Paper

Electrochemical Synthesis of Self-immolative Methylene Alkoxy Carbamate (MAC) to Facilitate Construction of Antibody–drug Conjugate Linkers with Alcohol-containing Payloads

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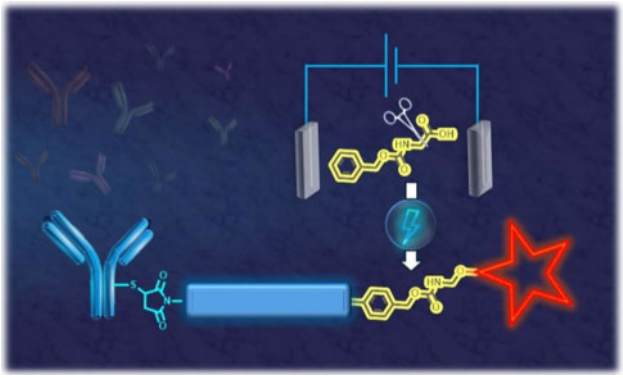
GRAPHICAL ABSTRACT



ABSTRACT

In the area of targeted drug delivery, the strategy of using methylene alkoxy carbamate (MAC) as a self-immolative unit in the linker construction for antibody–drug conjugate (ADC) has evolved as the most popular approach in recent time research. Reported synthetic routes for MAC linker construction are limited to very few options, which often suffer synthetic challenges with diverse sets of alcohol-containing payloads. An alternative concept of constructing MAC linkers, employing electrochemical decarboxylative coupling strategy, has been validated with a wide range of structurally different alcohols including primary, secondary, and tertiary analogues, using *N,N*-dimethylacetamide (DMA) as a solvent. The model study allows using alcohol as a limiting reactant, extending the opportunity of utilizing different alcohol-containing payloads to conjugate via MAC to construct ADCs.

Keywords antibody–drug conjugate (ADC), electro-organic synthesis, alcohol payloads, decarboxylative alkoxylation, methylalkoxycarbamates (MAC)

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Introduction

Over the last decade, antibody–drug conjugate (ADC) has evolved as one of the prime avenues in the area of cancer chemotherapy research, mostly because of the successful delivery of cytotoxic molecules directly and selectively into the tumor cells to achieve anticancer activity while avoiding adverse effects on other healthy cells. USFDA approval of several ADCs like Mylotarg Adcetris, Kadcyla, Besponsa, Polivy, Padcev, Enhertu, Trodelvy, Blenrep, Zynlonta, Tivdak, and Elahere[1] for the treatment of different oncology indications has inspired the growth in research around ADCs.

Advancement in antibody research has greatly influenced conceptualization of targeted drug delivery, expanding the scopes from cytotoxic payloads to PROTACs (degrader antibody conjugate [DAC])[2] and molecular glues (molecular glue antibody conjugate).[3]

A typical ADC design (**Fig. 1**) consists of three major components: (i) a biologically active payload (cytotoxic agent/PROTAC, etc.), (ii) an antibody delivery vehicle, and (iii) a linker to connect

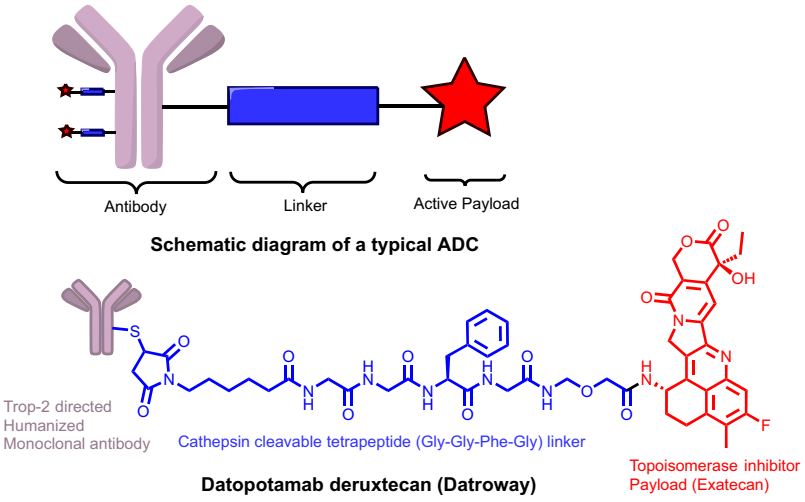


Fig. 1 Typical ADC design. Schematic Diagram and structural exemplification (Datroway).

these two moieties by covalent attachment, which enables selective cleavage followed by self-immolative release of the payload.[4] Cleavable linkers are mainly of three types, pH-sensitive, glutathione-sensitive and protease-sensitive. Acid labile linker present in Gemtuzumab ozogamicin (Mylotrag) is one of the early examples where it takes advantage of the lower pH in the endosomal (pH = 5–6) and lysosomal (pH = 4.8) compartments, to trigger hydrolysis of an acid labile hydrazone. The second release strategy exploits the higher concentrations of intracellular glutathione compared to plasma, enabling reduction of disulfide bridge. The third strategy utilizes the dominant proteases, found in a tumor cell lysosome for recognition and cleavage of a specific peptide sequence in the linker. Cathepsin-cleavable linkers such as "Val-Cit-PABC" linker[5] and analogous linkers such as Val-Ala, Phe-Lys, and Ala-Ala have been widely used to release the payloads. Other types of linkers take advantage of lysosomal and even extracellular enzymes such as glucuronidase and legumain to release amine-containing payloads[6–8] including auristatins, maytansinoids, PBD dimers, tubulysins, and nicotinamide phosphoribosyltransferase inhibitors.[9–13]

Several examples are available for linkers being typically attached to acids, amines and phenolic groups, however only limited reports of analogous cleavage systems for the release of alcohol-containing payloads are available.[14] Cleavable linker systems that release alcohol functionality are particularly or interest mainly because of two major reasons: (1) many biologically useful classes of payloads, like alkaloids and polyketides, contain multiple hydroxyl groups without having any suitable primary or secondary amines to attach with linker and (2) even when amine attachment to the payload is made, it often results in increase in polarity, adversely impacting payload permeability, and preventing either lysosomal escape and/or bystander activity on nearby cells.[15–17]

For the alcohol-containing agents as ADC payloads, a few synthetic strategies were explored earlier, for example, (1) synthesizing a carbonate of a camptothecin analogue, SN-38,[18] linked to monoclonal antibodies (mAbs) but were found to be labile under physiological conditions; (2) self-immolative dicarbamates[18–20]; and (3) ether linkages.[21] However, these approaches are limited in terms of scope, and only effective in releasing phenolic payloads. Regarding nonphenolic, alcohol-containing payloads, one of the most efficient strategies for the conjugation with antibodies was developed and reported by Kolakowski et al. from Seatle Genetics (**Fig. 2**, entry a) which involved methylene alkoxy carbamate (MAC), a self-immolative unit.[22] MAC construction for ADCs was successful in demonstrating an efficient release of alcohol drug surrogates by the action of β-glucuronidase. Use of MAC was an established process particularly in prodrug syntheses and used in several drug delivery strategies. One of the first applications involved an MAC-type unit to release 5-fluorouracil from a glucuronide prodrug.[23] Shabat et al. further developed the unit to release mercaptoacetic acid[24] and glucose.[25] Subsequently, Santi et al. used an MAC construct for β-elimination-promoted release of a potent cytotoxic agent SN-38.[26] Use of MAC to construct linkers is currently recognized as a highly popular process in DAC design and synthesis. Peter S. Dragovich et al.[27] from Genentech has successfully demonstrated delivery of chimeric BRD4 degraders where the researchers initially linked the BRD4 degrader to a cathepsin B cleavable peptidomimetic linker via an MAC moiety and then conjugated the linker-drug to an STEAP1-targeting mAb that contained two engineered Cys residues (LC-K149) to afford DAC (DAR ≈ 2.0) (**Fig. 2**, entry b).[23]

