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PAPER

Design, synthesis, and cyclization of 4-aminobutyric acid derivatives: potential candidates as self-immolative spacers†

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Self-immolative spacers have gained significant interest in recent years due to their utility in numerous prodrug, sensor and drug delivery systems. However, there are a very limited number of spacers that are capable of undergoing spontaneous and rapid reactions under mild conditions. To address this need, 4-aminobutyric acid derivatives were explored as a potential class of self-immolative spacers. Using a modular approach, eleven *N*- and α-substituted derivatives of 4-aminobutyric acid were synthesized, and their intramolecular cyclizations to γ-lactams were studied. Kinetics experiments were carried out at physiological pH and temperature, and the observed half-lives for the spacers ranged from 2 to 39 s, depending on the molecular structure. In addition, the pH dependence of the cyclization rate was also explored and it was found that cyclization still occurred rapidly at mildly acidic pH. Therefore, this class of compounds exhibits promise for incorporation into a variety of self-immolative systems where rapid cyclization reactions are desired.

Introduction

Chemical moieties capable of undergoing rapid and spontaneous intramolecular reactions in response to the cleavage of a capping or triggering unit are commonly referred to as self-immolative spacers.[1] In their typical form, these moieties comprise two reactive termini with a capping group or trigger as one terminus and the substrate of interest, such as a drug, fluorophore, or an additional spacer on the other terminus. Removal of the capping group results in an intramolecular reaction that ultimately results in the liberation of the substrate. As shown in Fig. 1, these intramolecular reactions generally involve electronic rearrangements such as 1,4, 1,6, or 1,8 elimination reactions[2,3] or cyclizations to form highly favored five- or six-membered rings.[4,5]

In recent years, the interest in self-immolative spacers has grown significantly as their application in various prodrug, sensor, and drug delivery systems has been explored. For example, the conjugation of self-immolative spacers to drug molecules has created inactive prodrugs that are converted to the free and active drugs by cleavage of the trigger upon exposure to an external stimulus.[6–15] They have also been used in the linkage of drug molecules to small molecule or antibody targeting moieties.[16–23] Sensors have been developed by using self-immolative spacers to conjugate reporter molecules such as fluorophores or imaging agents to peptide or enzyme sensitive triggers.[24–32] The use of these linkers in dendrimeric[33–37] and oligomeric[38,39] systems has also been explored, leading to an amplified release of drugs or reporter molecules.

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† Electronic supplementary information (ESI) available: General procedures and materials section, syntheses of compounds **6b**, **8c**, **9b**, **9c**, **10b**, **10c**, **11c**, **12a**, **12b**, **12c**, **12d**, **13b**, **13c**, **13d**, **17b**. ^{1}H and ^{13}C NMR spectra for all synthesis products, ^{1}H NMR spectra of compounds prior to and after cyclization, kinetics data. See DOI: 10.1039/c0ob00890g

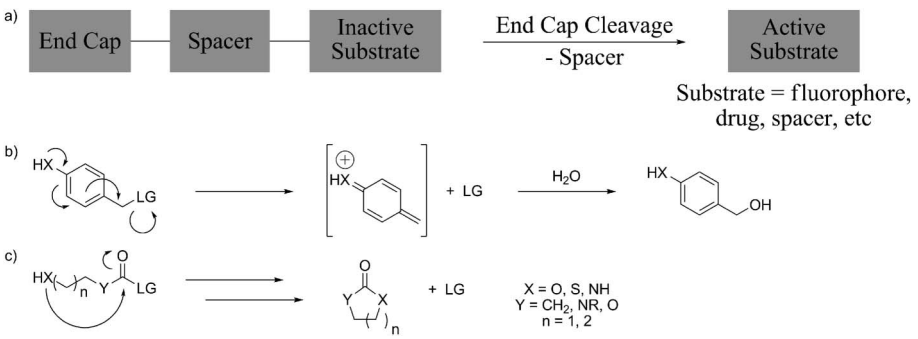


Fig. 1 a) Schematic of a self-immolative spacer; b) example of a 1,6 elimination reaction; c) example of a cyclization reaction.

Recently, our group and others have explored the development of linear polymers comprising self-immolative spacers. Shabat and coworkers have explored polymers based entirely on 1,6-elimination reactions as amplified reporters,[40] enzyme sensors,[41] and enzyme labels.[42] Moore and coworkers have prepared microcapsules based on cross-linked versions of similar polymers.[43] Our group has introduced cyclization spacers in alternation with elimination spacers as a means of controlling the polymer degradation rate, and have demonstrated that amphiphilic block copolymers such as **1** (Fig. 2) comprising one self-immolative block are capable of assembling into nanoparticles that degrade in a controlled manner to release their cargo.[44] Furthermore, we have also developed linear polymers such as **2**,[45] capable of degrading entirely by cyclization reactions in order to address the potential toxicity of the quinone methide intermediates that are produced during the 1,6-elimination reaction.[46]

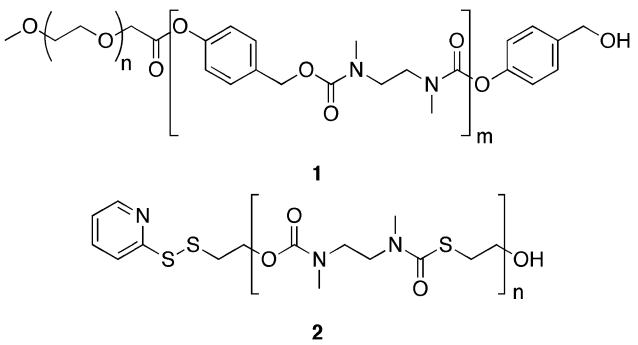


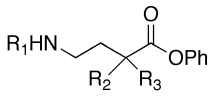
Fig. 2 Chemical structures of previously reported self-immolative polymers incorporating cyclization spacers.[44,45]

In order to fully exploit self-immolative spacers in these materials and other new applications, it is essential to have access to spacers that react at different rates. For example, the *N*,*N*′-dimethylethylenediamine spacer used in both polymers **1** and **2**, cyclized slowly at pH 7.4, which resulted in polymer degradation over a period of days to weeks. While several self-immolative spacers based on cyclization reactions have been reported, there are very few that cyclize rapidly under mild conditions.[1,4,47,48] To address this need and to develop a spacer that could potentially replace the *N*,*N*′-dimethylethylenediamine spacer in polymers such as **1** or **2**, we have investigated 4-aminobutyric acid derivatives as a potential new class of rapidly cyclizing self-immolative spacers. The recent incorporation of a 4-aminobutyric acid unit into an enzymatic detection probe suggested that this class of molecules may serve as rapidly cyclizing spacers.[24] However, there has not been a versatile synthetic strategy developed for the preparation of various analogues, nor a comprehensive study of their cyclization rates. Thus, described here is the design and synthesis of eleven different derivatives of 4-aminobutyric acid, and studies of their cyclizations.

