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From Ugi Multicomponent Reaction to Linkers for Bioconjugation

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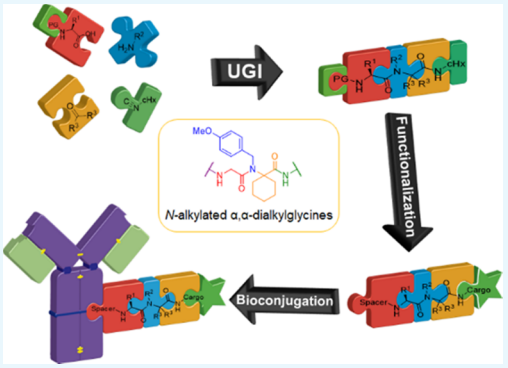
ABSTRACT: Bioconjugation is a key approach for the development of novel molecular entities with clinical applications. The biocompatibility and specificity of biomolecules such as peptides, proteins, and antibodies make these macromolecules ideal carriers for selective targeted therapies. In this context, there is a need to develop new molecular units that cover the requirements of the next generation of targeted pharmaceuticals. Here, we present the design and development of a versatile and stable linker based on a N-alkylated α,α-dialkyl dipeptide for bioconjugation, with a particular focus on antibody-drug conjugates (ADCs). Starting with the well-known Ugi multicomponent reaction, the convenient chemical modification of the prepared adducts allowed us the obtention of versatile bifunctional linkers for bioconjugation. A conjugation strategy was tested to demonstrate the efficiency of the linker. In addition, a novel cytotoxic anti-HER2 ADC was prepared using the Ugi-linker approach.



INTRODUCTION

The development of bioconjugation in recent years spans a broad range of bioconjugates, including biomolecules such as peptides, oligonucleotides, proteins, and antibodies, for biomedical applications. Due to the high potential of bioconjugates as medical treatments, the development of new methodologies that allow the synthesis of novel bioentities is desirable.[1,2]

Regarding antibody-drug conjugates (ADCs), considerable effort by the academic and industrial sectors has been channeled into designing antibodies armed with drugs, cytokines, toxins, and radionuclides, all of them with applications in cancer therapy.[3] The possibility of combining the favorable binding properties of monoclonal antibodies (mAbs) with the biocidal activities of potent cytotoxic agents promises to increase the therapeutic indexes of these antibodies.[4] At present, only four ADC products, namely, Adcetris,[5] Kadcyla,[6] Besponsa,[7] and Mylotarg,[8] have been approved for the treatment of certain types of cancer.

A growing number of parameters can be engineered into novel ADCs, including drug potency, targeting, and also appropriate linker selection. The linker is the most versatile aspect of the ADC as it contains the reactive group that governs the conjugation chemistry and serves as a chemical spacer that physically connects the drug payload to the antibody. The linker or handle can be modified in various ways to influence drug/linker characteristics (e.g., solubility) and ADC properties (e.g., potency, pharmacokinetics, therapeutic index, and efficacy in multidrug-resistant cells).[9]

Linkers can be subdivided into two categories: cleavable and non-cleavable. The acidic environment and proteases found in lysosomes and the reductive environment of the cytoplasm are some of the intracellular features exploited for drug release. Examples of cleavable linkers are those based on hydrazones,[10] disulfides,[11] or based on an enzymatically cleavable peptidic scaffold. Indeed, peptides can combine both systemic stability and rapid release of the drug inside the target cell. Optimized dipeptide-based linkers, such as those carrying the valine-citrulline sequence,[12] have shown promising results in terms of specificity and toxicity when compared to other labile linkers. As a result, the valine-citrulline platform is used for Adcetris and for several ADCs in clinical trials.[13]

In contrast, for non-cleavable linkers, it is assumed that the release of the drug takes place after internalization of the ADC in the target cell, which is followed by lysosomal degradation of the antibody to the amino acid level.[14] The bifunctional cross-linker succinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate is one of the most commonly used non-cleavable linkers in bioconjugation.[15,16] The choice of linker is often target- and drug-dependent as intracellular processes contribute to the generation of the fully active drug.

