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ARTICLE

Aziridine Based Electrophilic Handle for Asparatic Acid Ligation

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Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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A one-pot ligation strategy at aspartic acid junction has been developed by successfully incorporating aziridin-2,3-dicarboxylate to the *N*-side of a peptide fragment, affording *N*-aziridine appended peptide, which was ligated in solution phase with a variety of small peptide thio acids to afford native peptides, following ring-opening/peptidyl migration/desulfurization strategy. The reaction proceeds in highly regiospecific manner, and provides short native peptides in good isolable yields. A variety of aspartame based peptides were synthesized to showcase the generality of aziridine based ligation. Computational studies have also been performed to get insight about the reaction pathway.

Introduction

Peptide ligation is an ever-emerging field that targets to discover novel synthetic strategies for peptide synthesis. Several advancements have been witnessed in recent years that marked a notable impact to the current literature on peptide synthesis. The key strategy in peptide ligation involves the coupling of two peptide fragments in a chemo-selective manner. In this context, Kent's group reported pioneer work in exploring native chemical ligation (NCL) towards chemo-selective coupling of two unprotected peptide segments at cysteine junction.[1a-b] NCL methodology has revolutionized the area of protein synthesis, and served as powerful tool for constructing various protein targets such as glycoproteins, phosphoproteins, oncogenic KRas(G12V), ubiquitinated peptide conjugates, membrane protein, human chemokine hCCL21, erythropoietin, enzyme barnase, and many more.[2a-i] However, limitations associated with NCL strategy such as requirement of a cysteine residue at *N*-side of one of the peptide fragment, limits its usage. Thus, several modifications have been proposed to broaden the scope of NCL based peptide synthesis in the past decade. One such breakthrough was reported by Dawson's group, where desulfurization was introduced after ligation step for synthesizing non-cysteine containing proteins, exemplifying the ligation at alanine junction.[3] This strategy emerges an idea of generating cysteine surrogates by suitably placing the thiol auxiliary at natural amino acid templates that could be employed in peptide synthesis *via* ligation-desulfurization strategy. This ligation-desulfurization strategy has extended the scope of NCL towards the construction of peptides at specific amino acid junctions such as leucine,[4a-b] valine,[5a-b] phenylalanine,[6a-b] glutamic,[7] lysine,[8] proline,[9a-c] tryptophan[10] and aspartic acid.[11a-c] Along with NCL, other ligation strategies have also been emerged time-to-time to advance the peptide syntheses, namely Staudinger ligation[12a-d] that utilizes the scope of Staudinger reaction, KAHA ligation[13a-b] that ligates two peptide fragments, one having α-ketoacid at *C*-side and another having *O*-unsubstituted hydroxylamine at *N*-side of the peptide fragment and others. These emerged peptide synthetic strategies undoubtedly have provided significant advancements in this field, however they are associated with inherent challenges, and thus provide the scope of further advancements especially with vast array of complex amide functionalities.

In striking contrast, site-selective aziridine ring-opening has been explored reasonably in the area of peptide synthesis.[14a-d] In this context, Tam's group showcased the peptide ligation protocol by orthogonal coupling of two unprotected peptide fragments, one having thio acid at the *C*-side of peptide fragment, and another having β-bromo amino acid at *N*-side of peptide fragment. During this ligation process, an aziridine ring was formed that undergoes subsequent ring-opening by thiocarboxylate to afford α- and β- peptides.[15] However, no further investigations were reported based on this protocol. In 2011, Garner's group utilized the aziridine ring to ligate two peptide fragments *via* Cu(I)-mediated acylation, followed by hydrolysis to produce serine/threonine amino acid at ligation junction,[16a-b] however this moiety has not much explored as an electrophilic handle in peptide ligation. In order to expand the utilization of aziridine ring in peptide ligation at particular amino acid junction, we have recently exploited suitably derivatized aziridine moiety as cysteine surrogate in NCL, and exemplified ligation at phenylalanine and tryptophan junctions.[17] While extending the scope of this ligation methodology, in present work, we aimed to utilize inexpensive