Synthesis of MAC Linker

Popularity of having MAC linkers in prodrug design or ADCs encouraged synthetic organic chemists to find suitable synthetic procedures to construct MAC linkers. A few of the mostly popular and widely followed routes are: (1) converting the carbamate moiety to reactive N-chloromethyl carbamate using paraformaldehyde and TMSCl,[22] followed by Cl-substitution reaction with the OH-containing payload; (2) decarboxylative conversion of amino acid analogue to corresponding N-acetoxymethyl analogue, followed by reaction with alcohol[27]; (3) alkoxy carbamate

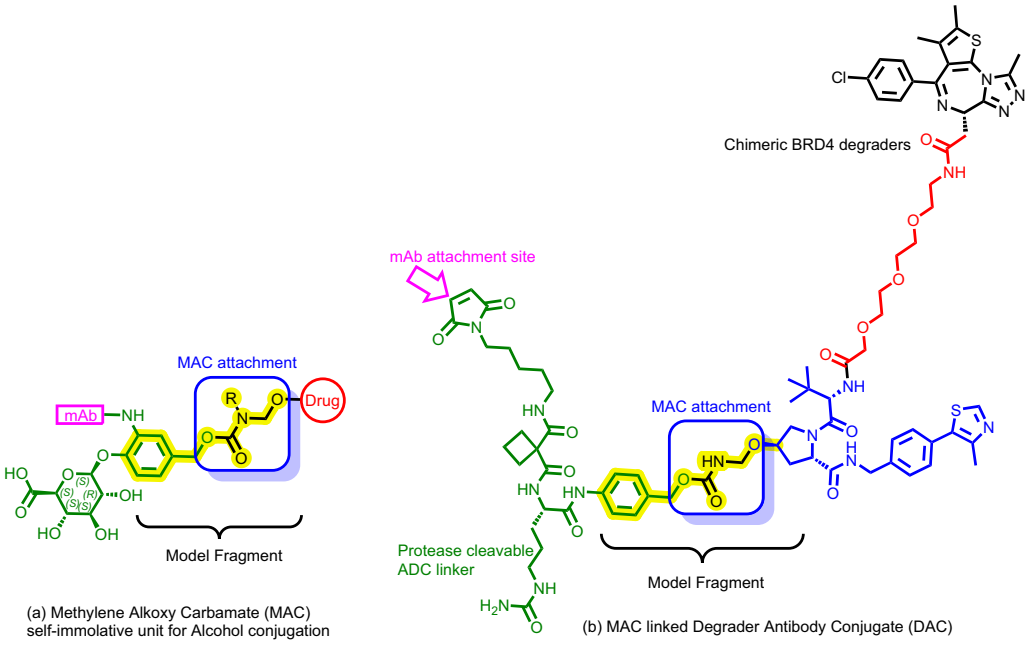


Fig. 2 (**a**) MAC attachment for the construction of β-glucuronidase cleavable ADCs with alcohol payloads. (**b**) MAC attaching cathepsin B cleavable peptidomimetic linker with BRD4 degrader via -OH of VHL ligand. Identifying the "model fragment".

synthesis employing a sequence of nitrile hydrozirconation, acylation, and alcohol addition[28]; or (4) converting the alcohol payload to corresponding alkoxy acetic acid followed by acyl azide formation and Curtius rearrangement (**Table 1**).[29]

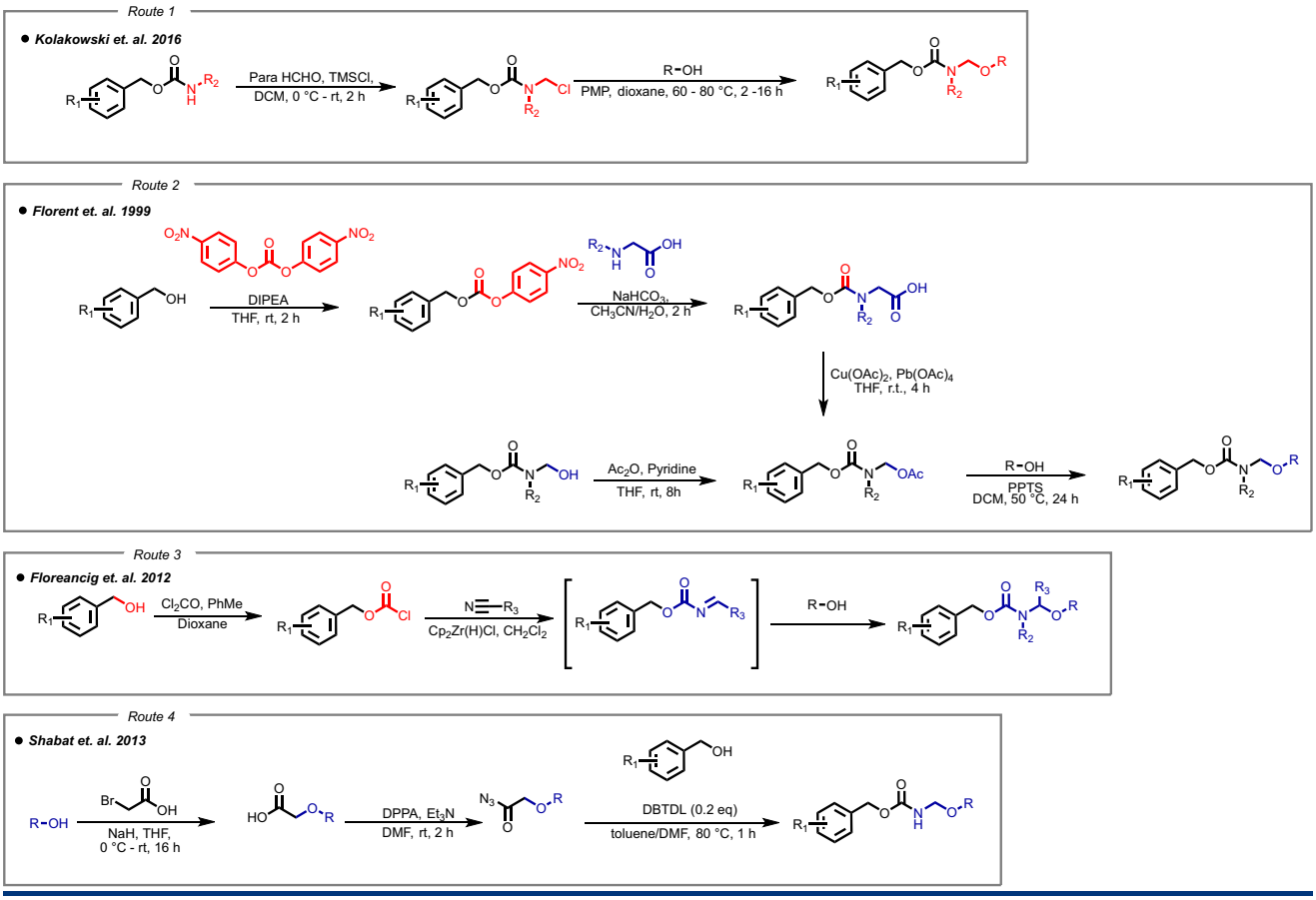
All these processes have limitations in terms of substrate scope and require optimizations for different sets of alcohol payloads. Increasing demand of ADC and DAC syntheses prompted us to explore newer ways of constructing MAC linkers useful for attaching wide range of payloads with -OH functionality.

