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Base-Catalyzed Phenol-Mannich Condensation of Preformed Cesium Iminodiacetate. The Direct Synthesis of Calcein Blue AM and Related Acyloxymethyl Esters

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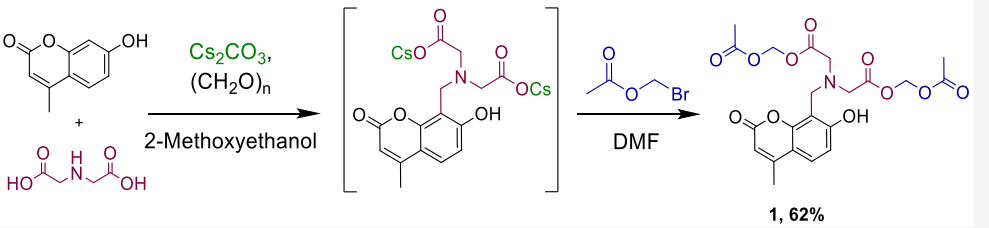

ABSTRACT: A rapid and highly practical one-flask procedure for the *positionally selective* preparation of (acyloxy)methyl *N*-(2-hydroxybenzyl)iminodiacetate and related diesters from iminodiacetic acid and phenols is described. The key to this multicomponent phenol-Mannich condensation resides in the use of cesium iminodiacetate as the reaction partner. This protocol has been applied in an unusually direct synthesis of the intracellular fluorescent dye Calcein blue AM, for which scant experimental and spectroscopic data are presently available.

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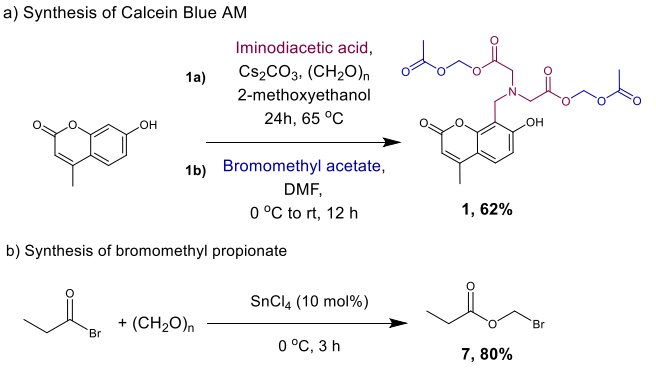
INTRODUCTION

Acyloxymethyl (AoM) esters serve as delivery and ultimately cellular entrapment vehicles for numerous intracellular fluorescent stains,[1a−e] and are also utilized in the synthesis of bioreversible prodrug conjugates. Labile (acetoxy)methyl (AM) dyes passively cross the cell membranes of viable cells. They are converted into the corresponding iminodiacetates by esterase cleavage, which are retained within the cell as their Ca^{2+} chelates, without compromising the cell membrane.[2] Acyloxymethyl esters are typically prepared by the alkylation of precursor iminodiacetic acids [as their Ag(I) or $(i\text{-Pr})_2EtNH^+$ salts] with the appropriate halomethyl ester.[1e,f] These alkylations often proceed in poor, to at best modest, isolated yields. We, therefore, sought an improved experimental protocol for anionic functionalization. Cesium carboxylates and *N*-sulfonamides have long been known to be excellent nucleophiles in S_N2 displacements,[3] and we have found the former can serve in significantly improved routes to AoM esters.[4] In this contribution, we report that dicesium iminodiacetate, generated in situ from iminodiacetic acid and inexpensive Cs_2CO_3 or 50% aqueous CsOH, undergoes efficient phenol-Mannich condensations with representative phenols, and that the resulting Cs salts can be directly alkylated with bromomethyl esters (or alternative halides) in DMF to furnish the corresponding diesters in respectable overall yields. A direct and highly efficient synthesis of the intracellular fluorescent dye Calcein blue AM, which relies on this method, is described (Scheme 1a). Significantly, although Calcein blue AM is commercially available, its price is exorbitant, ranging from $115 to $173/mg. Alternatively, Calcein blue[7c] could be converted to **1** in comparable yield using Cs_2CO_3/2-methoxyethanol followed by alkylation in DMF. In addition, we describe a markedly improved procedure for the preparation of bromomethyl esters, which involves the use of