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Electronic Supplementary Information (ESI) available: [Copies of original ^{1}H NMR, COSY, HMQC, HMBC and ^{13}C NMR spectral data are provided.
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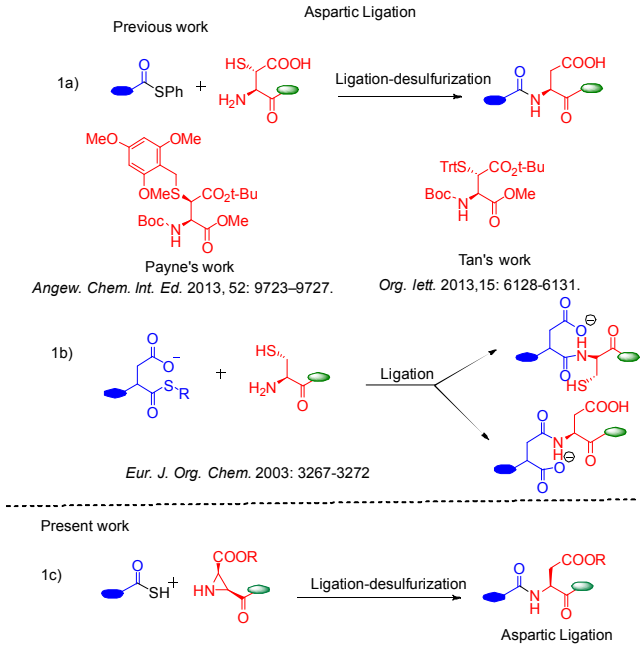
aziridin-2,3-dicarboxylate towards studying the scope of peptide ligation at aspartic acid junction.

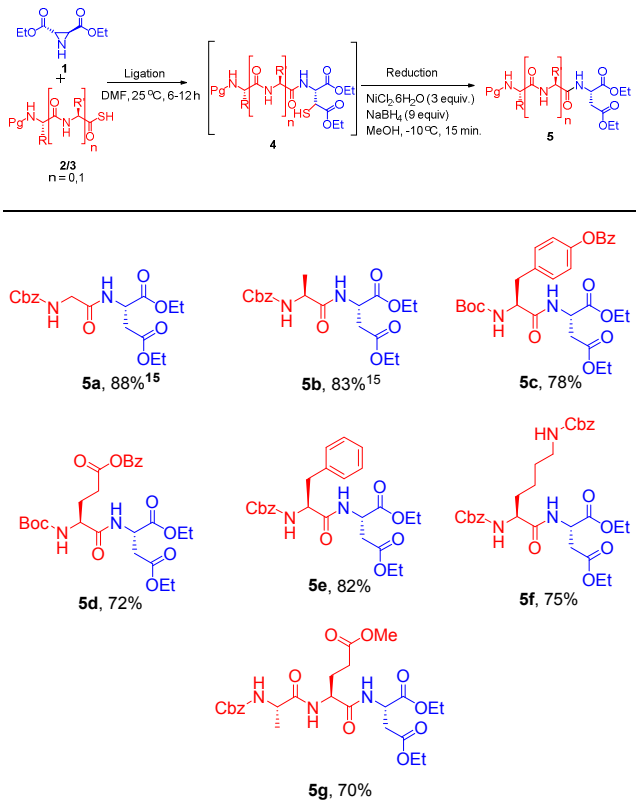
Previously reported peptide ligation methods at aspartic acid junction involves: (i) the use of *β*-thioaspartic acid unit at the *N*-side of peptide fragment, or (ii) the use of peptide fragment having aspartic acid at the *C*-side on NCL based ligation.[11] In context to the first method, Payne and Tan's group's independently showcased the use of differently protected *β*-thioaspartic acid unit as cysteine surrogate in producing a variety of aspartic acid based native peptides (Scheme 1a).[11b-c] However, there are numerous challenges involved in preparing *β*-thioaspartic acid such as the use of sophisticated sulfenylating agents, tedious and multi-step low-yielding protocols. While in the second method, competitive formation of isopeptides are observed during ligation process (Scheme 1b).[11a] Acknowledging the importance of aspartic acid unit in various naturally occurring peptides (RGD *etc*), and artificial sweetener aspartame (aspartyl-phenylalanine methyl), we have demonstrated the use of dialkyl (*S*,*S*)-aziridine-2,3-dicarboxylate as an electrophilic handle to obtain a series of aspartic acid based native peptides in a one-pot fashion *via* ligation-desulfurization (Scheme 1c).