Results and discussion

Design

The targets shown in Fig. 3 were designed with several aspects in mind. First, a phenyl ester was selected, as it would serve as a good model system for future applications. Many dyes,



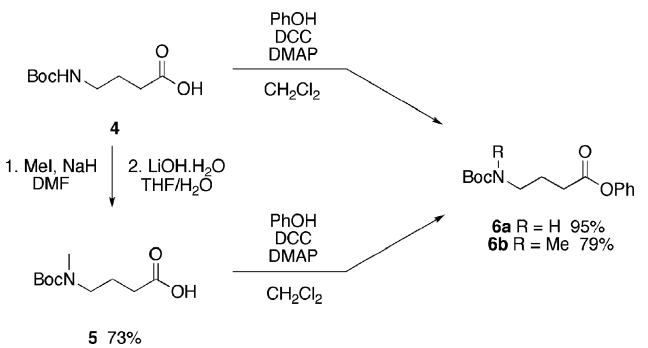
3a $R_1 = R_2 = R_3 = H$ **3b** $R_1 = Me$ $R_2 = R_3 = H$
3c $R_1 = R_2 = Me$ $R_3 = H$ **3d** $R_1 = Me$ $R_2 = allyl$ $R_3 = H$
3e $R_1 = Me$ $R_2 = Bn$ $R_3 = H$ **3f** $R_1 = R_2 = R_3 = Me$
3g $R_1 = Me$ $R_2 = R_3 = allyl$ **3h** $R_1 = Me$ $R_2 = R_3 = Bn$
3i $R_1 = Me$ $R_2 = -CH_2(CH_2)_2CH_2- = R_3$ (cyclopentyl)
3j $R_1 = R_2 = H$ $R_3 = OH$ **3k** $R_1 = Me$ $R_2 = H$ $R_3 = OH$

Fig. 3 Target 4-aminobutyric acid derivatives for kinetic studies.

such as fluorescein, Hoechst stain and umbelliferone, contain phenolic groups, along with the chemotherapy drug Topotecan. In addition, many other self-immolative spacers involve phenols,[1,3,5] thus allowing the new spacer to be readily alternated with other spacers, and incorporated into polymers analogous to **1**. Secondly, some *N*-methylated derivatives were targeted as this has previously been reported to significantly enhance the cyclization rate in the case of ethylenediamine derivatives.[49] Finally, substitution at the α position allowed us to examine the Thorpe-Ingold effect[50,51] and/or the reactive rotamer effect[52] on these compounds. It was also expected to slow any competing ester hydrolysis. To test the scope of these effects, the substitution pattern and the size of the substituents were varied from unsubstituted to an α,α-dibenzyl derivative. A single cyclopentyl ring was also incorporated to test the effect of a conformationally locked system. The 2-hydroxy derivatives were designed to provide insight into inductive effects.

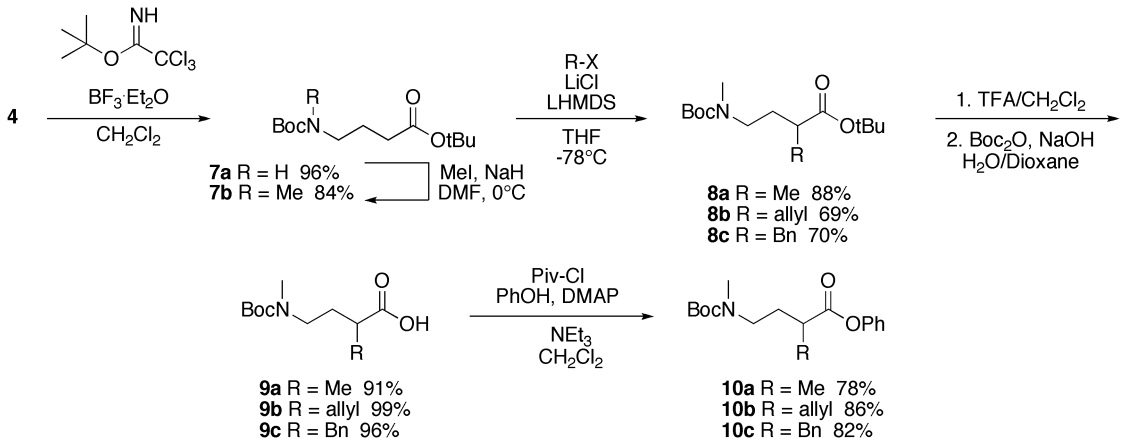
Synthesis

The synthesis of targets **3a–j** began with the previously reported Boc-protected 4-aminobutyric acid (**4**).[53] As shown in Scheme 1, the phenyl ester **6a** was obtained by coupling the acid **4** to phenol using DCC in the presence of DMAP. The *N*-methyl derivative was prepared by treating **4** with MeI and NaH, immediately followed by hydrolysis of the resulting methyl ester with LiOH. The resulting free acid **5** was then coupled to phenol using DCC and DMAP to obtain the desired Boc-protected phenyl ester **6b**.



Scheme 1 Synthesis of α-unsubstituted derivatives.

To prepare the α-monosubstituted compounds, the acid was first converted to a *t*-butyl ester **7a** using *t*-butyl-2,2,2-trichloroacetimidate in the presence of $BF_3 \cdot Et_2O$, and then *N*-methylation was performed as described above using MeI and NaH to provide **7b** (Scheme 2). Monoalkylated *t*-butyl esters **8a–c** were obtained by treatment of **7b** with LHMDS in the presence of LiCl at −78 °C, followed by the addition of the alkyl halide. In marked contrast to the formation of the benzyl and allyl derivatives



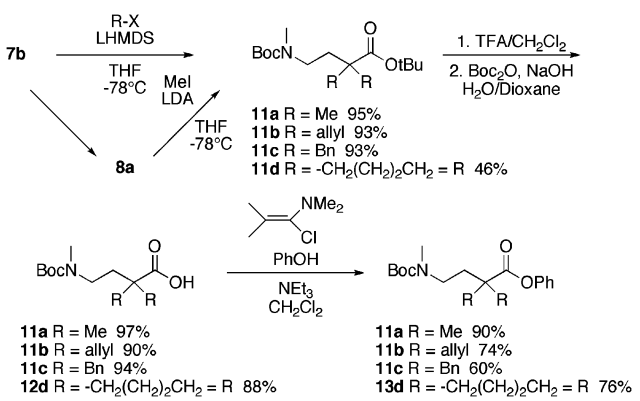
Scheme 2 Synthesis of α-monosubstituted derivatives.

which suffered from partial over-alkylation, monomethylation was cleanly achieved even when a large excess of both LHMDS and MeI were used, simplifying purification and increasing its yield relative to the other compounds. The substituted *t*-butyl esters were then converted to the free acids by first removing both the Boc group and the ester using TFA in CH_2Cl_2, and then reinstalling the Boc group on the amine. This process worked very efficiently for all substrates, with all products being obtained with yields in excess of 90%. The final step was formation of the phenyl ester. When DCC was used as described above for the preparation of **6a** and **b**, unsatisfactory yields of the desired products were obtained, likely due to steric hindrance at the α carbon. To circumvent this problem, the acid was converted to a mixed anhydride using pivaloyl chloride, and this anhydride was then reacted with phenol. This afforded phenyl esters **10a–c** in good yields, ranging from 78–86%.