With the advent and growing usage of electrochemistry[30] in organic transformation, we investigated the opportunity of utilizing the concept and figured out that a few scientific research groups have utilized electrochemistry for constructing MAC via an electrochemical decarboxylative alkoxylation of amino acids in alcohol solutions especially in methanol (**Table 2**).[31–36] Very recently, Cantillo et al. reported solvent-enabled electrochemical decarboxylative acetoxylation which can have a potential application in ADC molecule synthesis.[37] For MAC synthesis, all the existing electrochemical reports are limited to only a few alcohols like methanol and are reported to be used as solvent. For a practical application, the payload alcohol is required to be used in stoichiometric quantity as reactant. To validate our concept, we thoroughly investigated electrochemical MAC synthesis with various primary, secondary, and tertiary alcohols, using N-Cbz-protected amino acids as the model counterpart to resemble the substructure of any typical ADC molecule (**Fig. 2**, model fragment).

Herein, we disclose a model study enabling the synthesis of MAC linkers through electrochemical decarboxylative alkoxylation of amino acid analogues, with potential implications for the preparation of diverse ADCs incorporating alcohol-containing payloads.

As a starting point, N-Cbz glycine **1a** was used as a model substrate to study the formation of benzyl (methoxymethyl)carbamate **2a** with 10 equiv of methanol using DMA (3 mL) as solvent. The reaction was carried out in an undivided cell using graphite plate both as anode and cathode via a constant current electrolysis mode, by passing 70 mA of constant current to afford **2a** (**Table 3**, entry 1). **IKA Electrasyn 2.0** and **Eliteck Orgel 1.0** were used for the purpose, and the condition was achieved based on the reports[31–34] after an optimization of electric current to suit the requirement of using solvent and making the alcohol limiting. A quick optimization with polar aprotic solvents like DMF and MeCN was carried out; however, the yield of the final product was found to be less than the case with DMA (entries 3 and 4). Use of alcohol was fixed to 10 equiv, since a lower equivalence of alcohol (2 eq.) showed poor yield of **2a** (entry 2). The reaction time was optimized to 10 h (entry 5). Regarding the use of electrodes, porous graphite plates as both anode and cathode were found to be a good choice as other alternative metal electrodes like stainless steel and nickel plates were found to be incompatible when employed as anode (entries 6 and 7). On the contrary, stainless steel and nickel plates as cathode were found to be satisfactory (entries 8 and 9), although the yields were less. Further optimization was tried by adding 0.5 eq. of n-Bu_4NBF_4 to the reaction in order to decrease the acting potential (~9 V),[38] but a decremental effect on the product yield was noticed, which could be because of the greater ionization rate of supporting electrolyte[39] over methanol, thereby keeping large excess of methanol unreacted during the electrolysis (entry 10). This reaction was also performed as a control experiment in the absence of electric current by maintaining all the other reaction parameters and as expected, no formation of **2a** was observed, suggesting the key

Table 1 Conventional synthetic routes for MAC linker synthesis.



role of electrical energy in the electrooxidation of amino acid moiety (entry 11).

When the applied current was increased further to 100 mA, by maintaining the other parameters fixed, the yield of **2a** decreased drastically, probably because of over oxidation at such high current (**Table 3**, entry 12). After optimizing the conditions, we decided to explore the substrate scopes, altering the alcohol from MeOH to aliphatic alcohols like ethanol, n-propanol, n-butanol, and n-pentanol to observe successful conversions to **2b**, **2c**, **2d**, and **2e**, respectively, although a gradual decrease in yields (**Table 4**, entries 2a–2e) was observed.

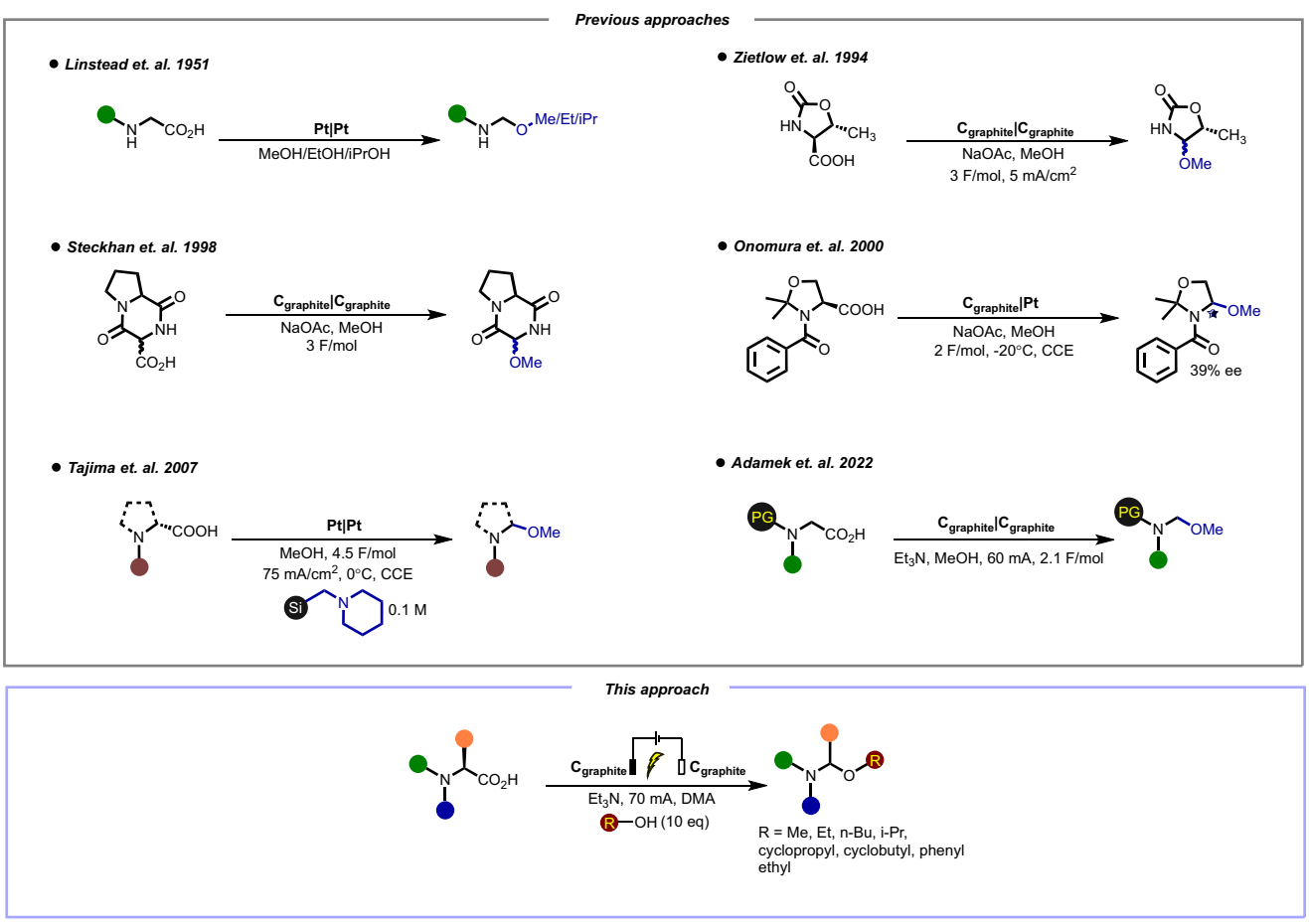
To extend the applicability, further exploration with secondary (isopropanol) and tertiary alcohols (tert-butanol) were used to obtain **2f** and **2g**, respectively (**Table 5**).

To our knowledge, MAC synthesis with classical nonelectrochemical route is not reported with any tertiary alkyl moiety. Decarboxylative electrochemical condition was successful in delivering methylene tert-butoxy carbamate analogue with moderate yield. In addition, the efficacy of the method was extensively tested by employing cyclic secondary alcohols like cyclobutanol, as the alkoxy coupling partner with Cbz glycine to afford carbamate **2h** (**Table 5**, entry 2h) successfully. Since classical nonelectrochemical conditions are generally not successful in synthesizing MAC with strained secondary alcohols like cyclopropanol, we checked the generality of the condition on cyclopropanol. As the existing electrochemical condition was not successful, temperature was raised to 90 °C to successfully deliver compound **2i**. After establishing the protocol's versatility with variety of alcohols, we focused on testing the method with structurally diverse protected amino acids. We employed N-Cbz-*L*-phenyl alanine **1aa** and successfully obtained three different alkoxy carbamates **2j–2l** with moderate to excellent yield via decarboxylative alkoxylation with phenyl ethanol, methanol and ethanol, respectively (**Table 6**, entries 2j–2l).