Scheme 1. Efficient Synthesis of Calcein Blue AM and Bromomethyl Propionate



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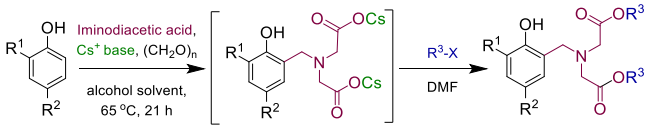
$SnCl_4$ as an efficient Lewis acid catalyst in place of $ZnCl_2$ (Scheme 1b).[5]

In a typical experimental procedure, iminodiacetic acid (1.2 equiv) was preliminarily converted to its cesium salt by pretreatment with Cs_2CO_3 (1.3 equiv) in 2-methoxyethanol at 80 °C, followed by the addition of the requisite phenol (1.0 equiv) and paraformaldehyde (1.1 equiv). The subsequent phenol-Mannich condensation was then conducted at 65 °C, followed by the removal of the solvent and water. Alkylation of the intermediate iminodiacetate dicesium salt was ultimately achieved in anhydrous DMF with the appropriate electrophile (2.5 equiv) to provide the desired *N*-(2-hydroxybenzyl)-iminodiacetate diester after standard column chromatography on silica gel.

As experimental notes, ethanol and methanol can serve as alternatives to 2-methoxyethanol as the initial solvent, but 2-propanol was found to be inferior. Temperatures of 65−80 °C were determined optimum for the initial dicesium salt formation as well as the subsequent phenol-Mannich condensation. In addition, the presence of excess water in the cases involving cesium hydroxide was found to slow the condensation reaction, resulting in decreased yields. A series of phenols were subjected to analogous base-catalyzed Mannich-type conditions using Cs_2CO_3 or commercial 50% aq. CsOH as bases, followed by alkylation of the resulting dicesium (arylmethyl)iminodiacetate salts in DMF. A compilation of these results is presented in Table 1.

Table 1. Scope of Sequential Phenol-Mannich Condensation/Alkylation

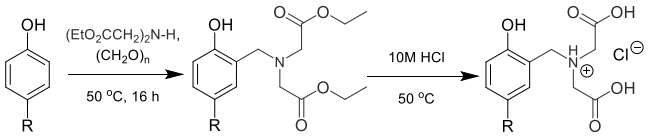


Entry	R^1	R^2	R^3−X	Yield (%)[a,b]
2a	-OMe	-Allyl	$MeCO_2CH_2Br$	69[a]
2b	-OMe	-Allyl	$EtCO_2CH_2Br$ (7)	50,[a] 64[b]
2c	-OMe	-Allyl	MEMCl	37,[a] 61[b]
3a	-OMe	$-CH_2NHCbz$	$MeCO_2CH_2Br$	34,[a] 78[b]
3b	-OMe	$-CH_2NHCbz$	$EtCO_2CH_2Br$ (7)	84[a]
4a	-H	-Br	$MeCO_2CH_2Br$	50,[a] 62[b]
4b	-H	-Br	MEMCl	49[a]
5a	-H	$-CH_2CH_2N_3$	$MeCO_2CH_2Br$	52[b]
6a	-Allyl	-H	$MeCO_2CH_2Br$	36,[a] 51[b]
6b	-Allyl	-H	$EtCO_2CH_2Br$ (7)	31,[a] 54[b]

[a]CsOH (6.2 M, 2.1 mmol) in EtOH (2.0 mL) was employed. [b]Cs_2CO_3 (1.3 mmol) in 2-methoxyethanol (2.0 mL) was employed.