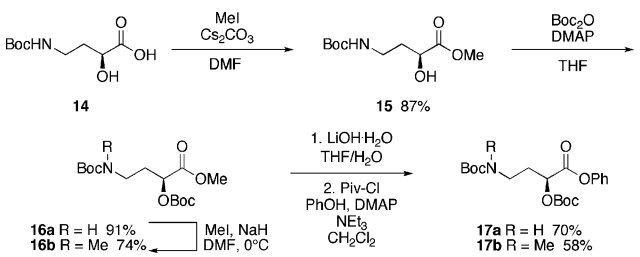
The α,α-dialkylated compounds were similarly derived from the intermediate **7b** (Scheme 3). Diallyl and dibenzyl *t*-butyl esters **11b** & **11c** were synthesized directly from **7b** by treatment first with 1 eq of LHMDS and then the alkyl halide followed an hour later by 2 eq of each, which gave clean conversion to the disubstituted products. Synthesis of the cyclopentyl ring was done similarly except with a single addition of the alkyl dihalide. Following the same protocol as allylation and benzylation to generate **11a** did not prove successful, and the products obtained were a mixture of the mono and disubstituted compounds. Resubjection of this material also did not prove successful, even after removal of byproducts. Similarly, **8a** could not be further methylated under these conditions. However, when the base was switched to LDA, the second methylation occurred cleanly, affording **11a** in very good yield. Curiously, when LDA was used on **7b**, a mixture of mono and dialkylated products was once again obtained, so it appears for this particular substrate the dimethyl *t*-butyl ester could only be obtained by doing successive methylations of **7b** and then **8a**, using LDA for the second methylation.

At this point a global deprotection and reinstallation of the Boc group was carried out, again producing the *N*-Boc acids in very good yields. To install the phenyl ester, it was evident that a mixed pivaloyl anhydride would be ineffective as there would be little steric differentiation between the two carbonyls of the anhydride. Therefore, the best option appeared to be conversion of the acid to an acid chloride. As the acid sensitivity of the Boc groups was incompatible with conventional methods for generating acid chlorides, the Ghosez reagent, 1-chloro-*N*,*N*,2-trimethylpropenylamine, which generates the acid chloride with no acidic byproducts, was used. This method proved quite effective, providing the desired phenyl esters in yields ranging from 60–90% after isolation.

As shown in Scheme 4, the synthesis of the α-hydroxy targets began with Boc-protected *S*-4-amino-2-hydroxybutyric acid[54] (**14**). This acid was treated with MeI in the presence of Cs_2CO_3 to afford the desired methyl ester **15** in very good yield. The next step was protection of the 2-hydroxy group, for which we selected a second Boc group. This group was chosen because it could be attached easily in high yields, and cleavage could occur simultaneously with the *N*-Boc group, thus removing an additional deprotection step from the reaction sequence. This Boc group was installed



Scheme 3 Synthesis of α,α-disubstituted derivatives.



Scheme 4 Synthesis of α-hydroxy substituted derivatives.

using Boc_2O in the presence of catalytic DMAP, affording di-Boc protected compound **16a** in 90% yield. To generate the target lacking an *N*-methyl group, **16a** was treated with LiOH to cleave the methyl ester, and the corresponding acid was converted to phenyl ester **17a** in 70% yield using the mixed anhydride method described above. The *N*-methyl derivative was synthesized by first methylating **16a** using MeI and NaH, and then following the same procedure as above to obtain the phenyl ester **17b**.

Kinetics

UV-visible spectroscopy was evaluated as a possible analytical tool for measuring the cyclization kinetics. The UV-visible spectra of phenol and a representative phenyl ester **6a** are shown in Fig. 4. While there is some overlap between the two spectra, at 276 nm the phenol is strongly absorbing while the phenyl ester is only weakly absorbing. Therefore, it was possible to perform kinetic studies measuring the phenol released upon cyclization by monitoring the absorbance at 276 nm.

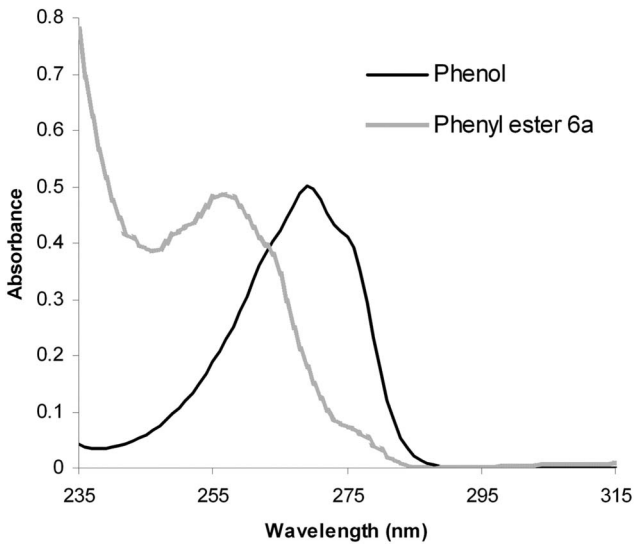


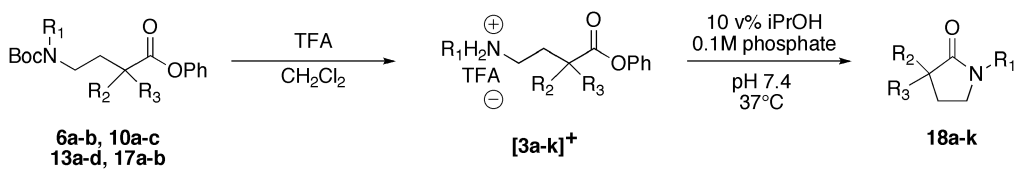
Fig. 4 UV-visible absorption spectra of phenol and a representative phenyl ester **6a**.