Scheme 1. Previous (1a-b) and present work (1c) on aspartic acid ligation

Results and Discussion

To investigate our studies, we first plan to perform the reaction between (*S*,*S*)-diethyl aziridine-2,3-dicarboxylate (**1a**) and Cbz-protected glycyl thioacid (**2a**) *via* recently explored one-pot ligation-desulfurization strategy.[17] Gratifyingly, the aziridine ring-opening followed by *S* to *N* acyl-migration proceeded smoothly at 25 °C using DMF without using any external additive, affording thiol substituted peptide intermediate **4a** in 4 h. Formation of **4a** was confirmed by HRMS. Intermediate **4a** when further exposed to reduction conditions yielded the corresponding aspartic acid appended dipeptide (**5a**) in 88% yield (Scheme 2). The scope of this strategy was now further extended to a variety of other amino thioacids (**2**) and peptide thioacids (**3**) to afford their corresponding aspartic acid containing di- and tripeptides (**5a-g**) (Scheme 2). All the products were obtained as single regioisomer in good-to-excellent yields (70-88%). In this overall process of ring-opening-migration and thiol reduction, the configuration at the other carbon of (*S*,*S*)-aziridine-2,3-dicarboxylate and the chiral carbon of amino acyl moieties are always retained. This was quite evident by the high diastereomeric excess as observed from the ^{1}H NMR spectra (>97% *de*) of the resultant peptides (**5a-g**). (Supporting Information)



Scheme 2. Aziridine based ligation to produce aspartic acid appended di- and tripeptides.

To further extend the scope of this strategy towards synthesizing aspartame appended peptides in solution phase, we plan to affix aziridine-2,3-dicarboxylate (**1a-b**) to the *N*-terminal of a phenylalanine moiety. Thus, alkaline hydrolysis of aziridine-2,3-dicarboxylates (**1a-b**) under controlled conditions in methanol was carried, which on coupling with L-Phe-OMe using DPPA as coupling reagent yielded aziridine tagged phenylalanine derivatives (**6a-b**) in 62-64% yields (Scheme 3).

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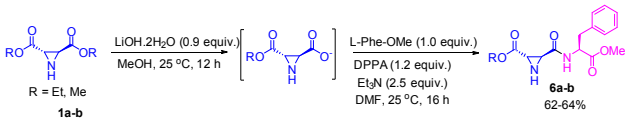
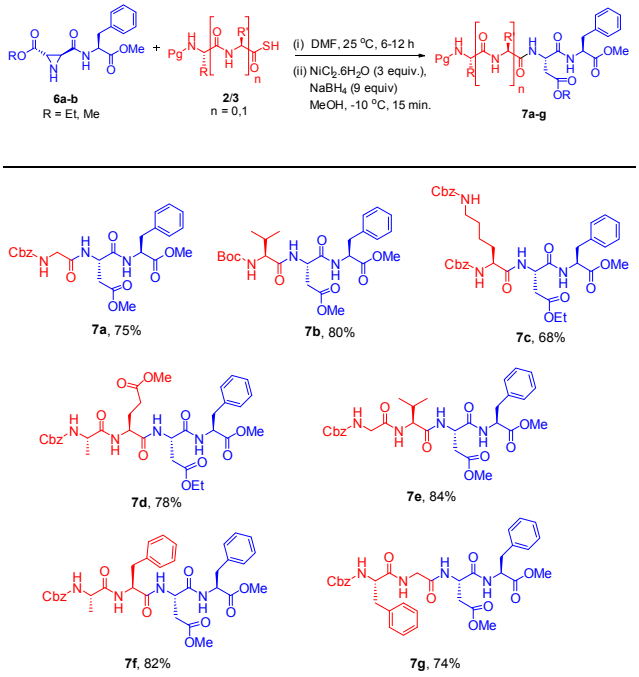


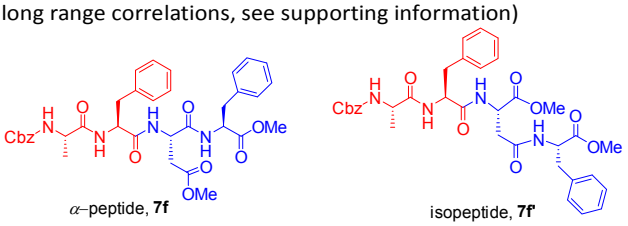
Scheme 3. Tagging of aziridine to *N*-side of phenylalanine methyl ester