In sharp contrast to *sodium* iminodiacetate, which gives inseparable mixtures of 2,6-di- and 2-monosubstituted products from 4-substituted phenols,[7a−c] the corresponding cesium salt provided *only* products derived from 2-substitution under the forgoing conditions. Moreover, for 2-allylphenol, substitution occurred only at the 6-position to the exclusion of the 4-substituted product. We have independently confirmed that limited positional selectivity is observed when Na^+ is used as the counterion.[7d] Previously, the use of diethyl iminodiacetate, although *under aprotic conditions* (PhMe, 110 °C), with 4-substituted phenols has also been reported to result in selective monosubstitution.[7a] It is also worthy of note that the resultant diethyl esters were subsequently converted to the desired iminodiacetic acids (as the HCl salts) using 10 M aqueous HCl under stringent conditions (Scheme 2).

Scheme 2. Previous Selective Synthetic Route to *N*-2-Hydroxybenzyl)iminodiacetic Acids[7a]



In summary, we have developed an efficient method for the direct synthesis of (acyloxy)methyl *N*-(2-hydroxybenzyl)-iminodiacetate and related diesters from simple phenols and iminodiacetic acid. Cesium carbonate serves as the essential base and Cs^+ cation source for this Mannich-type condensation, involving paraformaldehyde as the electrophilic coupling agent. This procedure obviates the earlier three-step preparative sequence using diethyl iminodiacetate.

EXPERIMENTAL SECTION

General Information. All chemical reagents were purchased from commercial sources and used as received without further purification unless noted otherwise. Solvents were certified ACS grade. All reactions were carried out under nitrogen using oven-dried glassware. Thin-layer chromatography (TLC) was performed on Silicycle glass-backed TLC plates, silica gel F-254 over a glass support, 250 μm thickness. Visualization was accomplished with UV light (254 nm) and/or potassium permanganate. All ^{1}H NMR and ^{13}C{^{1}H} NMR spectra were recorded on a Bruker 400 MHz Ascend AVANCE NEO NMR Spectrometer. Chemical shifts (δ) were reported in reference to solvent peaks (residue $CHCl_3$ at δ 7.28 ppm for ^{1}H and $CDCl_3$ at δ 77.0 ppm for ^{13}C{^{1}H}). LC-MS experiments were performed on an Agilent 1290 Infinity UPLC coupled to an Agilent 6538 Q-TOF mass spectrometer using a 2.1 mm × 100 mm Agilent Zorbax Eclipse Plus C18 column. Mobile phase A was 0.1% formic acid in water, and mobile phase B was 0.1% formic acid in acetonitrile. The gradient was as follows: initially 2% B, followed by a ramp to 98% B over 8 min, a hold at 98% B for 1.25 min before returning to 2% B, with a total run time of 10 min. The MS was operated in positive-ion electrospray mode with an m/z range of 50−1700 at 2 spectra/s.

General Procedure Using Cs_2CO_3. A 25 mL round-bottomed flask equipped with a magnetic stirring bar and yellow poly cap was charged with iminodiacetic acid (1.2 mmol, 1.2 equiv), cesium carbonate (1.3 mmol, 1.3 equiv), and anhydrous 2-methoxyethanol (2.0 mL) under nitrogen. The resulting mixture was heated to 80 °C in an oil bath for 3 h and then cooled to room temperature. The phenol of interest (1.0 mmol, 1.0 equiv) was added with stirring, followed by paraformaldehyde (1.1 mmol, 1.1 equiv). The resulting mixture was then heated to 65 °C in an oil bath for 21 h. The solvent was then removed on a rotary evaporator in vacuo, and final drying of the resulting solid in high vacuum at 60 °C in an oil bath for 12 h provided the corresponding iminodiacetic acid dicesium salt. The original 25 mL round-bottomed flask equipped with a magnetic stirring bar was charged with anhydrous DMF (3.0 mL) under nitrogen. The stirred reactant mixture was cooled to 0 °C in an ice bath, and the corresponding halide (2.5 mmol, 2.5 equiv) was added dropwise. The reactant mixture was stirred for 30 min at 0 °C and then stirred for an additional 12 h at room temperature. The DMF was removed on a rotary evaporator under a high vacuum. The resulting mixture was dissolved in water (3 mL) and extracted with EtOAc (3 × 5 mL). The organic layers were combined and washed with brine (1 × 5 mL). The combined organic layer was dried over Na_2SO_4 and filtered. The filtrate was evaporated, and the residue was purified by silica gel flash chromatography.