All of the target molecules were stored in their Boc-protected forms and were deprotected immediately prior to kinetic studies by treatment with TFA as shown in Scheme 5. Removal of the Boc group was verified by ^{1}H NMR spectroscopy. In most cases, protonation of the amine in the form of the TFA salt was sufficient to inhibit cyclization prior to and during NMR spectroscopy, but occasionally some cyclization was observed (see ESI†). The kinetic studies were performed by dissolving the deprotected substrates in *i*-PrOH, then diluting this solution ten-fold with 0.1 M pH 7.4 phosphate buffer. The measurements were carried out at the physiological temperature of 37 °C. The pH was verified after cyclization, and no change was observed. The linearity of $\ln[A]_0/[A]$ *versus* time graphs where [A] is the concentration of the starting material suggested that the cyclizations followed first order or pseudo first order kinetics (see ESI†). Rate constants were calculated as the slopes of the these graphs. The reported rate constants are the average of those obtained over a minimum of 3 experimental runs (Table 1). Reported errors correspond to the calculated standard deviations of these runs. From the average rate constant, the half-life for each spacer was also calculated. In each case, the structure of the cyclized product was verified by NMR spectroscopy and mass spectrometry (see ESI†). There was no evidence of background ester hydrolysis for any of the substrates.

Table 1 Rate constants and corresponding half lives for the intramolecular cyclizations of **3a–k**

Substrate	Rate constant (s^{-1})	Half-life (s)
3a	0.0179 ± 0.0035	39
3b	0.0708 ± 0.0090	9.8
3c	0.116 ± 0.014	6.0
3d	0.127 ± 0.017	5.5
3e	0.104 ± 0.024	6.7
3f	0.1334 ± 0.0031	5.2
3g	0.1338 ± 0.0059	5.2
3h	0.0276± 0.0024	25
3i	0.3516 ± 0.0026	2.0
3j	0.172 ± 0.022	4.0
3k	0.233 ± 0.013	3.0

All of the spacers cyclized quite rapidly with the half lives ranging from 2 to 39 s. When comparing **3a** and **3b**, it is clear that methylating the amine had a dramatic effect on the rate, with the half-life being reduced by a factor of approximately four. Consistent with the Thorpe-Ingold[50,51] and reactive rotamer effects,[52] α substitution further increased the rate of cyclization, as all of the monosubstituted spacers **3c**, **3d**, and **3e** reacted faster than the unsubstituted spacer **3b** and cyclized at similar rates. The α,α-disubstituted spacers **3f** and **3g** cyclized at similar rates to their monosubstituted analogues. However, the α,α-dibenzyl substituted spacer **3h** exhibited a nearly 4-fold decrease in rate, suggesting that steric crowding proximal to the ester impedes cyclization. In contrast, the α-cyclopentyl substituted compound **3i** exhibited the fastest rate of cyclization, indicating that conformational rigidity can play a role. Finally, both of the α-hydroxy substituted spacers **3j** and **3k** cyclized faster than the α-aliphatic substituted derivatives. Interestingly, the dramatic increase in rate caused by *N*-methylation that was observed for **3a** *versus* **3b** was not noted for **3j** *versus* **3k**. The rate did increase, indicating that there was still an effect, but it appears that the most significant contribution was from the α-hydroxy substituent.



Scheme 5 Deprotection and subsequent cyclization of 4-aminobutyric acid derivatives.

Table 2 Rate constants and corresponding half lives for the intramolecular cyclizations of **3i** at different pHs

pH	Rate constant (s^{-1})	Half-life (s)
7.4	0.3516 ± 0.0026	2.0
7.0	0.1822 ± 0.0188	3.8
6.0	0.0616 ± 0.0085	11
5.0	0.0199± 0.0015	35
4.0	0.0091 ± 0.0012	76

It was also of interest to investigate the effect of pH on the cyclization rate. This was of interest in considering the potential application of these new spacers in areas such as drug delivery. For example, certain drug delivery targets such as tumors,[55,56] inflamed tissues,[57] and intracellular compartments such as endosomes and lysosomes[58] are known to exhibit mildly acidic pHs and it would be desirable that the cyclization rate not be dramatically slowed in these environments. To explore this, the cyclization of the spacer **3i** was investigated at pHs 4.0, 5.0, 6.0 and 7.0 following the method previously described. As shown in Table 2, there was indeed a pronounced decrease in the cyclization rate with decreasing pH. Nevertheless, the cyclization was still rapid even at pH 4 with a half life of 76 s. In comparison, the *N*, *N*-dimethylethylenediamine spacer previously employed[44,45] has a reported half life of greater than 15 days at pH 4.2 and 37 °C. Therefore, these 4-aminobutyric acid spacers appear to be more promising for a wider range of physiological environments.

Conclusion

A new series of self-immolative spacers derived from 4-aminobutyric acid was developed. A modular synthetic approach was used for the preparation of eleven different derivatives. These derivatives allowed the effects of *N*-methylation and α-substitution to be explored. As expected, *N*-methylation led to enhanced cyclization rates. α-Substitution led to enhanced cyclization rates when the substituents were not too bulky but large groups such as benzyl slowed the cyclization. Electron withdrawing groups or conformationally restricted groups at the α position accelerated the rate. Overall, all of the target compounds exhibited rapid cyclization kinetics with half lives of less than one minute at pH 7.4 and 37 °C. In addition, cyclization still occurred rapidly at mildly acid pH. This suggests that these spacers should be of great utility in systems where a rapid release of the substrate even at acidic pHs is required. Furthermore, the versatile synthetic approach should allow the introduction of additional functionalities and also for their incorporation into a range of chemical systems, allowing for many new applications. Progress towards such applications is currently underway and will be reported in due course.

Experimental

General procedures and materials. All reagents were purchased from commercial sources and used without further purification unless otherwise noted. Anhydrous DMF and THF were obtained from a solvent purification system. Anhydrous CH_2Cl_2 and NEt_3 were distilled over CaH_2. Diisopropylamine was distilled over $MgSO_4$. Unless otherwise stated, all reactions were performed under a N_2 atmosphere using flame or vacuum-dried glassware. Column chromatography was performed using silica gel (0.063–0.200 mm size, 70–230 mesh). ^{1}H NMR spectra were obtained at 400 MHz and ^{13}C NMR spectra were obtained at 100 MHz. NMR chemical shifts are reported in ppm and are calibrated against residual solvent signals of $CDCl_3$ (δ 7.26, 77.36). Coupling constants are expressed in Hertz (Hz). Infrared spectra were obtained as films from CH_2Cl_2 on NaCl plates. High-resolution mass spectrometry (HRMS) was performed using a Finnigan MAT 8400 electron impact (EI) or a Micromass LCT electrospray ionization time-of-flight (ESI) mass spectrometer. UV-visible spectrscopy experiments were carried out using a Varian Cary 300 Bio UV-visible spectrophotometer.