We further extended our study with different amino acids from N-Cbz protected phenyl alanine to N-Cbz protected *L*-amino acids like proline, serine, and alanine under the standard condition and the resulting carbamates **2m, 2n**, and **2p** were effectively obtained with moderate yield (**Table 7**). We also altered the N-protecting group from Cbz to Fmoc and Boc and successfully demonstrated the robust nature of the condition affording **2o**, and **2q** from valine, and tyrosine, respectively, with 10 eq. of methanol (**Table 7**), by slightly decreasing the triethylamine equivalence for **2o**.

Moving beyond natural amino acids, we also explored the feasibility of the reaction on sterically hindered cyclopropyl analogue **1ag** and demonstrated a successful conversion to corresponding methoxy analogue **2r** with a satisfactory yield (**Table 8**). Additionally, survival of the strained cyclopropyl group accounts for the robustness of the method.

Table 2 Previous electrochemical approaches.



We did not restrict the validation only with amino acids, we further extended to dipeptide Fmoc Val-Ala OH **1ah** and successfully demonstrated the conversion to **2s** (**Table 9**). This study provided confidence in using the condition on peptides.

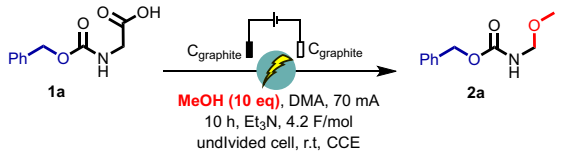
We also checked if the H- attached to N- atom of the amino acid has any role in the transformation. Accordingly, N-methyl Cbz protected glycine (**1ai**) was used to obtain **2t** with 10 eq. of isopropanol (**Table 10**, entry 2t) with moderate yield. This experiment clearly indicated the robust nature of the process.

From the mechanistic perspective, the electrochemical formation of carbamates from corresponding amino acid undergoes a well-known non-Kolbe electrolysis method[37] that commences via the anodic oxidation of the carboxylate ion **1x** resulting in decarboxylation of the amino acid and generation of a very stable iminium cation **2y**.[38] In the next step, a very fast nucleophilic alkoxide addition reaction to the sp^2 carbon atom attached to the iminium cation **2y** generates the target compound **2** (**Scheme 1**).[36] When started with chirally pure N-Cbz-*L*-phenylalanine **1aa**, we found the chiral purity of product **2l** to undergo a complete racemization, (*SI*, Page- S11). This observation supports the proposed mechanism as mentioned in **Scheme 1**.

To get insight of the mechanism, a control experiment was performed in the presence of radical quencher TEMPO. However, yield of **2a** was found to be consistent in the presence of 1 eq. of TEMPO (**Table 11**). This observation revealed that the electrochemical decarboxylative alkoxylation reaction proceeds through an ionic pathway. Based on the control experiment and previous literature reports,[37] it is evident that the electrochemical formation of carbamates from corresponding amino acid undergoes a well-known non-Kolbe electrolysis method that commences via the anodic oxidation of the carboxylate ion **1x** resulting in decarboxylation of the amino acid and generation of a very stable iminium cation **2y**.[38] In the next step, a very fast nucleophilic alkoxide addition reaction to the sp^2 carbon atom attached to the iminium cation **2y** generates the target compound **2** (**Scheme 1**).[36] When started with chirally pure N-Cbz-*L*-phenylalanine **1aa**, we found the chiral purity of product **2l** to undergo a complete racemization, (*SI*, Page- S11). This observation supports the proposed mechanism as mentioned in **Scheme 1**.

In order to expand the concept to have a practical application in constructing the MAC linker for an ADC molecule, it was required to be tested on a relevant model analogue **1aj**, which could easily be translated to deliver required ADCs. Accordingly, the electrochemical process applied on ADC building block **1aj** and successful formation of **2u** suggests a practical application of this strategy to synthesize MAC linkers with alcoholic payloads (**Table 12**).

Table 3 Optimization of the reaction conditions[a].



Ent	Deviations from the standard conditions	% Yield[b]
1	None	78
2	MeOH (2 eq.) with DMA as solvent	12
3	MeOH (10 eq.) with DMF as solvent	63
4	MeOH (10 eq.) with MeCN as solvent	50
5	2 h instead of 10 h	8
6	Stainless steel as anode instead of graphite	n.r.
7	Nickel as anode in place of graphite	n.r.
8	Stainless steel plate as cathode	67
9	Nickel plate as cathode	60
10	*n*-Bu_4NBF_4 (0.5 eq.) as supporting electrolyte	69
11	No current	n.r.
12	100 mA instead of 70 mA	27

[a]Optimized condition- **1a** (0.4 mmol) in DMA (3 mL), methanol (10 eq.), Et_3N (5 mol%), constant current (70 mA), undivided cell, graphite as anode and cathode.
[b]Isolated yield (no side product was found to form; lower yield corresponds to poor conversion from the starting materials); n.r. = no reaction.

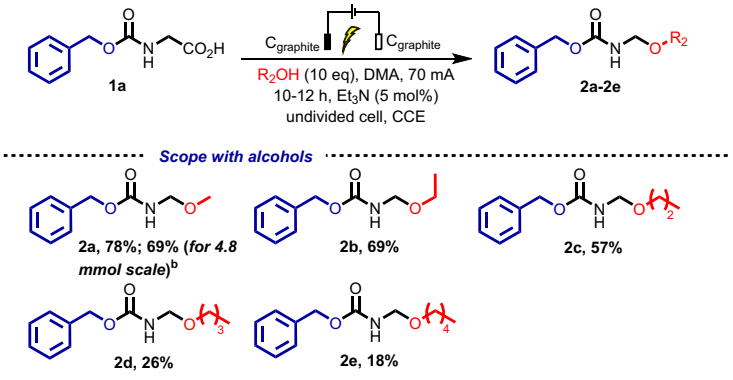
In conclusion, we have developed a novel route for synthesizing alkoxymethylene carbamates by electrochemical decarboxylation method, ideal for an application toward prodrug and ADC linker synthesis. A library of structurally diverse alkoxymethylene carbamate compounds was synthesized employing different varieties of alcohols. Reaction conditions were optimized to obtain a common strategy where use of equivalence of alcohol could be reduced to reactant level. The electrochemical procedure was successful in achieving MAC with strained secondary and tertiary alcohols that were challenging from classical synthetic conditions. The optimized reaction protocol, being operationally simple yet robust in nature, can be further extended to suit the MAC linker construction for ADC molecule synthesis. This novel electrochemical MAC synthesis option can be explored further to have real-life application in the field of ADC research.