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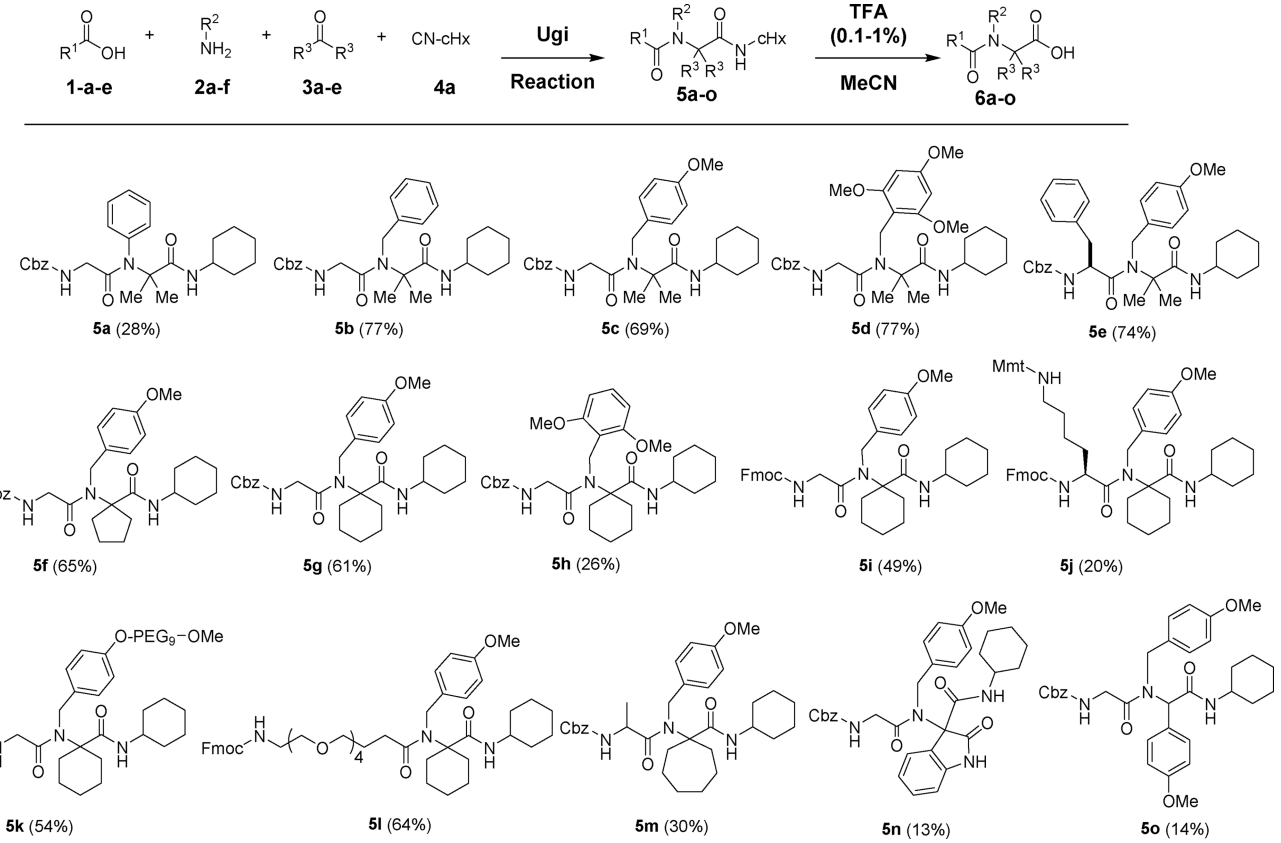
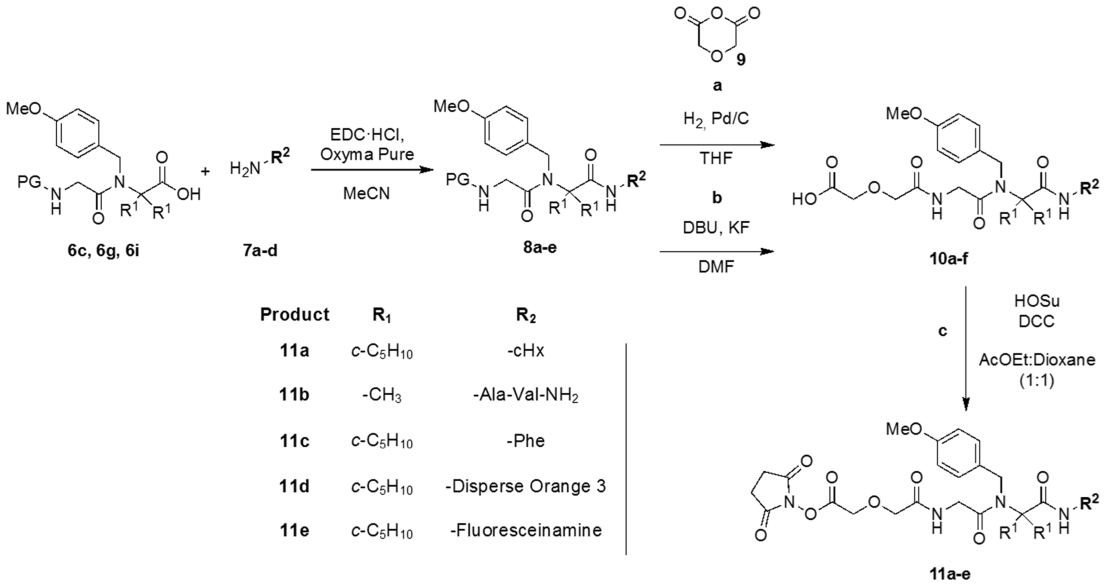
Scheme 1. Ugi Four-Component Reaction To Yield N-Alkylated α,α-Dialkylglycines (5a−n) (Yield in Brackets) Followed by C-Terminal Acidolysis. Compound5o Was Isolated as a By-Product of the Ugi Reaction with Isatin (See the Supporting Information). Mmt: 4-Monomethoxytrityl, Fmoc: 9-Fluorenylmethoxycarbonyl, Cbz: Carboxybenzyl, cHx: Cyclohexyl



