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Palladium-Mediated Approach to Coumarin-Functionalized Amino Acids

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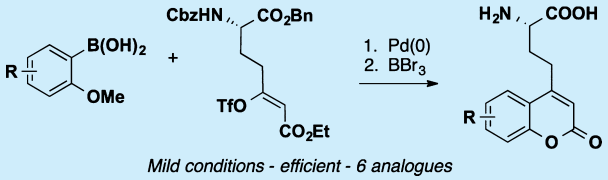
Supporting Information

ABSTRACT: Incorporation of the fluorogenic L-(7-hydroxycoumarin-4-yl)ethylglycine into proteins is a valuable biological tool. Coumarins are typically accessed via the Pechmann reaction, which requires acidic conditions and lacks substrate flexibility. A Pd-mediated coupling is described between *o*-methoxyboronic acids and a glutamic acid derived (*Z*)-vinyl triflate, forming latent coumarins. Global deprotection with BBr_3 forms the coumarin scaffold in a single step. This mild and scalable route yielded five analogues, including a probe suitable for use at lower pH.



The site-specific incorporation of fluorophore-containing unnatural amino acids (UAAs) into a protein of interest by genetic code expansion has emerged as a valuable alternate strategy to expressible fluorescent protein tags, for example, green fluorescent protein (GFP). When compared with large, protein-derived reporters (often greater than 20 kDa), fluorescent UAAs are significantly smaller in size and can be incorporated at the internal positions of a protein, whereas GFP is limited to the C- and N-termini.[1] Practically, UAAs can be introduced into a protein via engineered orthogonal tRNA/aminoacyl-tRNA synthetase pairs in response to the amber stop codon (UAG), strategically placed in a gene of interest.[2] The method is applicable to prokaryotic[3] and eukaryotic systems,[4] as well as living organisms.[5] Pioneering work by Schultz and co-workers demonstrated that L-(7-hydroxycoumarin-4-yl)-ethylglycine (**1**) could be selectively incorporated into a modified protein and used to probe urea-dependent denaturation.[6] UAA **1** exhibits favorable photophysical properties such as solvent- and pH-dependent fluorescence (dictated by the pK_a of the phenolic functionality: 7.8) and a reasonable quantum yield and Stokes shift. Consequently, it has proven a useful tool in chemical and molecular biology.[7] Given the application of **1**, it is somewhat surprising that analogues with varied aryl substitution patterns have not been extensively explored. This can be attributed to the typical methodology for accessing coumarin-4-yl-ethylglycines: the Pechmann condensation of resorcinol (**2**) and a glutamic acid-derived *β*-keto ester (**3**) (Figure 1).[8] In this approach, resorcinol is the only practical reaction partner for the keto ester, limiting the availability of analogues as a consequence. The strongly acidic and dehydrating conditions and requirement of superstoichiometric amounts of the aryl partner enforce further limitations. Furthermore, preparative HPLC is commonly used for the purification of **1**, which is hampered by solubility problems.[7c] Several alternate strategies have been reported that provide **1**, albeit as racemates.[9]

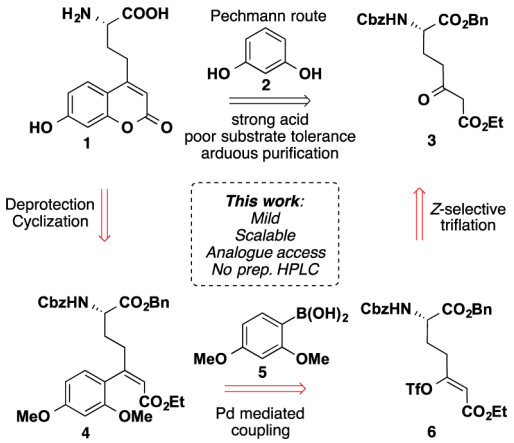


Figure 1. An alternate approach to the Schultz amino acid **1**.