Syntheses of all the carbamates **2a–2u** via electrochemical decarboxylative alkoxylation were performed via time-dependent constant current method using **Orgel 1.0** from Eliteck Industries Private Ltd. and **Electrasyn 2.0** from IKA. Then, 20 and 5 mL glass vials were used for electrochemical reaction. All the starting materials, alcohols, and reagents were procured from available commercial vendors and used without any further purification. All reactions were performed in oven-dried glass vials and round-bottom flasks. Progress of the reaction was monitored via TLC (Description—TLC silica gel 60 F_{254}, aluminum sheets 20×20 cm, layer thickness—175–225 µm) made by Merck. Spots were visualized under UV (254 nm) and by dipping under charring solutions ($KMnO_4$, ninhydrin, and PMA staining solution). Purification of the products was performed using Biotage Isolera 01 and Combi flash. Compound was loaded in 25 g and 40 g Biotage Sfar cartridges by making slurry in 230–400 mesh/60–120 mesh silica gel (dry method). Chiral SFC purification of product was performed on Pic-10-20 chiral SFC purification instrument made by Pic solution using YMC-Amylose C column with a cosolvent 0.5% isopropylamine in Isopropanol (flow rate—3 mL/min, injection volume—15 µL). 1H, ^{13}C NMR spectra were recorded on BRUKER AVANCE NEO 400 and 300 units (^1H NMR [400 MHz/300 MHz], ^{13}C NMR [100 MHz/75 MHz]) taking TMS (δ = 0 ppm) as internal standard. LCMS of the compounds were recorded in Agilent 1290 Infinity II (Column—CSH C-18 (30×2.1mm) 1.7 µm, UV range—210–350 nm, mobile phase A 0.1% HCOOH in H_2O, mobile phase B 0.1% HCOOH in MeCN). NMR splitting pattern singlet, doublet, triplet, quartet, heptet, doublet of doublet, doublet of triplet, and multiplet have been abbreviated as s, d, t, q, hept, dd, dt, and m, respectively.

General Electrochemical Procedure for the Synthesis of Benzyl (Methoxymethyl)carbamate 2a

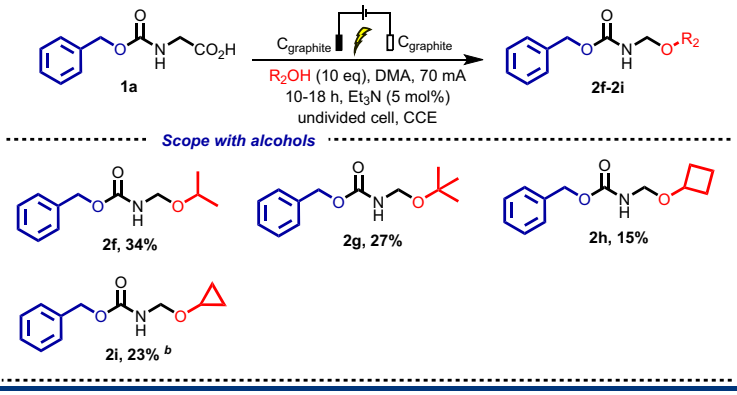
A 5 mL/20 mL IKA Electrasyn 2.0/Orgel 1.0 vial was charged with DMA (4 mL) solution of N-carbobenzyloxyglycine (N-Cbz Glycine) **1a** (250 mg, 1.2 mmol) and a stirring bar. Methanol (483 µL, 12 mmol) was then added to the solution while stirring followed by the dropwise addition of triethylamine (12.49 µL, 0.09 mmol). The solution was then kept stirring at ambient temperature for 5 min to ensure complete dissolution. Meanwhile, an IKA Electrasyn 2.0/Orgel 1.0 vial cap was fitted with graphite plate both as anode and cathode and was placed on the top of the reaction vial allowing the electrodes to dip inside the reaction solution. The reaction mixture was then electrolyzed at a constant current of 70 mA (4.2 F/mol) for 10 h, at ambient temperature (*SI*, Page S4, **Fig. S3**). Close monitoring of the reaction progression was performed via TLC (silica gel plate; eluent—2:3 EtOAc/hexanes). After the complete consumption of **1a**, as evident from TLC, the solution was transferred to a 100 mL separating funnel and diluted with DCM (20 mL) and water (10 mL). The electrodes were rinsed with DCM (10 mL) and collected. The organic layer was separated from the aqueous layer

Table 4 Substrate scope variations with aliphatic primary alcohols[a].



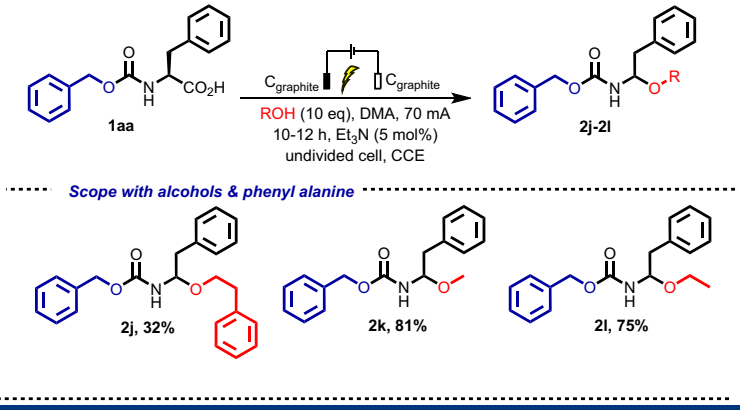
[a]Optimized condition- **1a** in DMA (3 mL), alcohol (10 eq.), constant current (70 mA), Et_3N (5 mol%), undivided cell, graphite as anode and cathode.
[b]Conditions for scale-up reaction- **1a** (4.8 mmol) in DMA (7 mL), constant current- 0.1 A for 24 h.

Table 5 Substrate scope variations with aliphatic secondary and tertiary alcohols[a].



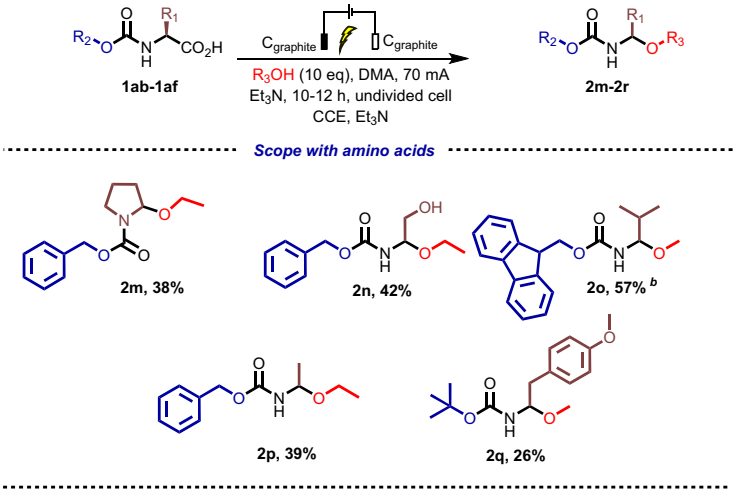
[a]Optimized condition- 1a in DMA (3 mL), alcohol (10 eq.), constant current (70 mA), Et_3N (5 mol%), undivided cell, graphite as anode and cathode.
[b]Heating at 90 °C.

Table 6 Substrate scope variations with different amino acids[a].



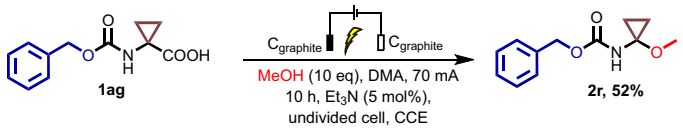
[a]Optimized condition- 1aa in DMA (3 mL), alcohol (10 eq.), constant current (70 mA), Et_3N (5 mol%), undivided cell, graphite as anode and cathode.

Table 7 Scope with amino acids[a].



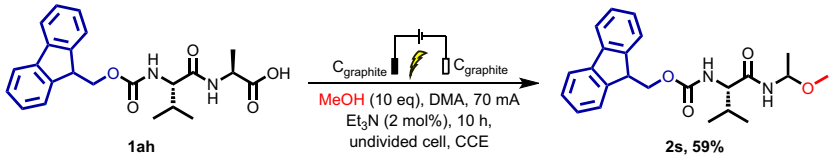
[a]Optimized condition- 1ab–1af in DMA (3 mL), alcohol (10 eq.), constant current (70 mA), Et_3N (5 mol%) undivided cell, graphite as anode and cathode.
[b]2 mol% of Et_3N.

Table 8 Scope with amino acids[a].