Synthesis of acid 5. To a flask containing **4**[53] (0.250 g, 1.23 mmol), DMF (12 mL) and MeI (0.23 mL, 3.69 mmol) were added, and the solution was cooled to 0 °C. NaH (0.128 g, 3.21 mmol) was suspended in DMF (1 mL) and the suspension was added dropwise to the reaction mixture. The resulting mixture was stirred for 4 h. A second portion of MeI (0.10 mL, 1.61 mmol) was then added followed by additional NaH (0.070 g, 2.92 mmol) suspended in DMF (0.5 mL) and the reaction mixture was stirred 2 h. The solvent was removed *in vacuo*, and the crude material was taken up in CH_2Cl_2 and poured onto H_2O. The product was extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and the solvent was removed *in vacuo*. The resulting oil was taken up in 1 : 1 THF : H_2O (12 mL), $LiOH{\cdot}H_2O$ (0.104 g, 2.48 mmol) was added and then the solution was stirred overnight. The solution was poured into 1 M HCl, and the product was extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and the solvent was removed. The crude product was purified by column chromatography (3 : 2 cyclohexane : EtOAc), yielding **5** (0.195 g, 73%) as a clear, colorless oil. ν_{max}/cm^{-1} 3205, 2980, 2937, 1738, 1698, 1490, 1457, 1401, 1367.^{1}H NMR ($CDCl_3$): δ 11.08 (br s, 1H), 3.35–3.22 (m, 2H), 2.85 (s, 3H), 2.36 (t, J = 7.2, 2H), 1.85 (quint, J = 6.8, 2H), 1.45 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 178.1, 155.9, 79.6, 47.9 & 47.5 (rotamers), 34.0, 31.0 & 30.8 (rotamers), 28.2, 22.7. HRMS: calc'd $[M]^+$ ($C_{10}H_{19}NO_4$): 217.1314. Found: (EI) 217.1318.

Synthesis of phenyl ester 6a and general DCC mediated esterification procedure. Compound **4**[53] (0.409 g, 2.01 mmol) was dissolved in CH_2Cl_2 (20 mL). Phenol (0.228 g, 2.45 mmol, 1.22 eq), DCC (0.623 g, 3.02 mmol, 1.5 eq) and DMAP (0.0236 g, 0.193 mmol, 0.09 eq) were added, and the solution was stirred for 1 h. The precipitate was filtered off and rinsed with CH_2Cl_2. The filtrate was poured into 1 M NaOH and the product was extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and the solvent was removed *in vacuo*. The crude material was purified by column chromatography (85 : 15 cyclohexane : EtOAc), affording **6a** (0.536 g, 95%) as a white solid. ν_{max}/cm^{-1} 3080, 3068, 3010, 2981, 2936, 2866, 1762, 1697, 1596, 1523, 1494, 1483, 1367. ^{1}H NMR ($CDCl_3$): δ 7.42–7.35 (m, 2H), 7.27–7.22 (m, 2H), 7.12–7.07 (m, 1H), 4.67 (br s, 1H), 3.27 (quartet, J = 6.6 Hz, 2H), 2.62 (t, J = 7.4, 2H), 1.95 (quint, J = 7.0 Hz, 2H), 1.46 (s, 9H).^{13}C NMR ($CDCl_3$): δ 171.7, 155.9, 150.5, 129.2, 125.7, 121.4, 79.1, 39.7, 31.5, 28.3, 25.2. HRMS: calc'd $[M+H]^+$ ($C_{15}H_{22}NO_4$): 280.1543. Found: (EI) 280.1549.

Synthesis of *tert*-butyl ester 7a. Compound **4**[53] (1.00 g, 4.92 mmol) was dissolved in CH_2Cl_2 (15 mL). *t*-Butyl-2,2,2-trichloroacetimidate (1.76 mL, 9.83 mmol, 2.0 eq) and $BF_3{\cdot}Et_2O$ (0.100 mL, 0.707 mmol, 0.14 eq) were added and the reaction mixture was stirred for 45 min. The solution was then filtered to remove the precipitate and the precipitate was rinsed with CH_2Cl_2. The filtrate was poured into a saturated solution of Na_2CO_3, and the product was extracted into CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and filtered, and the solvent was removed *in vacuo*. The material was taken up in cyclohexane and filtered a second time. The solvent was removed *in vacuo*, yielding **7a** (1.22 g, 96%) as a clear, colourless oil. ν_{max}/cm^{-1} 2980, 2937, 1718, 1716, 1525, 1456, 1393, 1368. ^1H NMR ($CDCl_3$): δ 4.73 (br s, 1H), 3.10 (quart, J-6.3 Hz, 2H), 2.22 (t, $J = 7.4$ Hz, 2H), 1.73 (quint, $J = 7.0$ Hz, 2H), 1.40 (s, 9H), 1.39 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 172.6, 155.7, 80.3, 79.0, 39.9, 32.8, 28.3, 28.0, 25.2. HRMS: calc'd $[M]^+$ ($C_{13}H_{25}NO_4$): 259.1784. Found: (EI) 259.1779.

Synthesis of *N*-methyl *tert*-butyl ester 7b. To a flask containing **7a** (1.11 g, 4.28 mmol) were added DMF (20 mL) and MeI (0.29 mL, 4.66 mmol), and the solution was cooled to 0 °C. NaH (0.103 g, 4.31 mmol) was suspended in DMF (2 mL) and added dropwise to the reaction mixture. The solution was stirred for 1 h, then a second equivalent each of MeI and NaH were added as above, and the solution was stirred overnight. The solution was poured into a 1 : 1 mixture of H_2O : saturated brine, and the product was extracted into CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and the solvent was removed *in vacuo*. Purification by column chromatography (19 : 1 cyclohexane : EtOAc → 9 : 1 cyclohexane : EtOAc) yielded **7b** (0.990 g, 84%) as a thin, colourless oil. ν_{max}/cm^{-1} 2979, 2937, 1731, 1700, 1482, 1458, 1395, 1367.^1H NMR ($CDCl_3$): δ 3.23 (t, $J = 7.4$ Hz, 2H), 2.85 (s, 3H), 2.22 (t, $J = 7.6$ Hz, 2H), 1.79 (quint, $J = 7.6$ Hz, 2H), 1.46 (s, 9H), 1.45 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 172.4, 155.6, 80.1, 79.1, 48.1 & 47.6 (rotamers), 34.0, 32.5, 28.3, 28.0, 23.3 & 22.9 (rotamers). HRMS: calc'd $[M]^+$ ($C_{14}H_{27}NO_4$): 273.1940. Found: (EI) 273.1947.