General Procedure Using CsOH. A 25 mL round-bottom flask equipped with a magnetic stirring bar and yellow poly cap was

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charged with iminodiacetic acid (1.0 mmol, 1.0 equiv) and anhydrous ethanol (2.0 mL) under nitrogen. Aqueous cesium hydroxide (6.2 M, 2.1 mmol, 2.1 equiv) was added dropwise with vigorous stirring. The phenol of interest (1 mmol, 1.0 equiv) was then added with stirring, followed by paraformaldehyde (1.1 mmol, 1.1 equiv). The resulting mixture was then heated at 65 °C in an oil bath for 21 h. Removal of the solvents in vacuo followed by azeotropic drying of the residue using anhydrous ethanol (3 × 5 mL) and final drying of the resulting solid in high vacuum at 60 °C in an oil bath for 12 h provided the corresponding iminodiacetic acid dicesium salt. The original 25 mL round-bottomed flask, equipped with its magnetic stirring bar, was charged with anhydrous DMF (3.0 mL) under nitrogen. The stirred reactant mixture was cooled to 0 °C in an ice bath, and the corresponding halide (2.5 mmol, 2.5 equiv) was added dropwise. The reactant mixture was stirred for 30 min at 0 °C and then stirred for an additional 12 h at room temperature. DMF was removed on a rotary evaporator under high vacuum. The resulting mixture was dissolved in water (3 mL) and extracted with EtOAc (3 × 5 mL). The organic layers were combined and washed with brine (1 × 5 mL). The combined organic layer was dried over Na_2SO_4 and filtered. The filtrate was evaporated, and the residue was purified by silica gel flash chromatography.

Calcien Blue AM (1). A 25 mL round-bottomed flask equipped with a magnetic stirring bar and yellow poly cap was charged with iminodiacetic acid (159 mg, 1.2 mmol, 1.2 equiv), cesium carbonate (424 mg, 1.3 mmol, 1.3 equiv), and anhydrous 2-methoxyethanol (2.0 mL) under nitrogen. The resulting mixture was heated at 80 °C in an oil bath for 3 h and then cooled to room temperature. 7-Hydroxy-4-methyl-2*H*-chromen-2-one (176 mg, 1.0 mmol, 1.0 equiv) was added with stirring, followed by paraformaldehyde (33 mg, 1.1 mmol, 1.1 equiv). The resulting mixture was then heated to 65 °C in an oil bath for 21 h. The solvent was then removed on a rotary evaporator followed by final drying of the resulting solid under high vacuum at 60 °C in an oil bath for 12 h to provide Calcein blue dicesium salt. To the original 25 mL round-bottom flask equipped with its magnetic stirring bar was added anhydrous DMF (3.0 mL) under nitrogen. The stirred mixture was cooled to 0 °C in an ice bath, and bromomethyl acetate[4] (243 μL, 2.5 mmol, 2.5 equiv) was added dropwise. The reactant mixture was stirred for 30 min at 0 °C and then for an additional 12 h at room temperature. The DMF was removed on a rotary evaporator under high vacuum, and the residue was diluted with water (3 mL) and extracted with EtOAc (3 × 5 mL). The organic layers were combined and washed with brine (1 × 5 mL). The combined organic layer was dried over Na_2SO_4 and filtered. The filtrate was evaporated, and the residue was purified by silica gel flash chromatography (hexanes/EtOAc 1:1) to furnish Calcein blue AM (**1**) as a viscous, colorless oil (288 mg, 62%): TLC R_f = 0.30 (SiO_2, hexanes/EtOAc 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.12 (s, 1H), 7.46 (d, J = 8.8 Hz, 1H), 6.87 (d, J = 8.8 Hz, 1H), 6.08 (s, 1H), 5.81 (s, 4H), 4.23 (s, 2H), 3.62 (s, 4H), 2.40 (s, 3H), 2.13 (s, 6H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 169.6, 169.5, 161.2, 160.7, 153.1, 153.0, 125.4, 113.7, 112.6, 111.1, 108.1, 79.6, 54.0, 47.8, 20.7, 18.8; HRMS (ESI/Q-TOF) m/z: [M + H]$^+$ calcd for $C_{21}H_{23}NO_{11}H$ 466.1344; found 466.1346.