We envisioned a practical synthesis of L-coumarin-4-ylethylglycines, where both the nature and position of aryl ring substituents could be varied outside of the strict prerequisites of the Pechmann condensation while conserving a similar disconnection. Obtaining access to analogues of **1** will expand the scope of coumarins as tools for chemical biology. Retrosynthetically, the *α*,*β*-unsaturated ester (**4**) was identified as the key intermediate toward these goals. We envisaged it could be accessed via a palladium-mediated cross coupling between a suitably substituted arylboronic acid (**5**) and a vinyl triflate (**6**) (Figure 1). Compound **6** can be derived from the commercially available *N*-Cbz-L-glutamic acid benzyl ester. Careful consideration of the phenolic and amino acid protecting groups would enable global deprotection and

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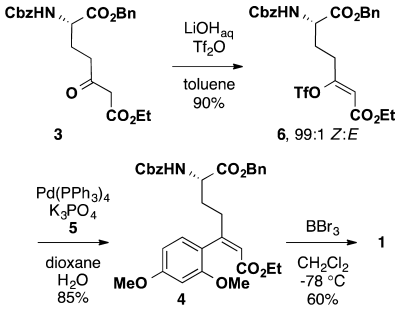
coumarin formation to be effected in a single reaction step. In this regard, the *N*-Cbz, benzyl ester, and phenolic methyl ether groups were chosen due to their lability upon exposure to boron tribromide. In order to avoid purification by preparative HPLC, we aimed that the product obtained in the final step could be purified by ion exchange chromatography.[7c,i]

The synthesis commenced with the formation of vinyl triflate **6** from *β*-keto ester **3** (Scheme 1), derived from the