Synthesis of α-methyl *tert*-butyl ester 8a. Compound **7b** (0.188 g, 0.687 mmol) was dissolved in THF (2 mL), and then LiCl (0.044 g, 0.104 mmol) was added and the solution was cooled to −78 °C. MeI (0.43 mL, 6.91 mmol) was added, followed by dropwise addition of LHMDS (1.0 M solution in THF, 2.00 mL, 2.00 mmol). The resulting solution was stirred for 30 min at −78 °C, then warmed to RT and stirred an additional 90 min. The reaction mixture was then poured into 1 M HCl and the product was extracted into EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and the solvent was removed *in vacuo*. The crude material was purified by column chromatography (19 : 1 cyclohexane : EtOAc) to afford **8a** (0.174 g, 88%) as a pale yellow oil. ν_{max}/cm^{-1} 2979, 2937, 1729, 1700, 1482, 1460, 1395, 1367.^1H NMR ($CDCl_3$): δ 3.37–3.11 (m, 2H), 2.84 (s, 3H), 2.31 (sextet, $J = 7.0$ Hz, 1H), 1.88 (sextet, $J = 7.0$ Hz, 1H), 1.63–1.51 (m, 1H), 1.46 (s, 9H), 1.45 (s, 9H), 1.15 (d, $J = 7.0$ Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 175.5, 155.6, 80.0, 79.2, 46.9 & 46.5 (rotamers), 37.9, 34.1, 31.7 & 31.2 (rotamers), 28.4, 28.0, 17.1. HRMS: calc'd $[M]^+$ ($C_{15}H_{29}NO_4$): 287.2097. Found: (EI) 287.2085.

Synthesis of α-allyl *tert*-butyl ester 8b and general monoalkylation procedure. Compound **7b** (0.204 g, 0.747 mmol) was dissolved in THF (3 mL), and then LiCl (0.048 g, 1.128 mmol) was added and the solution was cooled to −78 °C. LHMDS (1.0 M solution in THF, 0.93 mL, 0.93 mmol) was added slowly, and the solution was stirred 30 min. Allyl bromide (0.068 mL, 0.786 mmol) was then added dropwise, and the solution was stirred for 30 min at −78 °C, then warmed to RT and stirred for an additional 90 min. The reaction mixture was then poured into 1 M HCl, and the product was extracted into EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and the solvent was removed *in vacuo*. Purification by column chromatography (99 : 1 cyclohexane : EtOAc → 19 : 1 cyclohexane : EtOAc) to provide **8b** (0.162 g, 69%) as a thick, colorless oil. ν_{max}/cm^{-1} 2978, 2935, 1729, 1700, 1482, 1458, 1394, 1367. ^1H NMR ($CDCl_3$): δ 5.81–5.68 (m, 1H), 5.15–4.98 (m, 2H), 3.39–3.06 (m, 2H), 2.84 (s, 3H), 2.40–2.17 (m, 3H), 1.88–1.76 (m, 1H), 1.69–1.57 (m, 1H), 1.46 (s, 9H), 1.45 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 174.1, 155.5, 135.2, 116.7, 80.3, 79.2, 47.0 & 46.4 (rotamers), 43.3, 36.4, 34.1, 29.6 & 29.3 (rotamers), 28.3, 28.0. HRMS: calc'd $[M]^+$ ($C_{17}H_{31}NO_4$): 313.2253. Found: (EI) 313.2259.

Synthesis of acid 9a and general deprotection-*N*-Boc reprotection procedure. Under an air atmosphere, a flask was charged with **8a** (0.108 g, 0.376 mmol), and then 1 : 1 TFA:CH_2Cl_2 (2 mL) was added and the solution was stirred for 2 h. The solvent was then removed *in vacuo*, and CH_2Cl_2 was added and removed 3 times to help further remove residual TFA. After thorough drying, the material was taken up in 1 : 1 dioxane : 0.5 M NaOH solution (2 mL), and the pH was adjusted to approximately 12 using 1 M NaOH. Di-*t*-butyldicarbonate (0.470 mmol, 1.25 eq) was added, and the solution was stirred overnight. The material was poured onto 1 : 1 1 M HCl : saturated brine, and the product was extracted into EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and the solvent was removed *in vacuo*. The crude material was purified by column chromatography (85 : 15 cyclohexane : EtOAc → 70 : 30 cyclohexane : EtOAc) to provide **9a** (79.5 mg, 91%) as a clear, colorless oil. ν_{max}/cm^{-1} 3472, 3237, 2979, 2941, 1701, 1687, 1488, 1467, 1403, 1368. ^1H NMR ($CDCl_3$): δ 10.24 (br s, 1H), 3.52–3.11 (m, 2H), 2.85 (s, 3H), 2.46 (sextet, $J = 7.0$ Hz, 1H), 2.02–1.82 (m, 1H), 1.71–1.55 (m, 1H), 1.46 (s, 9H), 1.23 (d, $J = 7.4$ Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 181.3, 156.0, 79.9, 46.7, 36.7, 34.0, 31.2, 28.3, 17.0. 3472, 3237, 2979, 2941, 1701, 1687, 1488, 1467, 1403, 1368. HRMS: calc'd $[M]^+$ ($C_{11}H_{21}NO_4$): 231.1471. Found: (EI) 231.1464.

Synthesis of phenyl ester 10a and general pivaloyl chloride mediated esterification procedure. The acid **9a** (0.181 g, 0.783 mmol) was dissolved in CH_2Cl_2 (8 mL). Freshly distilled NEt_3 (0.27 mL, 1.94 mmol) and pivaloyl chloride (0.12 mL, 0.975 mmol) were added and the solution was stirred for 30 min. Phenol (0.115 g, 1.22 mmol) and DMAP (0.012 g, 98.2 μmol) were added and the solution was stirred overnight. The solution was poured into 1 M HCl and the product was extracted into CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and the solvent was removed. The crude material was purified by column chromatography (9 : 1 cyclohexane : EtOAc) to provide **10a** (0.187 g, 78%) as a colorless oil. ν_{max}/cm^{-1} 3040, 2979, 2940, 1758, 1695, 1495, 1481, 1398, 1366. ^1H NMR ($CDCl_3$): δ 7.39 (dd, J = 7.8 & 8.2 Hz, 2H), 7.23 (dd, J = 7.0 & 7.4 Hz, 1H), 7.09 (d, $J = 7.8$ Hz, 2H), 3.37–3.23 (m, 2H), 2.88 (s, 3H), 2.70 (sextet, $J = 7.0$ Hz, 1H), 2.16–2.03 (m, 1H), 1.80–1.67 (m, 1H), 1.47 (s, 9H),

1.36 (d, J = 7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 174.9, 155.6, 150.7, 129.3, 125.6, 121.4, 79.3, 46.7 & 46.3 (rotamers), 37.0, 31.6 & 31.0 (rotamers), 28.3, 17.1. HRMS: calc'd $[M+H]^+$ ($C_{17}H_{26}NO_4$): 308.1856. Found: (EI) 308.1860.