These two aziridine tagged phenylalanine derivatives (**6a-b**) were then explored as electrophilic handles for different amino thioacids **2** and peptide thioacids **3** to afford aspartame containing tri- and tetrapeptides (**7a**-**g**) in good-to-excellent yields *via* our optimized one-pot ligation-desulfurization strategy (Scheme 4). All the isolated native peptides were completely characterized by detailed spectroscopic analysis. Interestingly, here again the products were obtained as single regioisomers with retention of configuration at stereocentres.



Scheme 4. Synthesis of aspartame containing native peptides.

To ascertain the formation of *α*-isomer (**7f**) (formed by selectively ring opening at *C*-2 position) over isopeptide (**7f'**) (that would have been formed by selectively ring opening at C-3 position), COSY, HSQC and HMBC studies (Figure 1) were performed on one of the representative peptide, Cbz-L-Ala-L-Phe-L-Asp(OMe)-L-Phe-OMe (**7f**). All the proton and carbon peaks of tetrapeptide, Cbz-L-Ala-L-Phe-L-Asp(OMe)-L-Phe-OMe (**7f**) were assigned *via* COSY and HSQC spectrum respectively (Supporting Information for COSY and HSQC). Long range ^{1}H-^{13}C correlation (HMBC) spectra implied the correlation of Asp-OCH_3 (δ 3.59 ppm) protons with Asp-CO ester peaks (δ 171.7 ppm), which in turn correlates with CH_2 protons of aspartic acid (δ 2.58 and 2.73 ppm). These specific correlations along with other spectroscopic data confers the compound to be *α*-peptide **7f** but not isopeptide **7f'**. (For detailed study on other long range correlations, see supporting information)



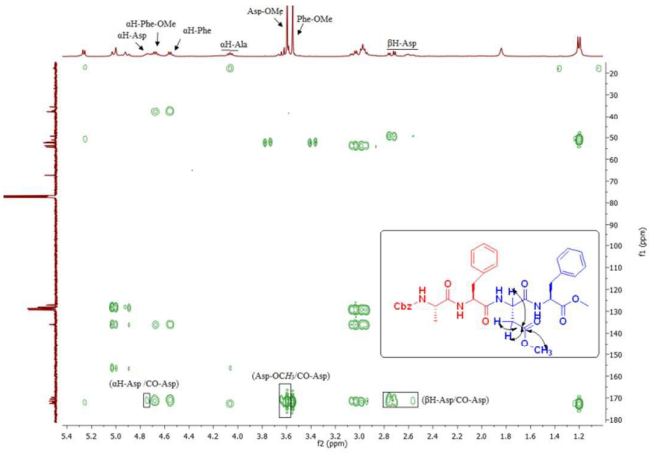
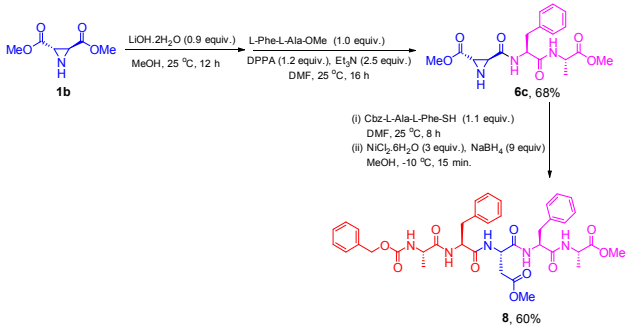


Figure 1. HMBC spectrum of tetrapeptide, **7f** (only selective correlations are indicated)

Finally, we applied the designed aziridine-mediated ligation protocol for preparing a pentapeptide in solution phase. For this purpose, aziridine appended dipeptide (**6c**) was synthesized in a one-pot two step procedure by hydrolyzing aziridine-2,3-dicarboxylate **1b**, followed by coupling with dipeptidyl ester (L-Phe-L-Ala-OMe). This aziridine handled dipeptide **6c** was subjected to one-pot ligation-desulfurization strategy, using dipeptidyl thioacid (Cbz-L-Ala-L-Phe-SH) to yield aspartame based peptapeptide (**8**) in 60% yield. (Scheme 5) This extended experiment further confirms the scope of presented aziridine-mediated one-pot ligation-desulfurization method for peptide synthesis.