Bis(acetoxymethyl) 2,2′-((5-Allyl-2-hydroxy-3-methoxybenzyl)azanediyl)diacetate (2a). Subjection of 4-allyl-2-methoxyphenol (153 μL, 1.0 mmol, 1.0 equiv) to the general CsOH procedure with the addition of bromomethyl acetate (245 μL, 2.5 mmol, 2.5 equiv) as the electrophile afforded 314 mg of the title compound (69%) as a viscous, clear oil after silica gel flash chromatography. (hexanes/EtOAc 2:1): TLC R_f = 0.20 (SiO_2, hexanes/EtOAc 2:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.44 (s, 1H), 6.60 (d, J = 1.7 Hz, 1H), 6.37, (d, J = 1.7 Hz, 1H), 5.86 (ddt, J = 16.9, 10.3, 6.7 Hz, 1H), 5.70 (s, 4H), 5.02−4.96 (m, 2H), 3.90 (s, 2H), 3.80 (s, 3H), 3.54 (s, 4H), 3.21 (d, 2H, J = 6.7 Hz), 2.05 (s, 6H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 169.5, 169.4, 147.9, 144.5, 137.7, 131.0, 121.5, 121.0, 115.6, 112.3, 79.4, 56.0, 55.5, 53.4, 39.7, 20.7. Spectral data are consistent with those previously reported.[4]

((2,2′-((5-Allyl-2-hydroxy-3-methoxybenzyl)azanediyl)bis(acetyl))bis(oxy))bis(methylene) Dipropionate (2b). Subjection of 4-allyl-2-methoxyphenol (153 μL, 1.0 mmol, 1.0 equiv) to the general Cs_2CO_3 procedure with the addition of bromomethyl propionate (243 μL, 2.5 mmol, 2.5 equiv) as the electrophile afforded 314 mg of the title compound (64%) as a viscous, clear oil after silica gel flash chromatography. (hexanes/EtOAc 3:1): TLC R_f = 0.20 (SiO_2, hexanes/EtOAc 2:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1H), 6.69 (d, J = 1.7 Hz, 1H), 6.46 (d, J = 1.7 Hz, 1H), 5.95 (ddt, J = 16.9, 10.3, 6.7, 1H), 5.81 (s, 4H), 5.10−5.05 (m, 2H), 3.98 (s, 2H), 3.89 (s, 4H), 3.63 (s, 4H), 3.30 (d, J = 6.6 Hz, 2H), 2.41 (q, J = 7.6 Hz, 4H), 1.18 (t, J = 7.6 Hz, 6H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 173.0, 169.5, 147.9, 144.6, 137.7, 130.9, 121.5, 121.0, 115.6, 112.3, 79.4, 56.0, 55.5, 53.4, 39.7. 27.3, 8.7; HRMS (ESI/Q-TOF) m/z: [M + H]$^+$ calcd for $C_{23}H_{31}NO_{10}H$ 482.2021; found 482.2022.