Synthesis of α,α-dimethyl *tert*-butyl ester 11a. A flask was charged with freshly distilled $NH(iPr)_2$ (0.25 mL, 1.77 mmol) and THF (1 mL), and the solution was cooled to −78 °C. BuLi (2.5 M solution in hexane, 0.69 mL, 1.73 mmol) was added and the solution was stirred for 15 min. This solution was then transferred *via* canula to a flask containing **8a** (0.165 g, 0.574 mmol) and LiCl (0.041 g, 0.967 mmol) in THF (2 mL) and the solution was stirred for 15 min. MeI (0.22 mL, 3.53 mmol) was added dropwise and the solution was stirred overnight. The reaction mixture was then poured onto 1 M HCl, and the product was extracted into EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and the solvent was removed *in vacuo*. The product was further purified by column chromatography (19 : 1 cyclohexane : EtOAc) to afford **11a** (0.165 g, 95%) as a pale yellow oil. ν_{max}/cm^{-1} 2979, 2953, 1700, 1654, 1480, 1458, 1394, 1367. ^{1}H NMR ($CDCl_3$): δ 3.25–3.12 (m, 2H), 2.84 (s, 3H), 1.75–1.66 (m, 2H), 1.46 (s, 9H), 1.45 (s, 9H), 1.60 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 176.6, 155.5, 79.9, 79.1, 45.4, 41.2, 37.8, 33.9, 28.4, 27.9, 25.1. HRMS: calc'd $[M]^+$ ($C_{16}H_{31}NO_4$): 301.2253. Found: (EI) 301.2246.

Synthesis of α,α-diallyl *tert*-butyl ester 11b and general di-alkylation procedure. Compound **7b** (0.232 g, 0.849 mmol) was dissolved in THF (4 mL) and the solution was cooled to −78 °C. LHMDS (1.0 M solution in THF, 0.85 mL, 0.85 mmol) was added slowly and the solution was stirred for 15 min. Allyl bromide (0.074 mL, 0.855 mmol) was added dropwise, and the solution was stirred for 1 h. A second addition of LHMDS (1.70 mL, 1.70 mmol) and allyl bromide (0.13 mL, 1.73 mmol) was performed and the solution was stirred overnight. The solution was poured into 1 M HCl and the product was extracted into EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and the solvent was removed *in vacuo*. Further purification by column chromatography (97 : 3 cyclohexane : EtOAc) afforded **11b** (0.280 g, 93%) as a clear, colorless oil. ν_{max}/cm^{-1} 3081, 2979, 2936, 1723, 1700, 1483, 1458, 1393, 1367. ^{1}H NMR ($CDCl_3$): δ 5.83–5.66 (m, 2H), 5.15–5.05 (m, 4H), 3.27–3.10 (m, 2H), 2.82 (s, 3H), 2.30 (d, J = 7.4 Hz, 4H), 1.76–1.68 (m, 2H), 1.46 (s, 18H). ^{13}C NMR ($CDCl_3$): δ 174.4, 155.4, 133.4, 118.3, 80.6, 79.3, 47.7, 44.6 & 44.2 (rotamers), 39.0, 34.0, 32.2 & 31.8 (rotamers), 28.4, 28.0. HRMS: calc'd $[M]^+$ ($C_{20}H_{35}NO_4$): 353.2566. Found: (EI) 353.3557.

Synthesis of α-cyclopentyl *tert*-butyl ester 11d. Compound **7b** (0.142 g, 0.519 mmol) was dissolved in THF (25 mL) and the solution was cooled to −78 °C. LHMDS (1.0 M soln in THF, 0.84 mL, 0.84 mmol) was added and the solution was stirred for 15 min. 1,4-Dibromobutane (0.070 mL, 0.586 mmol) was added dropwise and the solution was stirred 30 min. A second equivalent of LHMDS was added and the solution was stirred overnight. The reaction mixture was poured into 1 M HCl and the product was extracted 3 times with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and the solvent was removed *in vacuo*. Purification by column chromatography (98 : 2 cyclohexane : EtOAc → 97 : 3 cyclohexane : EtOAc) yielded **11d** (0.078 g, 46%) as a clear, colorless oil. ν_{max}/cm^{-1} 2976, 2953, 2875, 1724, 1701, 1482, 1456, 1393, 1367. ^{1}H NMR ($CDCl_3$): δ 3.22–3.08 (m, 2H), 2.83 (s, 3H), 2.14–2.02 (m, 2H), 1.86–1.74 (m, 2H), 1.69–1.58 (m, 4H), 1.53–1.39 (m, 2H), 1.46 (s, 9H), 1.45 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 176.3, 155.4, 79.8, 79.0, 52.7, 46.2 & 45.9 (rotamers), 35.6, 36.1, 34.0, 28.4, 27.9, 24.8. HRMS: calc'd $[M+H]^+$ ($C_{18}H_{34}NO_4$): 328.2482. Found: (EI) 328.2482.

Synthesis of phenyl ester 13a and general Ghosez reagent mediated esterification procedure. Compound **12a** (0.153 g, 0.624 mmol) was dissolved in CH_2Cl_2 (6 mL). 1-Chloro-*N*,*N*,2-trimethylpropenylamine (0.12 mL, 0.907 mmol) was added and the solution was stirred for 1 h. Phenol (0.118 g, 1.25 mmol) and distilled NEt_3 (0.18 mL, 1.29 mmol) were added and the solution was stirred overnight. The reaction mixture was poured into 1 M HCl and the product was extracted into CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and the solvent was removed. Purification by column chromatography (97 : 3 cyclohexane : EtOAc) yielded **13a** (0.181 g, 90%) as a colorless oil. ν_{max}/cm^{-1} 3107, 3078, 3073, 2978, 2934, 2898, 1752, 1699, 1594, 1494, 1485, 1471, 1428, 1394, 1368. ^{1}H NMR ($CDCl_3$): δ 7.39 (dd, J = 7.8 & 8.2 Hz, 2H), 7.25 (dd, J = 8.2 & 7.4 Hz, 1H), 7.07 (d, J = 7.8 Hz, 2H), 3.37–3.29 (m, 2H), 2.88 (s, 3H), 1.97–1.87 (m, 2H), 1.47 (s, 9H), 1.38 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 175.7, 155.4, 150.8, 129.3, 125.6, 121.4, 79.3, 45.3 & 45.0 (rotamers), 41.1, 37.8 & 37.0 (rotamers), 34.1, 28.4, 25.1. HRMS: calc'd $[M+H]^+$ ($C_{18}H_{28}NO_4$): 322.2013. Found: (EI) 322.2013.

Synthesis of methyl ester 15. Compound **14** (3.31 g, 15.1 mmol) was dissolved in DMF (150 mL). Cs_2CO_3 (6.17 g, 18.9 mmol) was added, and then MeI (0.99 mL, 15.9 mmol) was added slowly and the solution was stirred for 90 min. The reaction mixture was poured into 1 : 1 H_2O : saturated brine, and the product was extracted into CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and the solvent was removed *in vacuo*. Column chromatography (7 : 3 cyclohexane : EtOAc → 1 : 1 cyclohexane : EtOAc) yielded **15** (3.05 g, 87%) as a yellow oil. ν_{max}/cm^{-1} 3390, 2980, 2952, 1754, 1697, 1527, 1455, 1394, 1368. ^{1}H NMR ($CDCl_3$): δ 4.89 (br s, 1H), 4.26 (dd, J = 3.9 & 8.1 Hz, 1H), 3.79 (s, 3H), 3.43–3.20 (m, 2H), 2.07–1.96 (m, 1H), 1.89–1.77 (m, 1H), 1.44 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 174.9, 156.2, 79.1, 68.4, 52.1, 36.5, 33.7, 28.1. HRMS: calc'd $[M+H]^+$ ($C_{10}H_{20}O_5$): 234.1336. Found (EI) 234.1335.