Scheme 2. Bifunctionalization of Ugi Linkers and Activation with *N*-Hydroxysuccinimide (HOSu) for Bioconjugation. Reaction (a): One-Pot Cbz Hydrogenolysis and Amine Acylation with Diglycolic Anhydride (9) for Compounds8a−c. Reaction (b): Simultaneous Fmoc Group Elimination and Resultant Amine Acylation with 9 for Compounds8d and 8e. See the Supporting Information for Structures and Reaction Yields



Product	R_1	R_2
11a	c-C_5H_{10}	-cHx
11b	-CH_3	-Ala-Val-NH_2
11c	c-C_5H_{10}	-Phe
11d	c-C_5H_{10}	-Disperse Orange 3
11e	c-C_5H_{10}	-Fluoresceinamine

The classical Ugi reaction is a four-component coupling reaction (U-4CR) between an amine, a carbonyl compound (aldehyde or ketone), a carboxylic acid, and isocyanide, which yield a N-alkylated α,α-dialkylglycine when ketone is used as the carbonyl compound.[17] This 4-CR is one of the most important isocyanide-based multicomponent reactions to access peptide-like structures. It has been widely used in modern synthetic chemistry[18−24] and tentatively used for

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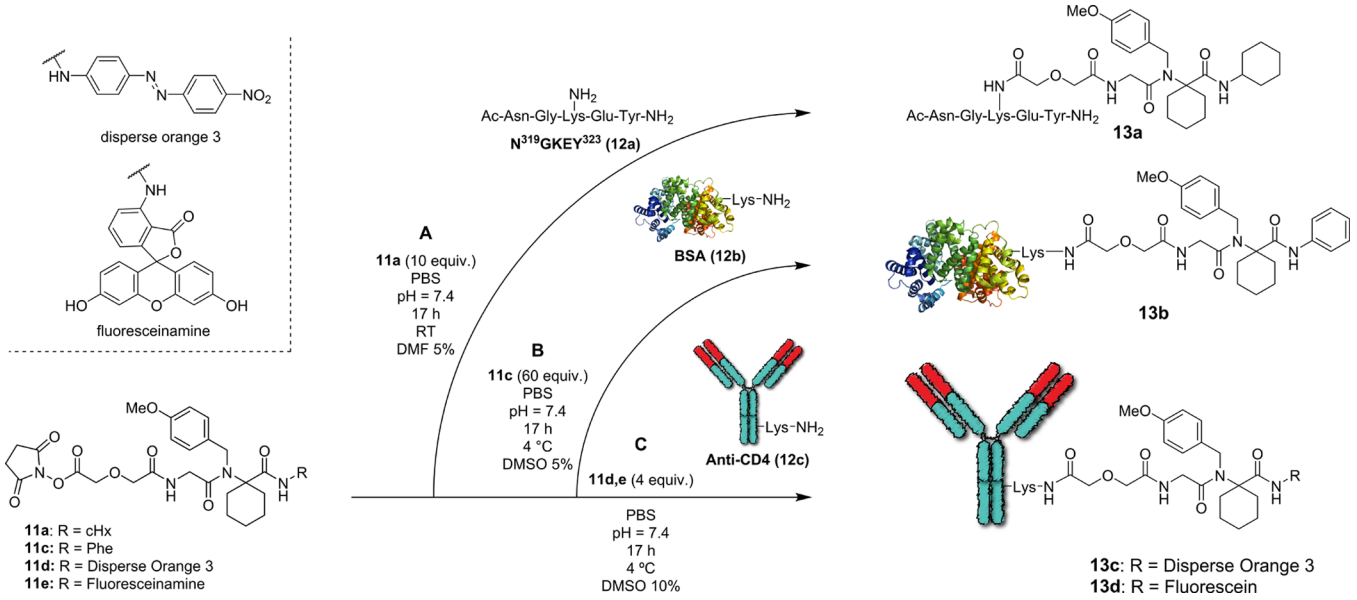
Scheme 3. Lysine-Mediated Bioconjugation with the Activated*N*-Hydroxysuccinic Ester Ugi Adducts to (A) Trastuzumab Pentapeptide, (B) BSA, and (C) Anti-CD4



direct bioconjugation.[18,25−28] In this regard and given the stability that N-alkylated α,α-dialkylglycines (Ugi adducts) confer to the peptidic scaffold, our attention was drawn to Ugi adducts as linkers for bioconjugation.

Here, we report the design of an efficient and robust strategy to synthesize and apply trialkylglycine derivatives (Ugi adducts) as versatile linkers for bioconjugation.

RESULTS AND DISCUSSION

Synthesis of N-Alkylated α,α-Dialkyl Glycines. The starting point for the general strategy was focused on the synthesis of trialkylglycine derivatives. To this end, we performed a one-pot synthesis, under Ugi conditions, of a small library of 1,4-dicarbonylic compounds based on α,α-dialkylglycines (Ugi adducts, Scheme 1), through the condensation of a carboxylic acid, a primary amine, a ketone, and cyclohexyl isocyanide. In this regard, all the Ugi reactions furnished the desired products **5a−n** in moderate to high yields and without evidence of amino acid racemization for compounds **5e** and **5j**. Compound **5o** was isolated as a by-product of the Ugi reaction with isatin (**5n**).