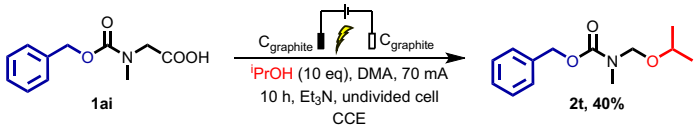
[a]Optimized condition- **1ag** in DMA (3 mL), methanol (10 eq.), constant current (70 mA), Et_3N (5 mol%), undivided cell, graphite as anode and cathode.

Table 9 Scope with dipeptide[a].

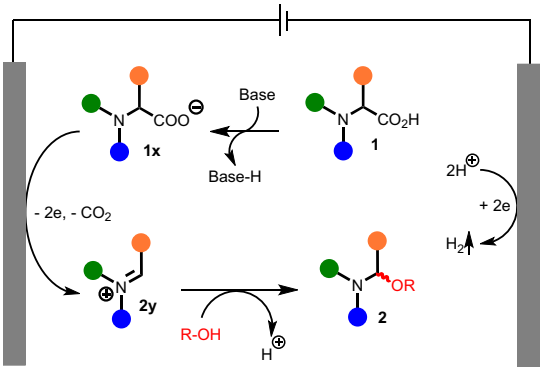


[a]Optimized condition- **1ah** in DMA (3 mL), methanol (10 eq.), constant current (70 mA), Et_3N (2 mol%), undivided cell, graphite as anode and cathode.

Table 10 Scope with N-substituted amino acid[a].



[a]Optimized condition- **1ai** in DMA (3 mL), isopropanol (10 eq.), constant current (70 mA), undivided cell, graphite as anode and cathode.



Scheme 1 **Plausible mechanism.**

using DCM (2×20 mL) and the combined portion was collected over anhydrous sodium sulfate. The organic layer was concentrated in vacuo and purified by flash chromatography using PE/EA (2:1) (eluent) with 230–400 mesh silica gel in Biotage Isolera instrument. The purified fractions were collected and concentrated under reduced pressure to afford benzyl (methoxymethyl)carbamate **2a** (182 mg, 78%)[40] as a colorless liquid.

Benzyl (Methoxymethyl)carbamate 2a

^1H NMR ($CDCl_3$, 400 MHz) δ ppm 7.32–7.46 (m, 5 H), 5.52 (br s, 1 H), 5.16 (s, 2 H), 4.66 (br d, J = 7.13 Hz, 2 H), 3.36 (s, 3 H), 1.64 (s, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.4, 136.1, 128.6, 128.3, 128.2, 73.7, 67.1, 55.7.

Benzyl (Ethoxymethyl)carbamate 2b

According to the standard method mentioned above, constant current electrolysis of ((benzyloxy)carbonyl)glycine **1a** (210 mg, 1.00 mmol) in the presence of ethanol (10 eq.) yielded benzyl (ethoxymethyl)carbamate **2b** (144.4 mg, 69%) as a colorless liquid. ^1H NMR ($CDCl_3$, 400 MHz) δ ppm 7.30–7.45 (m, 5 H), 5.50 (br s, 1 H), 5.16 (s, 2 H), 4.71 (d, J = 7.13 Hz, 2 H), 3.58 (q, J = 7.00 Hz, 1 H), 3.47–3.64 (m, 1 H), 1.22 (t, J = 7.00 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.4, 136.2, 128.6, 128.2, 72.2, 67.0, 63.6, 15.1;

Benzyl (Propoxymethyl)carbamate 2c

According to the standard method mentioned above, constant current electrolysis of ((benzyloxy)carbonyl)glycine **1a** (240 mg, 1.15 mmol) in the presence of *n*-propanol (10 eq.) yielded benzyl (propoxymethyl)carbamate **2c** (146 mg, 57%) as a colorless liquid. ^1H NMR ($CDCl_3$, 400 MHz) δ ppm 7.31–7.42 (m, 5 H), 5.51 (br s, 1 H), 5.16 (s, 2 H), 4.71 (d, J = 7.00 Hz, 2 H) 3.47 (t, J = 6.69 Hz, 2 H), 1.55–1.64 (m, 2 H), 0.93 (t, J = 7.44 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.4, 136.2, 128.6, 128.2, 72.4, 70.0, 67.0, 22.9, 10.6.

Benzyl (Butoxymethyl)carbamate 2d

According to the standard method mentioned above, constant current electrolysis of ((benzyloxy)carbonyl)glycine **1a** (240 mg, 1.15 mmol) in the presence of n-butanol (10 eq.) yielded benzyl (butoxymethyl)carbamate **2d** (62.6 mg, 26%) as a colorless liquid. ^1H NMR ($CDCl_3$, 400 MHz) δ ppm 7.31–7.46 (m, 5 H), 5.47 (br s, 1 H), 5.16 (s, 2 H), 4.71 (d, J = 7.13 Hz, 2 H), 3.51 (t, J = 6.57 Hz, 2 H), 1.53– 1.61 (m, 4 H), 1.38 (dq, J = 14.93, 7.32 Hz, 2 H), 0.93 (t, J = 7.38 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 136.2, 128.6, 128.2, 72.4, 68.1, 67.0, 31.7, 19.3, 13.8.

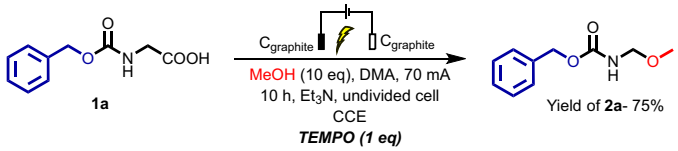
Benzyl ((Pentyloxy)methyl)carbamate 2e

According to the standard method mentioned above, constant current electrolysis of ((benzyloxy)carbonyl)glycine **1a** (220 mg, 1.05 mmol) in the presence of n-pentanol (10 eq.) yielded benzyl ((pentyloxy)methyl)carbamate **2e** (47.5 mg, 18%) as a colorless liquid. ^1H NMR ($CDCl_3$, 400 MHz) δ ppm 7.32–7.40 (m, 5 H), 5.50 (br s, 1 H), 5.16 (s, 2 H), 4.71 (d, J = 7.13 Hz, 2 H), 3.50 (t, J = 6.63 Hz, 2 H), 1.55–1.61 (m, 2 H), 1.22–1.42 (m, 4 H), 0.89–0.94 (m, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.3, 136.2, 128.6, 128.2, 72.4, 68.4, 67.0, 29.3, 28.3, 14.0.

Benzyl (Isopropoxymethyl)carbamate 2f

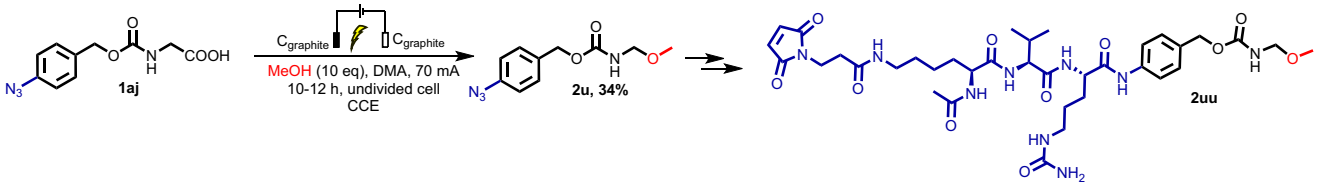
According to the standard method mentioned above, constant current electrolysis of ((benzyloxy)carbonyl)glycine **1a** (205 mg, 1.00 mmol) in the presence of isopropanol

Table 11 Control experiment[a].



[a]Optimized condition- **1a** in DMA (3 mL), methanol (10 eq.), TEMPO (1 eq.), constant current (70 mA), undivided cell, graphite as anode and cathode.

Table 12 Application to build potential ADC building block[a].