Bis((2-methoxyethoxy)methyl)-2,2′-((5-allyl-2-hydroxy-3-methoxybenzyl)azanediyl)diacetate (2c). Subjection of 4-allyl-2-methoxyphenol (153 μL, 1.0 mmol, 1.0 equiv) to the general Cs_2CO_3 procedure with the addition of 2-Methoxyethoxymethyl chloride (283 μL, 2.5 mmol, 2.5 equiv) as the electrophile afforded 296 mg of the title compound (61%) as a viscous, yellow oil after flash chromatography. (EtOAc/hexanes 2:1): TLC R_f = 0.30 (SiO_2, EtOAc/hexanes 2:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.83 (s, 1H), 6.59 (d, J = 1.7 Hz, 1H), 6.38 (d, J = 1.7 Hz, 1H), 5.85 (ddt, J = 16.8, 10.2, 6.7 Hz, 1H), 5.30 (s, 4H), 5.01−4.96 (m, 2H), 3.91 (s, 1H), 3.79 (s, 1H), 3.73−3.71 (m, 4H), 3.53 (s, 4H) 3.48−3.46 (m, 4H), 3.30 (s, 6H), 3.20 (d, J = 6.7 Hz, 2H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 170.3, 148.0, 144.8, 137.8, 130.8, 121.5, 121.3, 115.5, 112.3, 89.8, 71.5, 69.8, 59.1, 56.0. 55.6, 53.6, 39.7. Spectral data are consistent with those previously reported.[4]

Bis(acetoxymethyl) 2,2′-((5-((((Benzyloxy)carbonyl)amino)-methyl)-2-hydroxy-3-methoxybenzyl)azanediyl)diacetate (3a). Subjection of **8** (287 mg, 1 mmol, 1.0 equiv; see Supporting Information) to the general Cs_2CO_3 procedure with the addition of bromomethyl acetate (245 μL, 2.5 mmol, 2.5 equiv) as the electrophile afforded 447 mg of the title compound (78%) as a viscous, clear oil after silica gel flash chromatography. (hexanes/EtOAc 1:1): TLC R_f = 0.50 (SiO_2, hexanes/EtOAc 2:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (s, 1H), 7.39−7.28 (m, 5H), 6.80 (s, 1H), 6.57 (s, 1H), 5.78 (s, 4H), 5.16 (s, 2H), 5.10 (s, 1H), 4.29 (d, J = 5.8 Hz, 2H), 3.98 (s, 2H), 3.86 (s, 3H), 3.62 (s, 4H), 2.13 (s, 6H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 169.6, 169.4, 156.3, 148.2, 145.7, 136.5, 129.4, 128.5, 128.2, 121.2, 120.9, 111.4, 79.5, 66.8, 56.1, 55.32, 53.4, 45.0, 20.7; HRMS (ESI/Q-TOF) m/z: [M + H]$^+$ calcd for $C_{27}H_{32}N_2O_{12}H$ 577.2028; found 577.2034.

((2,2′-((5-((((Benzyloxy)carbonyl)amino)methyl)-2-hydroxy-3-methoxybenzyl)azanediyl)bis(acetyl))bis(oxy))bis-(methylene) Dipropionate (3b). Subjection of **8** (287 mg, 1.0 mmol, 1.0 equiv) to the general CsOH procedure with the addition of bromomethyl propionate (243 μL, 2.5 mmol, 2.5 equiv) as the electrophile afforded 334 mg of the title compound (84%) as a viscous, clear oil after silica gel flash chromatography. (hexanes/EtOAc 1:1): TLC R_f = 0.30 (SiO_2, Hexanes/EtOAc 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H), 7.39−7.33 (m, 5H), 6.80 (s, 1H), 6.57 (s, 1H), 5.81 (s, 4H), 5.16 (s, 2H), 5.08 (s, 1H), 4.29 (d, J = 5.8 Hz, 2H), 3.98 (s, 2H), 3.87 (s, 3H), 3.62 (s, 4H), 2.41 (q, J = 7.5 Hz, 4H), 1.17 (t, J = 7.5 Hz, 6H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 173.1, 169.4, 156.4, 148.2, 145.8, 136.5, 129.4, 128.5, 128.2, 121.2, 120.8, 111.4, 96.1, 79.5, 66.8, 56.1, 55.3, 53.4, 45.0, 27.3, 8.7; HRMS (ESI/Q-TOF) m/z: [M + H]$^+$ calcd for $C_{29}H_{36}N_2O_{12}H$ 605.2341; found 605.2340.