Synthesis of *O*-Boc methyl ester 16a. Compound **15** (0.491 g, 2.11 mmol) was dissolved in THF (20 mL). Di-*t*-butyldicarbonate (0.577 g, 2.64 mmol) and DMAP (0.030 g, 0.25 mmol) were added and the solution was stirred for 1 h. The solution was then poured into 1 M HCl and the product was extracted into CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and the solvent was removed *in vacuo*. Purification by column chromatography (9 : 1 cyclohexane : EtOAc) afforded **16a** (0.635 g, 91%) as a thick, colorless oil. ν_{max}/cm^{-1} 3408, 2982, 2958, 1749, 1718, 1521, 1458, 1369. ^{1}H NMR ($CDCl_3$): δ 4.90 (br s, 1H), 4.80 (dd, J = 5.0 & 7.0 Hz, 1H), 3.64 (s, 3H), 3.23–3.04 (m, 2H), 2.02–1.83 (m, 2H), 1.37 (s, 9H), 1.31 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 170.3, 155.4, 152.5, 82.7, 78.9, 72.0, 52.0, 36.2, 31.1, 28.1, 27.3. HRMS: calc'd $[M+H]^+$ ($C_{15}H_{28}NO_7$): 334.1866. Found: (EI) 334.1874.

Synthesis of *N*-methyl *O*-Boc methyl ester 16b. Compound **16a** (0.133 g, 0.399 mmol) was dissolved in DMF (2 mL), and the solution was cooled to 0 °C. MeI (0.25 mL, 4.01 mmol) was added,

and NaH (0.010 g, 0.429 mmol) suspended in DMF (0.5 mL) was added dropwise to the reaction mixture. The solution was stirred for 1 h and then a second equivalent of NaH was added as above and the solution was stirred a second hour. The reaction mixture was then poured onto 1 : 1 1 M HCl : saturated brine, and the product was extracted into CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and the solvent was removed *in vacuo*. Further purification by column chromatography (96 : 4 cyclohexane : EtOAc) yielded **16b** (0.103 g, 74%) as a colourless oil. ν_{max}/cm^{-1} 2981, 2937, 1748, 1700, 1483, 1460, 1396, 1369. ^1H NMR ($CDCl_3$): δ 4.81 (dd, J = 4.3 & 8.6 Hz, 1H), 3.71 (s, 3H), 3.56–3.31 (m, 1H), 3.26–3.15 (m, 1H), 2.80 (s, 3H), 2.14–1.89 (m, 2H), 1.44 (s, 9H), 1.40 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 170.4, 155.4, 152.7, 82.9, 79.5, 72.0, 52.2, 44.9, 34.5 & 34.2 (rotamers), 29.5 & 29.1 (rotamers), 28.2, 27.5. HRMS: calc'd $[M+H]^+$ ($C_{16}H_{30}NO_7$): 348.2017. Found: (EI) 348.2015.

Synthesis of phenyl ester 17a and general procedure for conversion from a methyl to phenyl ester. Under an air atmosphere, a flask was charged with **16a** (0.168 g, 0.503 mmol) and the material was dissolved in 1 : 1 THF–H_2O (5 mL). $LiOH{\cdot}H_2O$ (0.0264 g, 1.25 eq) was added, and the solution was stirred overnight. The reaction mixture was then poured into 1 : 1 1 M HCl : saturated brine and the product was extracted into CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and the solvent was removed *in vacuo*. The flask was then fully evacuated, refilled with N_2, and the material was dissolved in CH_2Cl_2 (5 mL). Pivaloyl chloride (0.077 mL, 1.24 eq) and NEt_3 (0.18 mL, 2.6 eq) were added and the solution was stirred for 30 min. Phenol (0.0724 g, 1.53 eq) and DMAP (0.0073 g, 0.12 eq) were then added and the solution was stirred overnight. The reaction mixture was poured into 1 M HCl and the product was extracted into CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and the solvent was removed *in vacuo*. The crude material was purified by column chromatography (9 : 1 cyclohexane : EtOAc) to yield **17a** (0.140 g, 70%) as a colorless oil. ν_{max}/cm^{-1} 3460, 2983, 2962, 1747, 1695, 1653, 1521, 1495, 1369. ^1H NMR ($CDCl_3$): δ 7.37 (t, J = 7.4 Hz, 2H), 7.23 (t, J = 7.4 Hz, 1H), 7.11 (d, J = 7.8 Hz, 2H), 5.11 (dd, J = 5.1 & 7.4 Hz, 1H), 4.84 (br s, 1H), 3.45–3.27 (m, 2H), 2.31–2.13 (m, 2H), 1.51 (s, 9H), 1.44 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 168.8, 155.7, 152.8, 150.1, 129.4, 126.1, 121.2, 83.3, 79.4, 72.3, 36.5, 31.3, 28.3, 27.6. HRMS: calc'd $[M+H]^+$ ($C_{20}H_{30}NO_7$): 396.2017. Found: (EI) 396.2016.

Kinetic studies. Absorption spectra for phenol and [**3a**]·TFA were obtained by preparing a 1 mg mL^{-1} solution of each in 1 M HCl and measuring the absorbance between 320 and 230 nm. To measure the cyclization rate, the Boc protected compound was dissolved in 1 : 1 TFA : CH_2Cl_2 (approximately 4 mL) and the resulting solution was stirred for 2 h. The solvent was removed *in vacuo*, and CH_2Cl_2 was added and removed 3 times to remove residual TFA, after which the flask was fully evacuated. The material was then suspended in H_2O, frozen in liquid nitrogen, and dried on a lyophilizer. 1.5 mg of the target **3a–k** was then dissolved in 0.2 mL of iPrOH and the solution was preheated to 37 °C. This solution was then added with stirring to 1.8 mL of buffer solution already in the cuvette in the spectrometer at 37 °C. The change in absorbance at 276 nm with respect to time was measured. The absorbance at t = 0 was taken as 0% conversion, while the absorbance value after the absorbance had stabilized was taken as 100% conversion (verified by NMR). From this, the % conversion with respect to time was calculated. To obtain the first order rate constants, $\ln[A]_0/[A]$ *versus* time was plotted where $[A]_0/[A]$ effectively corresponds to 100/(100% conversion). Phosphate buffers (0.1 M) were used for pHs 6.0, 7.0, and 7.4. Acetate buffers (0.1 M) were used for pHs 4.0 and 5.0.

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