All Ugi derivatives were subjected to a C-terminal amide acidolysis with trifluoroacetic acid (TFA) solutions. As expected, the rate of acidolysis in the 1,4-dicarbonyl systems depends of the N-alkyl group and of the dialkylic substituents. Thus, the introduction of cyclic compounds into the dipeptidic scaffold forced the approximation of the central carbonyl group to the C-terminal amide by an angle reduction, and consequently, the acidolysis took place faster than with the acyclic compounds. Although most of the corresponding carboxylic acids were obtained from fast (**5d**, **5i**, and **5m**) to extremely slow (**5a** or **5o**) reaction times, only **6c**, **6e**, **6g**, and **6i−l** were quantitatively isolated (Supporting Information). In this regard, we continued our studies with **6c**, **6g**, and **6i** derivatives.

C-Terminal Functionalization. As a drug model, several aliphatic and aromatic amines were successfully incorporated into the free C-terminal carboxylic acid using an excess of the soluble carbodiimide (EDC·HCl) as the coupling agent, in the presence of Oxyma Pure as the additive, furnishing the corresponding amides in moderate to high yields after purification (Scheme 2). Of note, as the C-terminal lacks epimerizable α-proton, the adducts will not undergo racemization.

N-Terminal Functionalization. After removing Cbz or Fmoc from the Ugi adducts, amine functionalization is needed to anchor the amines present in the biomolecule. In this regard, a convenient approach to anchor the reactive amines of peptides and proteins is via amide bond formation. To this end, we functionalized the N-terminal of our dipeptidic scaffolds.

As a first approach for the Cbz-protecting group, palladium-catalyzed hydrogenolysis was done to yield the corresponding amines for further functionalization (see the Supporting Information). However, we detected diketopiperazine formation, which may hamper the reaction yield, so an alternative approach was developed. To avoid this side reaction, optimization was performed by a one-pot deprotection and acylation reaction. First, the Cbz hydrogenolysis was performed followed by in situ amine acylation using diglycolic anhydride (Scheme 2, reaction a). This strategy gave the desired derivatives **10a−d** in excellent yields and without further purification of the crude products (see the Supporting Information).

In the same manner, the Fmoc-protected dipeptides (**8d** and **8e**) were functionalized with the diglycolic moiety (Scheme 2, reaction b). To this end, simultaneous elimination and acylation of the protecting group were attempted. For Fmoc substrates, the N-terminal protecting group was removed using a strong and non-nucleophilic base (DBU), and potassium fluoride and diglycolic anhydride were added to trap the amine released, thereby allowing simultaneous Fmoc elimination and amine acylation. Although the reaction yields were low compared with the Cbz analogs (see the Supporting Information), the use of the Fmoc group was crucial when the amine attached to the C-terminal was hydrogen-sensitive.

To determine the stability of these Ugi adducts, compound **10b** (1 mg/mL) was incubated in male human serum type AB

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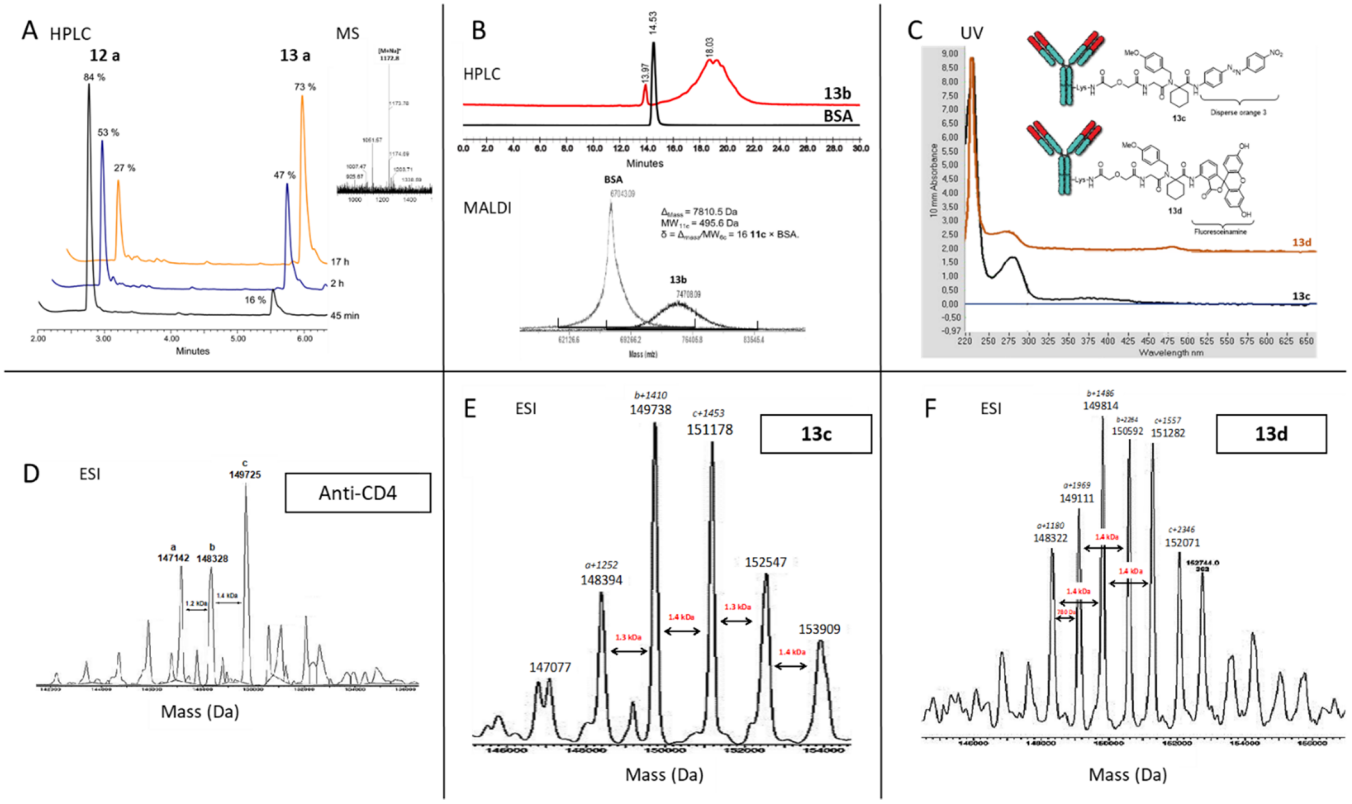