Scheme 1. Synthesis of the Schultz Coumarin 1

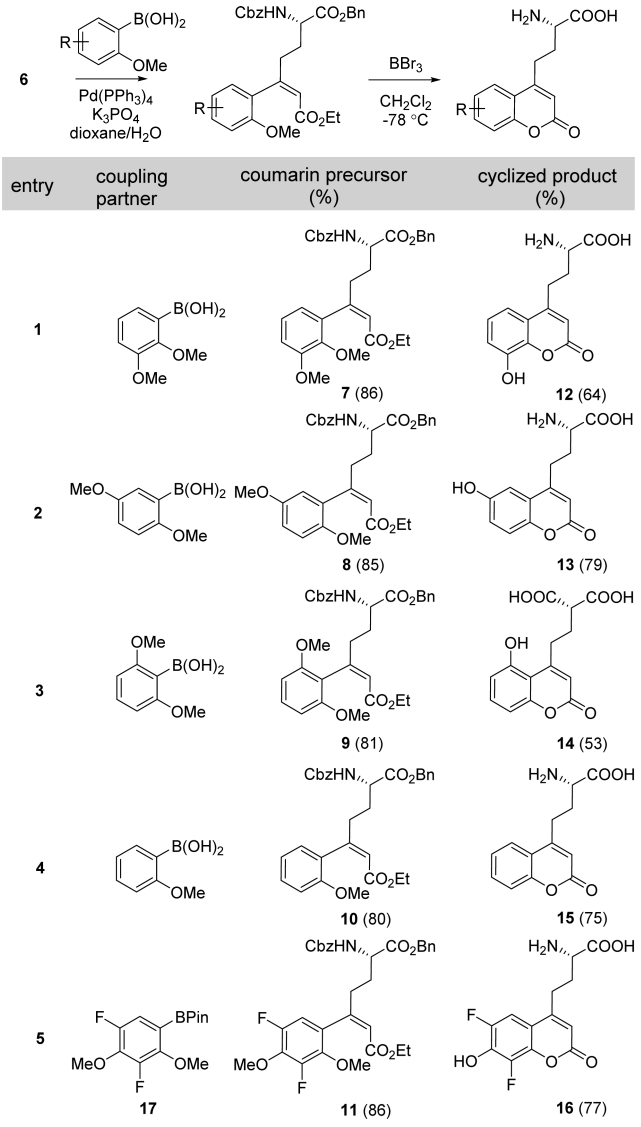


corresponding glutamic acid in 85% yield (see Supporting Information). As the *Z* isomer of **6** was desired for ring closure to form the coumarin motif, we applied the modified Schotten–Baumann-type conditions of Babinski et al., where the enolate geometry, and therefore stereoselectivity of the enol triflate formation, is controlled by the choice of base.[10] Initial experiments using LiOH and trifluoromethanesulfonic anhydride provided crude **6** as a 9:1 mixture of the *Z*/*E* isomers, respectively. Further tuning of the reaction conditions, including slow addition and lowering the internal reaction temperature to 0–5 °C, resulted in a 99:1 *Z*/*E* ratio and a yield of 90% after chromatography. The importance of temperature control was most pronounced during large-scale (≥20g) preparations of **6**. Attempts to selectively synthesize the isomeric *E*-**6** ($(Me)_4NOH$, hexanes[10]) to test if the *E* isomer could isomerize and undergo coumarin formation during BBr_3 treatment were not successful.

With intermediate *Z*-**6** in hand, we subjected it to cross coupling with 2,4-dimethoxyphenylboronic acid (**5**). A number of conditions and reagent combinations were screened, and the operationally simple and economical conditions of $Pd(PPh_3)_4$ (5 mol %) and K_3PO_4 in 1,4-dioxane/water at 50 °C gave the most satisfying results. Catalyst loading could be further reduced to 2.5 mol % without diminishing coupling performance and could be performed on gram scale (1.11 g of triflate, 85% yield). As we could access the linear coumarin precursor **4** on a practical scale, we sought to identify conditions for the global deprotection/cyclization step to complete the synthesis. The challenges of this reaction involved optimizing the amount of BBr_3 required, formation of the coumarin scaffold, and developing a practical purification method. Solutions of **4** in dichloromethane were cooled to −78 °C, treated with varying amounts of BBr_3, and slowly warmed to ambient temperature before reverse quenching into ice water. It was found that 5.4 equiv of BBr_3 was sufficient for global deprotection and cyclization, resulting in a sole product, **1**. Purification of the reaction product was complicated by the large amounts of boric acid formed during reaction quenching. To remedy this, the biphasic mixture from the quench was concentrated to a small volume and the resulting slurry applied directly to a Dowex 50 column (H^+ form, 300 mesh, 40 g wet resin per g product). Elution of the boric acid byproduct with water, followed by a gradient of aq NH_4OH, provided the coumarin amino acid **1** in 60% isolated yield after lyophilization, avoiding preparative HPLC. The Mosher's amide of **1** was prepared, and no extensive epimerization was observed (see the Supporting Information).

To further elucidate the scope of the coumarin synthesis, we performed the cross coupling of **6** with a number of commercially available *o*-methoxy-substituted arylboronic acids (Table 1, entries 1–4). Here, all derivatives employed

Table 1. Substrate Scope of Coumarin Synthesis



performed well, with the yields for **7**–**10** in the range of 80–86%. The BBr_3-initiated deprotection/cyclization of these linear motifs furnished the coumarins **12**–**15**. Varying the position and presence of the phenolic substituent provides analogues to probe the structure–activity relationship (SAR) of the different aminoacyl-tRNA synthetase mutants during amber suppression screening and mutagenesis.

As the fluorescent properties of coumarin **1** are influenced by the acidity of the C-7 phenol, it would be useful to access analogues where the phenolic pK_a is lowered.[9b,11] An UAA with these properties would be advantageous for studies of biological

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components that operate under acidic conditions, i.e., the endosomal and lysosomal pathways (pH ≈ 5). Fluorine substituents at the 6 and 8 positions of 7-OH-coumarin have been shown to significantly reduce the phenolic pK_a while improving photostability.[11] Toward these goals, the difluorinated boronic acid pinacol ester **17** was synthesized from 2,3,4,5-tetrafluoronitrobenzene (35% over four steps, see the Supporting Information). Both the coupling and coumarin formation reactions proceeded smoothly (86 and 77%, respectively) to afford the fluorinated coumarin **16**.