Bis(acetoxymethyl) 2,2′-((5-Bromo-2-hydroxybenzyl)-azanediyl)diacetate (4a). Subjection of 4-bromophenol (173 mg, 1.0 mmol, 1.0 equiv) to the general Cs_2CO_3 procedure with the addition of bromomethyl acetate (245 μL, 2.5 mmol, 2.5 equiv) as the electrophile afforded 288 mg of the title compound (62%) as a viscous, clear oil after silica gel flash chromatography. (hexanes/EtOAc 3:1): TLC R_f = 0.30 (SiO_2, hexanes/EtOAc 3:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.06 (s, 1H), 7.32 (dd, J = 2.5, 8.6 Hz, 1H), 7.1 (d, J = 2.5 Hz, 1H), 6.80 (d, J = 8.6 Hz, 1H), 5.81 (s, 4H), 3.97 (s, 2H), 3.61 (s, 4H), 2.16 (s, 6H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$,) δ 169.5, 156.5, 132.5, 132.1, 122.8, 118.6, 111.1, 79.4, 55.5, 53.4, 20.7;

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HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ calcd for $C_{17}H_{20}BrNO_9H$ 462.0394; found 462.0397.

Bis((2-methoxyethoxy)methyl) 2,2′-((5-Bromo-2-hydroxybenzyl)azanediyl)diacetate (4b). Subjection of 4-bromophenol (173 mg, 1.0 mmol, 1.0 equiv) to the general CsOH procedure with the addition of 2-methoxyethoxymethyl chloride (283 μL, 2.5 mmol, 2.5 equiv) as the electrophile afforded 241 mg of the title compound (49%) as a viscous, clear oil after silica gel flash chromatography. (hexanes/EtOAc 3:1): TLC R_f = 0.30 (SiO_2, hexanes/EtOAc 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.24 (s, 1H), 7.22 (dd, J = 2.4, 8.6 Hz, 1H), 7.03, (d, J = 2.4 Hz, 1H), 6.70 (d, J = 8.6, 1H), 5.32 (s, 4H), 3.89 (s, 2H), 3.74−3.72 (m, 4H), 3.51 (s, 4H), 3.50−3.47 (m, 4H), 3.31 (s, 6H); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 170.4, 156.6, 132.4, 132.0, 123.2, 118.6, 111.0, 90.0, 71.5, 69.9, 59.1, 55.5, 53.7; HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ calcd for $C_{19}H_{28}BrNO_9H$ 494.1020; found 494.1020.

Bis(acetoxymethyl) 2,2′-((5-(2-Azidoethyl)-2-hydroxybenzyl)azanediyl)diacetate (5a). Subjection of 4-(2-azidoethyl)phenol[8] (163 mg, 1.0 mmol, 1.0 equiv) to the general Cs_2CO_3 procedure with the addition of bromomethyl acetate (245 μL, 2.5 mmol, 2.5 equiv) as the electrophile afforded 235 mg of the title compound (52%) as a viscous, clear oil after silica gel flash chromatography. (hexanes/EtOAc 2:1): TLC R_f = 0.50 (SiO_2, hexanes/EtOAc 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.00−6.97 (s, 1H), 6.78−6.75 (m, 2H), 5.71 (s, 4H), 3.90 (s, 2H), 3.53 (s, 4H), 3.36 (t, J = 7.1 Hz, 2H), 2.71 (t, J = 7.1 Hz, 2H), 2.05 (s, 6H); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 169.5, 169.4, 156.1, 130.05, 130.00, 120.8, 116.9, 79.4, 56.0, 53.3, 52.6, 34.5, 20.6; HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ calcd for $C_{19}H_{24}N_4O_9H$ 453.1616; found 453.1622.