Figure 1. Conjugate characterization. (A) HPLC and HPLC-MS analysis of peptide **12a** conjugation at 45 min, 2 h, and 17 h reaction times. (B) HPLC and MALDI analysis of BSA and conjugate **13b**. (C) UV spectra of **13c** and **13d**. (D−F) ESI-MS analysis for deglycosylated anti-CD4 and conjugates **13c** and **13d**, respectively.

and Hank's Balanced Salt solution (HBSS) (9:1) at 37 °C for 24 h. The reaction was monitored by HPLC from 30 min to 24 h of incubation. After 24 h of treatment, no evidence of degradation of compound **10b** was found, thereby indicating the stability of the linker in human serum. Compounds **10a−c** were also treated with a milder acidolytic aqueous mixture (MES 0.1 M at pH = 4.8), and as expected, they did not undergo C-terminal amide cleavage. This inherent stability supports that the tested Ugi adducts are suitable non-cleavable linkers for bioconjugation.

Bioconjugation. To achieve the bioconjugation through the ε-amino (lysine) or N-terminal α-amino groups of biomolecules, we activated the carboxylic acids (**10a−h**) through *N*-hydroxysuccinimide esterification (Scheme 2, reaction c). This was achieved by treating the free carboxylic acid with an equimolecular amount of HOSu and a slight excess of dicyclohexylcarbodiimide as the coupling agent in a mixture of AcOEt:dioxane (1:1) as the solvent. The solvent mixture caused the precipitation of dicyclohexyl urea and facilitated its elimination from the reaction crude product by filtration, quantitatively yielding the activated HOSu esters (**11a−e**).

With the activated *N*-hydroxysuccinic esters in hand (**11a−e**), the bioconjugation to a peptide, a protein, and a monoclonal antibody was tackled. First, these esters were conjugated to a Lys-containing peptide. For this purpose, the pentapeptide $N^{319}GKEY^{323}$ (**12a**), selected from the heavy chain of the humanized IgG1 monoclonal antibody trastuzumab (anti-HER2), was manually synthesized by solid-phase peptide synthesis using the Fmoc/tBu strategy to yield the N-acetyl and C-amidated pentapeptide (see the Supporting Information). The Lys conjugation was performed by adding the activated carboxylic acid (10 equiv.) in DMF (5%) to a solution of the synthesized peptide in PBS at pH = 7.4 for 17 h at room temperature (Scheme 3A). HPLC and HPLC-MS analyses (Figure 1A) revealed the formation of conjugate **13a** (73% HPLC conversion).

After observing efficient lysine conjugation in a peptidic sequence, we moved on to address bioconjugation in a more complex system. For this purpose, we bound compound **11c** to bovine serum albumin protein (BSA, **12b**) (Scheme 3B). Analysis of the resulting BSA-conjugate **13b** (Figure 1) showed, as expected for lysine-mediated ligation, a heterogeneous conjugation to the lysine of BSA (broad HPLC peak observed). Moreover, the conjugation was efficient. Mass analysis determined that 16 molecules of **11c** were attached to the BSA surface when 1.7 equiv. of compound **11c** × one molecule of BSA (total 60 equiv.) were used. To test the stability of the conjugate, **13b** was treated with a MES 0.1 M buffer at pH = 4.8 at 37 °C for 24 h. No appreciable hydrolysis was observed.