The spectroscopic properties of coumarins **1** and **16** were investigated in phosphate buffer at pH 2.8−8.0. Monitoring the effect of pH on absorption (360 nm) allowed us to determine the pK_a of compound **16** as 4.8 (see the SI; pK_a of **1**: 7.8). While the absorption spectra of both compounds changed at the different pH's (Figure S1), the shape and wavelength range of the fluorescence spectra were independent of pH throughout the measured range, indicating that emission only occurs from one ionization form of the compounds (Figures S2 and S3). Both compounds displayed high quantum yields, relatively long lifetimes, and well-separated emission peaks (Table 2). In contrast to compound **1** that showed spectroscopic differences only at pH ≥ 6, difluorinated compound **16** showed marked differences in absorbance at pH 4−6, resulting in varied fluorescence intensity (Figure 2). This demonstrates its potential as a wavelength-ratiometric pH probe for application in low pH environments (Figure S5).

Table 2. Spectroscopic Data for Compounds 1 and 16 in Phosphate Buffer

compd	pH	λ_{max} (nm)	ε (M^{-1} cm^{-1})	em_{max} (nm)	Φ_f	τ (ns)
1	2.8	320	11900	456	0.68	5.5
	8.0	362	11500	455	0.72	5.5
16	2.8	320	10000	458	0.68	5.5
	8.0	362	14900	457	0.67	5.6

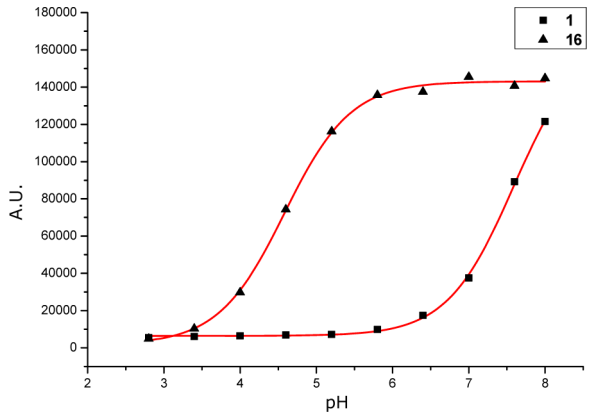

Figure 2. Emission at 460 nm of compounds **1** and **16** at pH 2.8−8.0 when excited at 360 nm. A.U. = arbitrary units.

In conclusion, we describe a novel route to coumarin-functionalized amino acids for application in genetic code expansion technologies. Traditionally, the Pechmann cyclization has been used to access non-natural amino acids such as **1**, which operates under harsh conditions and is not amenable to analogue exploration. We circumvented these problems via the palladium-mediated coupling of 2-methoxyarylboronic acid with a glutamic acid derived vinyl triflate **6** (99:1 *Z*/*E*) to access the latent precoumarin **4**. Due to the careful selection of the phenolic and amino acid protecting groups, treatment of the linear compound **4** with BBr_3 initiated the removal of all protecting groups and effected lactonization to construct the coumarin motif. To demonstrate the versatile and mild nature of this reaction sequence, coumarins **12**−**16**, which differ by the phenolic substitution of their aryl rings, were synthesized. Of importance, coumarins **1** and **12**−**16** could be obtained on a useful scale without relying on preparative HPLC purification. It is envisaged these analogues will provide insight into SAR involved in aminoacyl-tRNA synthetase mutagenesis and be useful as fluorescent probes to investigate biological processes that occur in lower pH environments.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b00854.

Experimental procedures; ^1H, ^{13}C and ^{19}F NMR spectra for novel compounds; spectroscopic studies (PDF)

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Notes

The authors declare no competing financial interest.

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