[a]Optimized condition- **1aj** in DMA (3 mL), methanol (10 eq.), constant current (70 mA), Et_3N (5 mol%), undivided cell, graphite as anode and cathode.

(10 eq.) yielded benzyl (isopropoxymethyl)carbamate **2f** (76 mg, 34%) as a colorless liquid.[41] ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.38–7.49 (m, 5 H) 7.28 (s, 1 H), 5.44 (br s, 1 H), 5.15 (s, 2 H), 4.73 (br d, *J* = 7.00 Hz, 2 H), 3.79–3.85 (m, 1 H), 1.21 (s, 3 H) 1.19 (s, 3 H)

Benzyl (*Tert*-Butoxymethyl)carbamate 2g

According to the standard method mentioned above, constant current electrolysis of ((benzyloxy)carbonyl)glycine **1a** (215 mg, 1.03 mmol) in the presence of tert-butanol (10 eq.) yielded benzyl (tert-butoxymethyl)carbamate **2g** (66 mg, 27%) as a colorless liquid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.31–7.41 (m, 5 H), 5.39 (br s, 1 H), 5.14 (s, 2 H), 4.72 (d, *J* = 7.00 Hz, 2 H), 1.64 (s, 1 H), 1.27 (s, 9 H), ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.8, 136.3, 128.5, 128.2, 73.7, 66.9, 66.4, 28.0.

Benzyl (Cyclobutoxymethyl)carbamate 2h

According to the standard method mentioned above, constant current electrolysis of ((benzyloxy)carbonyl)glycine **1a** (200 mg, 0.96 mmol) in the presence of cyclobutanol (10 eq.) yielded benzyl (cyclobutoxymethyl)carbamate **2h** (56.5 mg, 15%) as a colorless liquid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.32–7.40 (m, 5 H), 5.42 (br s, 1 H), 5.11–5.18 (m, 2 H), 4.66 (d, *J* = 7.2 Hz, 2 H), 4.10 (quin, *J* = 7.2 Hz, 1 H), 2.19–2.28 (m, 2 H), 1.90–2.03 (m, 2 H), 1.62–1.76 (m, 1 H), 1.47–1.57 (m, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 128.6, 128.2, 77.3, 77.0, 76.7, 30.7, 12.8.

Benzyl (Cyclopropoxymethyl)carbamate 2i

According to the standard method mentioned above, constant current electrolysis along with heating (90 °C) of the DMA (3 mL) solution ((benzyloxy)carbonyl)glycine **1a** (215 mg, 1.03 mmol) in the presence of cyclopropanol (10 eq.) yielded benzyl (tert-butoxymethyl)carbamate **2i** (52 mg, 23%) as a colorless liquid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.38–7.40 (m, 5 H), 5.55 (br s, 1 H), 5.16 (s, 2 H), 4.74 (br d, *J* = 7.25 Hz, 2 H), 3.34–3.40 (m, 1 H), 0.58–0.67 (m, 2 H), 0.44–0.52 (m, 2 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 136.2, 128.8, 128.6, 128.2, 77.4, 77.0, 76.7, 72.5, 67.1, 51.1, 5.4.

Benzyl (1-Phenethoxy-2-phenylethyl)carbamate 2j

According to the standard method mentioned above, constant current electrolysis of ((benzyloxy)carbonyl)phenylalanine **1aa** (205 mg, 0.68 mmol) in the presence of phenyl ethanol (10 eq.) yielded benzyl (1-phenethoxy-2-phenylethyl)carbamate **2j** (82 mg, 32%) as yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.29–7.37 (m, 6 H), 7.16–7.28 (m, 9 H), 5.28 (dt, *J* = 10.26, 5.38 Hz, 1 H), 5.09 (s, 2 H), 5.00 (br d, *J* = 10.01 Hz, 1 H) 3.78–3.86 (m, 1 H) 3.64–3.73 (m, 1 H) 2.94 (d, *J* = 5.50 Hz, 2 H) 2.80–2.88 (m, 2 H) 1.60 (s, 1 H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 155.8, 138.9, 138.5, 135.9, 129.8, 129.1, 129.0, 128.7, 128.6, 128.4, 128.0, 126.1, 82.1, 68.9, 63.7, 39.2, 36.1.

Benzyl (1-Methoxy-2-phenylethyl)carbamate 2k

According to the standard method mentioned above, constant current electrolysis of ((benzyloxy)carbonyl)phenylalanine **1aa** (205 mg, 0.68 mmol) in the presence of methanol (10 eq.) yielded benzyl (1-methoxy-2-phenylethyl)carbamate **2k** (157 mg, 81%) as a white solid.[42] ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 2.95 (d, *J* = 5.48 Hz, 1 H) 2.90–2.98 (m, 1 H) 3.35 (s, 3 H) 4.93– 5.03 (m, 1 H) 5.10 (s, 2 H) 5.12–5.22 (m, 1 H) 7.20–7.27 (m, 3 H) 7.27 (s, 1 H) 7.28–7.40 (m, 7 H).

Benzyl (1-Ethoxy-2-phenylethyl)carbamate 2l

According to the standard method mentioned above, constant current electrolysis of ((benzyloxy)carbonyl)phenylalanine **1aa** (205 mg, 0.68 mmol) in the presence of ethanol (10 eq.) yielded benzyl (1-ethoxy-2-phenylethyl)carbamate **2l** (153 mg, 75%) as a white solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 7.30–7.41 (m, 6 H), 7.28–7.30 (m, 3 H), 7.21–7.28 (m, 4 H), 5.21–5.30 (m, 1 H), 5.09 (s, 2 H), 4.99–5.06 (m, 1 H), 3.62–3.72 (m, 1 H), 3.44–3.58 (m, 1 H), 2.92–2.99 (m, 2 H), 1.57 (s, 2 H), 1.17 (t, *J* = 7.0 Hz, 3 H), ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.5, 137.2, 129.4, 129.1, 128.6, 128.5, 128.3, 128.2, 128.0, 128.0, 125.3, 67.0, 58.5, 50.6, 18.4; HRMS (APPI$^+$/APCI$^+$, Q-ToF) *m/z*: [M+H]$^+$ calcd for $C_{18}H_{21}NO_3$: 300.1600; Found: 300.2210.benzyl 2-ethoxypyrrolidine-1-carboxylate **2m.**[43]

According to the standard method mentioned above, constant current electrolysis of ((benzyloxy)carbonyl)-L-proline **1ab** (200 mg, 0.80 mmol) in the presence of ethanol (10 eq.) yielded benzyl 2-ethoxypyrrolidine-1-carboxylate **2m** (76 mg, 38%) as a colorless liquid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.29–7.43 (m, 5 H), 5.24–5.38 (m, 1 H), 5.11–5.22 (m, 2 H), 3.58–3.79 (m, 2 H), 3.51–3.58 (m, 1 H), 3.32–3.47 (m, 1 H), 2.03– 2.19 (m, 1 H), 1.87–1.98 (m, 2 H), 1.78 (td, *J* = 12.44, 5.38 Hz, 1 H), 1.10–1.30 (m, 3 H).

Benzyl (1-Ethoxy-2-hydroxyethyl)carbamate 2n

According to the standard method mentioned above, constant current electrolysis of ((benzyloxy)carbonyl)-L-serine **1ac** (200 mg, 0.84 mmol) in the presence of ethanol (10 eq.) yielded benzyl (1-ethoxy-2-hydroxyethyl)carbamate **2n** (84 mg, 42%) as a colorless liquid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 7.33–7.40 (m, 6 H) 7.28–7.33 (m, 1 H) 5.69–6.02 (m, 1 H) 5.03– 5.24 (m, 3 H) 5.01–5.25 (m, 1 H) 3.93– 4.10 (m, 1 H) 3.69–3.79 (m, 2 H) 3.45–3.65 (m, 1 H) 1.26 (t, *J* = 7.05 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 155.2, 135.8, 128.6, 128.4, 128.3, 128.1, 81.0, 67.4, 64.6, 58.5, 18.4.