Going a step further, conjugation to anti-CD4, a monoclonal antibody (Scheme 3C), was performed. A solution of activated **11d** (4 equiv.) and **11e** (4 equiv.) in DMSO was added separately to a solution of anti-CD4 (1.5 and 1.0 mg/mL for **11d** and **11e**, respectively) in PBS at pH 7.4 and was left to react for 17 h at 4 °C. The excess linker was then removed using a size-exclusion column, and the fractions obtained were characterized (Figure 1C−F and Supporting Information). UV analysis of **13c** and **13d** (Figure 1C) revealed the variation of the UV spectra at 375 and 490 nm, respectively, thereby confirming the conjugation. This finding indicates that the

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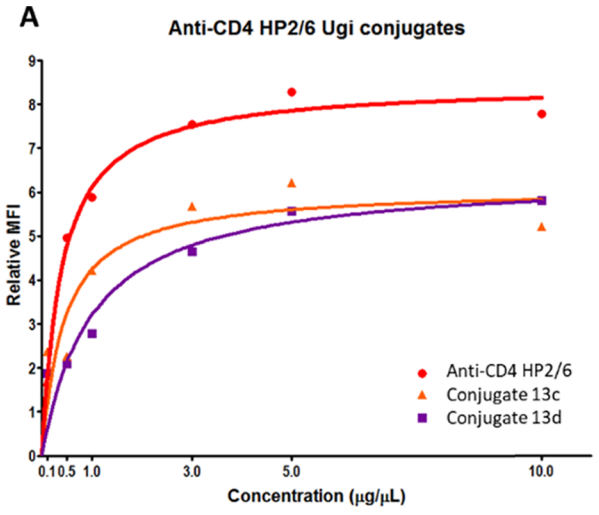
conjugated fluorophores were attached to the antibody. Using ESI-MS and after antibody deglycosylation with PNGase F, we detected at least two molecules of **11c** and **11d** attached to the antibody.

Antibody Affinity Is Not Hampered by Conventional Amine Bioconjugation. To determine whether the conjugation of **11c** and **11d** to the anti-CD4 HP2/6 mAbs affected the binding to their epitopes, we performed an antibody binding analysis with the Jurkat cell line that constitutively expresses CD4 (Figure 2). We used increasing concentrations



B

	AC_{50} (μg/mL)	Max MFI (%)
Anti-CD4	0.38	100
Conjugate 13c	0.53	59
Conjugate 13d	0.57	58

Figure 2. (A) Cell binding analysis by flow cytometry of anti-CD4 and conjugates **13d** and **13c** to Jurkat cells. The cell line was incubated with the indicated concentrations of mAb or the conjugates **7d** and **7c** followed by incubation with FITC-labeled goat anti-mouse IgG antibodies. The mean fluorescence intensity (MFI) ratio was calculated using the MFI value of an irrelevant mAb (basal MFI value) as reference. (B) AC_{50} and Max MFI (%) for mAb anti-CD4 and its conjugates (**13c** and **13d**). AC_{50}, calculated as the antibody concentration (AC) needed to achieve 50% of the highest MFI value for each conjugate.

(0.1−10 μg/mL) of the naked antibody (anti-CD4 HP2/6 mAbs) and its corresponding conjugates (**13c** and **13d**). Accordingly, **13c** and **13d** were still active against CD4 with a slight reduction in binding affinity compared to the anti-CD4 mAb (AC_{50} 0.53 and 0.57 μg/mL compared to 0.38 μg/mL, respectively). This result suggests that **13c** and **13d** coat more than 50% of all available CD4 molecules on the cell surface. This decrease in binding affinity could result from lysine conjugation, which may affect the antigen-binding site or even the constant region of the antibody, which the secondary antibody recognizes.

Anti-HER2 ADCs Linked with UGI Adducts Show Specific Targeting against HER2$^+$ Cancer Cells. As a final step to validate these linkers in a biological setting, we conjugated an antitumoral tubulin-targeting payload with trastuzumab, a therapeutic antibody. The potential of the resulting ADC to inhibit cell growth was tested, as was its selectivity against tumor cells expressing the trastuzumab target, that is, the receptor tyrosine-protein kinase erbB-2, also known as HER2. The marine molecule PM050489,[29] which binds tubulin with pM affinity and causes cell death with potencies in the same sub-nM,[30] was used to generate the derivative PM120028, which was modified to incorporate the Ugi adduct decorated with a maleimide functionality to allow conjugation at Cys residues in trastuzumab.

The resulting ADC, named MI150011 (structure shown in Figure 3), was tested for its capacity to impair cell growth in four human breast cancer cell lines, namely, SK-BR-3 (HER2 positive), HCC-1954 (HER2 positive), MDA-MB-231 (HER2 negative), and MCF-7 (HER2 negative). MI150011 yielded IC_{50} values in the nM range against the HER2-positive lines, whereas its potency decreased around 1 order of magnitude against HER2-negative cells (Figure 3).

Although the potency of MI150011 was significantly lower than that of the initial PM120028 molecule, its greater activity in HER2-positive lines indicates a degree of selectivity that was absent in the latter. This selectivity demonstrates that MI150011 shows adequate stability in solution and that its conjugation to the antibody does not preclude the ability of the latter to interact with its antigen, hence validating the use of this linker for the preparation of novel therapeutic entities.