Bis(acetoxymethyl) 2,2′-((3-Allyl-2-hydroxybenzyl)-azanediyl)diacetate (6a). Subjection of 2-allylphenol (130 μL, 1.0 mmol, 1.0 equiv) to the general Cs_2CO_3 procedure with the addition of bromomethyl acetate (245 μL, 2.5 mmol, 2.5 equiv) as the electrophile afforded 215 mg of bis(acetoxymethyl) 2,2′-((3-allyl-2-hydroxybenzyl)azanediyl)diacetate (51%) as a viscous, clear oil after silica gel flash chromatography. (hexanes/EtOAc 3:1): TLC R_f = 0.30 (SiO_2, hexanes/EtOAc 2:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.03 (s, 1H), 7.12 (dd, J = 7.4, 1 Hz, 1H), 6.87, (dd, J = 7.4, 1 Hz, 1H), 6.76 (dd, J = 14.9, 7.4, 1H), 6.06 (ddt, J = 16.9, 10.3, 6.7, 1H), 5.81 (s, 4H), 5.12−5.05 (m, 2H), 4.01 (s, 2H), 3.62 (s, 4H), 3.45 (d, J = 6.6 Hz, 2H), 2.15 (s, 6H); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 169.6, 169.5, 154.9, 137.0, 130.1, 127.8, 127.6, 120.4, 119.2, 115.3, 79.4, 56.1, 53.4. 33.9, 20.7; HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ calcd for $C_{20}H_{25}NO_9H$ 424.1602; found 424.1604.

((2,2′-((3-Allyl-2-hydroxybenzyl)azanediyl)bis(acetyl))bis-(oxy))bis(methylene) Dipropionate (6b). Subjection of 2-allylphenol (130 μL, 1.0 mmol, 1.0 equiv) to the general Cs_2CO_3 procedure with the addition of bromomethyl propionate (243 μL, 2.5 mmol, 2.5 equiv) as the electrophile afforded 141 mg of the title compound (54%) as a viscous, clear oil after silica gel flash chromatography. (hexanes/EtOAc 3:1): TLC R_f = 0.30 (SiO_2, hexanes/EtOAc 2:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.04 (s, 1H), 7.11 (dd, J = 6.5, 1 Hz, 1H), 6.86, (dd, J = 6.5, 1 Hz, 1H), 6.76 (dd, J = 14.9, 7.5, 1H), 6.04 (ddt, J = 16.7, 10.1, 6.7, 1H), 5.82 (s, 4H), 5.11−5.05 (m, 2H), 4.01 (s, 2H), 3.62 (s, 4H), 3.45 (d, J = 6.6 Hz 2H), 2.42 (q, J = 7.5 Hz, 4H), 1.18 (t, J = 7.5 Hz, 6H); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 173.0, 169.6, 154.9, 137.1, 130.1, 127.8, 127.6, 120.5, 119.2, 115.3, 79.4, 56.0, 53.4, 33.9, 27.2, 8.7; HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ calcd for $C_{22}H_{29}NO_9H$ 452.1915; found 452.1923.

Bromomethyl Propionate (7).[9] A 25 mL round-bottomed flask equipped with a magnetic stirring bar and an N_2 inlet was charged with propionyl bromide (4 mL, 45 mmol, 1.0 equiv) and stannic chloride (60 μL, 0.5 mmol, 0.1 equiv). Paraformaldehyde (1.3 g, 45 mmol, 1.0 equiv) was added in small portions to the stirred reactant mixture at 0 °C. The reactant mixture was allowed to stir in the ice bath for an additional 3 h while gradually warming to r.t. The resulting mixture was purified by flash distillation (12 Torr, 40 °C) to give 7 as a yellow liquid. (6.0 g, 80%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.83 (s, 2H), 2.43 (q, J = 7.5 Hz, 2H), 1.19 (t, J = 7.5 Hz, 3H); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 172.3, 57.1, 27.5, 8.6.

■ ASSOCIATED CONTENT

Data Availability Statement

The data underlying this study are available in the published article and its Supporting Information.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.3c00155.

Expermental procedure of **8**, safety details, and NMR spectra of new compounds. (PDF)

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Notes

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