(9H-Fluoren-9-yl)methyl (1-Methoxy-2-methylpropyl)carbamate 2o

According to the standard method mentioned above, constant current electrolysis of (((9H-fluoren-9-yl)methoxy)carbonyl)-L-valine **1ad** (225 mg, 0.66 mmol) in the presence of ethanol (10 eq.) and trimethylamine (0.02 eq.) yielded (9H-fluoren-9-yl) methyl (1-methoxy-2-methylpropyl)carbamate **2o** (122 mg, 57%) as a white solid.[44] ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.80 (d, *J* = 7.5 Hz, 2 H), 7.63 (d, *J* = 7.3 Hz, 2 H), 7.43 (td, *J* = 7.4, 0.6 Hz, 2 H), 7.32–7.37 (m, 2 H), 7.25–7.32 (m, 1 H), 4.94 (br d, *J* = 10.1 Hz, 1 H), 4.64 (dd, *J* = 10.4, 5.9 Hz, 1 H), 4.51–4.58 (m, 1 H), 4.45–4.51 (m, 1 H), 4.23– 4.29 (m, 1 H), 3.32 (s, 3 H), 1.84 (dq, *J* = 13.2, 6.7 Hz, 1 H), 1.62 (br s, 1 H), 0.97 (d, *J* = 6.8 Hz, 3 H), 0.93 (d, *J* = 6.8 Hz, 3 H).

Benzyl (1-Ethoxyethyl)carbamate 2p

According to the standard method mentioned above, constant current electrolysis of ((benzyloxy)carbonyl)-L-alanine **1ae** (225 mg, 1.01 mmol) in the presence of ethanol (10 eq.) and trimethylamine (0.05 eq.) yielded (9H-fluoren-9-yl)methyl (1-methoxy-2-methylpropyl)carbamate **2p** (88 mg, 39%) as a white solid.[45] ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.38–7.40 (m, 5 H), 5.40 (br s, 1 H), 5.12–5.14 (m, 2 H), 5.06–5.09 (m, 1 H), 3.63– 3.69 (m, 1 H), 3.51–3.55 (m, 1 H), 1.37 (d, J = 5.75 Hz, 3 H), 1.19–1.21 (m, 3 H).

Tert-Butyl (1-Methoxy-2-(4-methoxyphenyl)ethyl)carbamate 2q

According to the standard method mentioned above, constant current electrolysis of (*S*)-2-((tert-butoxycarbonyl)amino)-3-(4-methoxyphenyl)propanoic acid **1af** (210 mg, 0.71 mmol) in the presence of methanol (10 eq.) and trimethylamine (0.05 eq.) yielded tert-butyl (1-methoxy-2-(4-methoxyphenyl)ethyl)carbamate **2q** (52 mg, 26%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.15–7.20 (m, 2 H), 6.84–6.88 (m, 2 H), 5.01–5.07 (m, 1 H), 4.77–4.79 (m, 1 H), 3.81 (s, 3 H), 3.35 (s, 3 H), 2.86–2.90 (m, 2 H), 1.44 (s, 9 H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ ppm 162.7, 158.2, 130.7, 129.0, 126.3, 114.4, 114.1, 113.8, 83.8, 55.3, 51.0, 28.0.

Benzyl (1-Methoxycyclopropyl)carbamate 2r

According to the standard method mentioned above, constant current electrolysis of 1-(((benzyloxy)carbonyl)amino)cyclopropane-1-carboxylic acid **1ag** (220 mg, 0.93 mmol) in the presence of methanol (10 eq.) yielded benzyl (1-methoxycyclopropyl) carbamate **2r** (107 mg, 52%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.33–7.39 (m, 5 H), 5.73 (br s, 1 H), 5.59–5.83 (m, 1 H), 5.14 (br s, 2 H), 3.36 (s, 3 H), 1.10–1.18 (m, 2 H), 1.00 (br s, 2 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.5, 136.2, 128.6, 128.2, 67.8, 66.9, 54.1, 14.7

(9H-fluoren-9-yl)methyl((2S)-1-((1-methoxyethyl)amino)-3-methyl-1-oxobutan-2-yl)carbamate 2s

According to the standard method mentioned above, constant current electrolysis of (((9H-fluoren-9-yl)methoxy)carbonyl)-L-valyl-L-alanine **1ah** (220 mg, 0.54 mmol) in the presence of methanol (10 eq.) and triethylamine (0.02 eq.) yielded (9H-fluoren-9-yl)methyl ((*2S*)-1-((1-methoxyethyl)amino)-3-methyl-1-oxobutan-2-yl)carbamate **2s** (126 mg, 59%) as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm 8.17–8.34 (m, 1 H), 7.90 (d, J = 7.5 Hz, 2 H), 7.75 (br d, J = 7.2 Hz, 1 H), 7.42 (t, J = 7.5 Hz, 2 H), 7.29–7.37 (m, 2 H), 5.02–5.14 (m, 1 H), 4.19–4.32 (m, 3 H), 3.77–3.92 (m, 1 H), 3.32 (s, 3 H), 3.12–3.19 (m, 3 H), 2.51 (dt, J = 3.6, 1.8 Hz, 3 H), 1.89–2.05 (m, 1 H), 1.18–1.26 (m, 3 H), 0.82–0.96 (m, 6 H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.9, 156.6, 144.3, 141.2, 128.1, 127.5, 125.8, 120.6, 77.5, 66.1, 60.8, 54.9, 47.1, 30.4, 21.5, 21.4, 19.8, 19.1, 18.6.

Benzyl (Isopropoxymethyl)(methyl)carbamate 2t

According to the standard method mentioned above, constant current electrolysis of N-((benzyloxy)carbonyl)-N-methylglycine **1ai** (210 mg, 0.94 mmol) in the presence of isopropanol (10 eq.) yielded benzyl (isopropoxymethyl)(methyl)carbamate **2t** (89 mg, 40%) as a colorless liquid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm 7.35–7.39 (m, 4 H), 7.29–7.34 (m, 1 H), 5.13 (s, 2 H), 4.73 (s, 2 H), 3.64 (sept, J = 6.09 Hz, 1 H), 2.89 (s, 3 H), 1.09 (s, 3 H), 1.08 (s, 3 H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 154.9, 136.6, 128.5, 128.1, 127.9, 68.8, 68.1, 67.2, 32.6, 22.3, 22.1.

4-Azidobenzyl (Methoxymethyl)carbamate 2u

According to the standard method mentioned above, constant current electrolysis of (((4-azidobenzyl)oxy)carbonyl)glycine **1aj** (102 mg, 0.41 mmol) in the presence of methanol (10 eq.) and triethylamine (0.05 eq.) yielded 4-azidobenzyl (methoxymethyl)carbamate **2u** (33 mg, 34%) as a yellow liquid. ^{1}H NMR ($CDCl_3$, 400 MHz,) δ ppm 7.35–7.41 (m, 2 H), 7.01–7.07 (m, 2 H), 5.50 (br s, 1 H), 5.12 (s, 2 H), 4.65 (br d, J = 7.13 Hz, 2 H), 3.36 (s, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) 156.3, 140.1, 132.9, 129.9, 119.2, 73.7, 66.4, 55.7.

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Statements and Additional Information

Conflict of Interest The authors declare that they have no conflict of interest.

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Contributors' Statement M.B.: Conceptualization, Data curation, Formal analysis, Investigation, Resources, Validation, Writing - original draft. S.N.: Conceptualization, Data curation, Investigation, Project administration, Supervision, Writing - original draft, Writing - review & editing. C.K.: Conceptualization, Project administration, Supervision, Writing - review & editing. S.K.M.: Funding acquisition, Project administration, Writing - review & editing.

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