CONCLUSIONS

Here, we described an efficient strategy to synthesize and apply trialkylglycine derivatives (Ugi adducts) as linkers for bioconjugation. Correspondingly, a small library of 15 N-alkylated α,α-dialkylglycines were synthesized in moderate to high yields by an Ugi multicomponent reaction and conveniently characterized. These adducts led to the C-terminal carboxylic group under TFA solutions in organic solvents. However, they are totally stable under low acidic conditions in aqueous media, thereby making them strong candidates as non-cleavable linkers for bioconjugation due to their intrinsic stability.

The demonstrated ease with which both the C- and N-termini of the designed dipeptidic-based linkers can be selectively modified in different manners reveals an interesting versatile system with broad applicability in the field of bioconjugation. As shown, these linkers allow the introduction of distinct kinds of amines, as drug models, to the C-termini using standard coupling conditions. Even more interesting is that the lack of epimerizable α-proton could allow the use of stronger coupling reagents. In addition, the reactive amino group at the N-termini could allow straightforward modification for further activation for conjugation. Here, we identified an interesting strategy involving the simultaneous elimination of the protecting group and acylation with a symmetric anhydride (one-pot reaction) that yielded a free carboxylic acid available for bioconjugation. All the reaction conditions were optimized to finally obtain high reaction yields and purity of the crude products.

Furthermore, we found that mild conjugation strategies for amine and cysteine amino acids through different biomolecules were successful and did not affect the integrity or properties of the biomolecules. Despite the conjugation process, the ADCs synthesized did not lose their ability to bind to CD4 in Jurkat cells in comparison to the naked antibody. Going one step further, we prepared a novel cytotoxic anti-HER2 ADC using one of the Ugi linkers described herein. The cell viability studies with this ADC showed specificity toward HER2-

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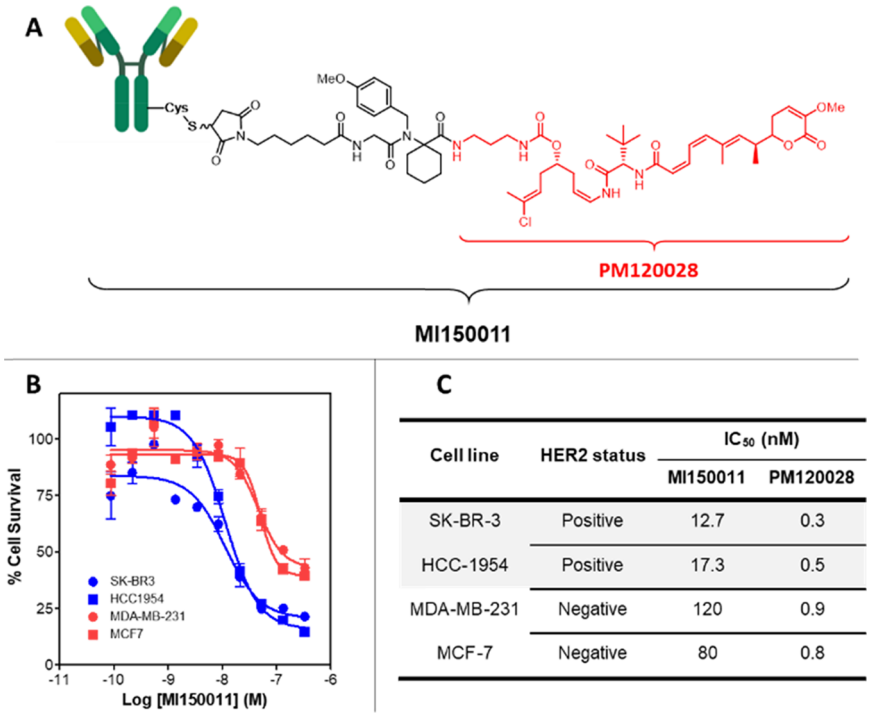

C

Cell line	HER2 status	IC_{50} (nM)	
		MI150011	PM120028
SK-BR-3	Positive	12.7	0.3
HCC-1954	Positive	17.3	0.5
MDA-MB-231	Negative	120	0.9
MCF-7	Negative	80	0.8

Figure 3. (A) Structure of the ADC **MI150011** and the initial molecule PM120028. (B) Dose−response curves showing the effect of MI150011 on cell survival after 72 h of incubation with breast cancer cell lines. Error bars correspond to the standard deviation of triplicate samples. (C) IC_{50} values for MI150011 and PM120028 in cell lines with different HER2 expression levels.

positive cell lines, thereby confirming the utility of Ugi adducts to prepare new linkers for conjugation.

EXPERIMENTAL SECTION

Ugi Multicomponent Reaction. The corresponding ketone (1 equiv. or excess) and the primary amine (1 equiv.) were mixed for 15 min at room temperature in the presence of Na_2SO_4. Liquid ketones were used as a reaction solvent. For high-boiling-point or solid ketones, 2 mL of MeOH was added to the reaction. Then N-protected amino acid (1 equiv.) was added, and the mixture was stirred for 10 min. After that, cyclohexyl isocyanide (1 equiv.) was added to the mixture, which was left to react for 1 h to 2−3 weeks, depending on the progression of the reaction, at room temperature and in the dark to avoid side reactions. The suspension was then filtered, and the solvent was removed under vacuum. Finally, the crude was purified through chromatographic techniques or by recrystallization to obtain the final product with adequate purity. All starting amines were commercially available, except 4-MeO-PEG_9-benzylamine (**2k**), which was previously prepared by the ether formation between 4-cyanophenol and the bromo-PEGylated derivative followed by nitrile reduction to yield 4-MeO-PEG_9-benzylamine (two steps, 58%, see the Supporting Information).

