 was added and the organic phase was washed with H_2O. The aqueous phase was back-extracted once with CH_2Cl_2. The combined organic phases were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The crude was dissolved in CH_2Cl_2 and as little MeOH as possible, and was fixed on Celite. The product was purified by automated silica gel flash column chromatography (CH_2Cl_2–EtOAc, 100:0 → 9:1), and isolated as a yellow solid; yield: 30 mg (39%).

Mp 215 °C; R_f = 0.26 (silica gel; CH_2Cl_2–EtOAc, 95:5).

IR (neat): 3332, 1718, 1605, 1527, 1419, 1238, 1145 cm^{-1}.

^1H NMR (400 MHz, DMSO-d_6): δ = 8.09 (t, *J* = 6.2 Hz, 1 H, H-8), 7.47 (d, *J* = 8.7 Hz, 1 H, H-3), 7.38–7.18 (m, 5 H, H-10, H-11, and H-12), 6.38 (dd, *J* = 8.7, 2.3 Hz, 1 H, H-2), 6.27 (d, *J* = 2.2 Hz, 1 H, H-1), 6.03 (t, *J* = 1.3 Hz, 1 H, H-4), 5.25 (d, *J* = 1.4 Hz, 2 H, H-5), 4.24 (d, *J* = 6.1 Hz, 2 H, H-9), 3.95 (t, *J* = 7.4 Hz, 4 H, H-6), 2.35 (quint, *J* = 7.4 Hz, 2 H, H-7).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 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 $C_{21}H_{21}N_2O_4$: 365.1496; found: 365.1493.

Anal. Calcd for $C_{21}H_{20}N_2O_4$: 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

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