Acidolysis of Ugi Adducts. The protected amides **5a−o** (1 mg/mL in MeCN) were treated with 0.1−1% TFA solutions. Some molecules needed mixtures of 0.1−1% TFA in H_2O/MeCN (1:1) (see the Supporting Information).

C-Terminal Functionalization. The free C-terminal carboxylic acid dipeptide (1 equiv.) was treated with the corresponding amine (1 to 5 equiv.) using EDC·HCl (from 1 equiv. to excess) as the coupling reagent and Oxyma Pure (1 to 9 equiv.) as an additive in MeCN or DMF as the solvent. The reaction led to carboxylic consumption, as monitored by HPLC. The solvent was removed under reduced pressure. The solid was dissolved in AcOEt (25 mL), and the organic layer was washed with water (3 × 20 mL), saturated Na_2CO_3 (3 × 20 mL), and brine (3 × 20 mL) and dried over $MgSO_4$, and the solvent was then removed. When needed, the final product was isolated by automatic purification on a prepacked Redisep Rf Gold C_{18} 13g column by using H_2O/MeCN from 90:10 to 0:100 over 20 min. The collected fractions were lyophilized to yield **8a−e**.

One-Pot Protecting Group Removal and Amine Acylation. Cbz: The Cbz-protected dipeptide (**5d**, **5g**, **5m**, or **8a−c**) was dissolved in dry THF. Diglycolic anhydride (1 equiv.) and Pd/C catalyst (10% weight) were then added, and the system was purged with N_2 and H_2. The reaction was stirred under a H_2 atmosphere at room temperature until complete Cbz elimination. The reaction was followed by HPLC. The solution was filtered over Celite, and the solvent was removed under reduced pressure to yield the N-terminal acylated dipeptides.

Fmoc: The Fmoc-protected compound **8d** or **8e** (1 equiv.) in DMF, diglycolic anhydride (1 equiv.), KF supported on Al_2O_3 (3 equiv.), and DBU (1.2 equiv.) were added, and the mixture was stirred under a N_2 atmosphere until complete Fmoc elimination. The mixture was then filtered, and the solvent was removed under reduced pressure. The crude was purified on a prepacked Redisep Rf Gold C_{18} 13g column using H_2O/MeCN from 90:10 to 0:100 over 20 min. The fractions were collected and lyophilized obtaining the N-acylated compound **10g** or **10h**.

HOSu Activation. A solution of the corresponding carboxylic acid (**10a−f**) in dioxane/AcOEt (1:1) was treated with *N*-hydroxysuccinimide (1−2 equiv.) and DCC (1−2 equiv.). The reaction was stopped when the starting COOH disappeared (HPLC). The solution was filtered over Celite and

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then passed through a 0.2 μm filter. The solvent was removed under reduced pressure. The products were used for conjugation without further purification.

Bioconjugation. A solution of the activated OSu esters **11a−e** (1.5−60 equiv. per lysine in the biomolecule) in DMF/DMSO (2−10%) was added to a biomolecule-containing solution (0.5−11 mg/mL) in PBS (pH = 7.4). The mixture was left to react for 17−24 h. The excess unreacted molecules were then removed by PD-10 desalting column purification using water as the eluent. Next, the protein-containing fractions were collected and characterized.

Binding Affinity Experiments. Jurkat (5×10^5) cells were first incubated with 50 μg/mL γ-globulin for 10 min and then with the anti-CD4 HP2/6 monoclonal antibody (mAb) or the corresponding conjugates at the indicated concentrations for 1 h on ice. Cells were then washed with PBS twice and incubated with 10 μg/mL FITC-labeled goat anti-mouse IgG antibodies (BD Biosciences) for 30 min on ice. Finally, they were washed twice and resuspended in PBS for their analysis by flow cytometry using a FACSCanto II cytometer.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c00099.

Synthetic and analytical details (methodologies, HPLC, NMR, UV−vis, ESI-MS, and SDS-PAGE) (PDF)

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Author Contributions

I.R.-T. did the experimental synthesis of Ugi adducts and bioconjugation and data analysis. G.P.-C., B.S.-C., F.S.-M., and J.M.Z performed binding affinity experiments and data analyses of the results. C.C. and J.M.D. did the synthesis and data analysis of the ADC MI150011 and its component molecule PM120028. I.R.-T, H.R., and F.A. wrote the paper with contribution and revision of all the other authors and designed the experiments. H.R. and F.A. supervised the project. F.A., C.C., J.M.Z., and F.S.-M. conceived the project MarinMab.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

ADC, antibody-drug conjugate; Mmt, 4-monomethoxytrityl; Fmoc, 9-fluorenylmethoxycarbonyl; Cbz, carboxybenzyl; cHx, cyclohexyl

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