Scheme 5. Aziridine based ligation to produce aspartic acid appended pentapeptide

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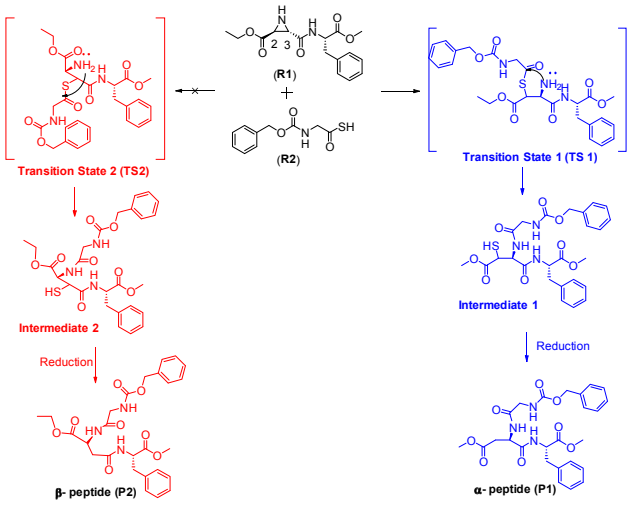
Computational Analysis

To get an insight into reaction mechanism, we performed computational experiments to understand the relative energies in the reaction pathway. The systematic representation of attack of thioacid to aziridine **6a** is represented in Scheme 6.



Scheme 6. Schematic representation of peptide formation *via* aziridine-mediated ligation

The techniques employed include a full conformation search followed by scoring of the conformers based on the spatial distances between the reactive atoms. The full conformation search was performed considering rotatable bonds, and the calculations were executed by using the MMX force field, in PCMODEL v.9.3 software.[18a] The best pre-organized conformer for each compound is obtained. Figure 3 presents the optimized geometry obtained at the BP86/def-TZVP[19] level of theory for the reactants (R), transition states (TS), and product (P) for reactions shown in Scheme 6. As the reaction goes from the transition state to the product, the lone pair of nitrogen attacks the carbonyl group of O=C–S followed by acyl migration and the C-N bond becomes completely formed. The reaction process can also be monitored by observation of the changes in the energy levels. In the transition state, the density map mainly delocalizes to the N and C atoms. In the product, the density map mainly focuses on the C atom. Some spin density also delocalizes into the nearby connected methyl and carbonyl group. The optimized geometry, relative energies, dipole moment and HOMO-LUMO gap were also calculated for intramolecular rearrangement as indicated in Figure 2.

We further investigated the minimum-energy conformations of the radicals, in which the substitution group and the thiol group are always in *trans* configuration. The optimized geometry and energies for all of the structures are given in the Figure 2 & 3.

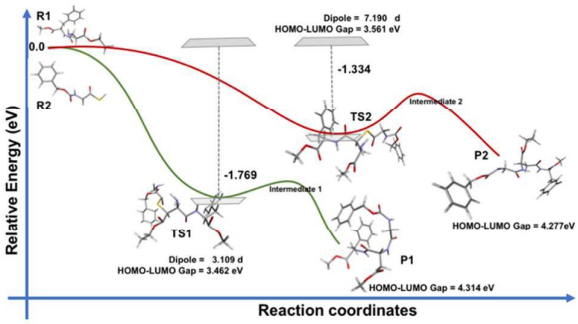


Figure 2. Energy profile diagram showing the optimized geometries, relative energies, dipole moment and HOMO-LUMO gap of possible transition states/intermediates of the reaction pathways

Figure 3 shows the optimized geometry of transition state. As the reaction progress from the reactant to the transition state, the distance between the C and N atoms changes substantially from 4.606 Å to 3.651 Å in TS1, and the bond length of C-N changes from 4.397 Å to 3.684 Å with respect to the energy profile calculations. The frontier molecular orbitals of a compound are at the "frontier" of electron occupation—the highest-energy occupied and lowest-energy unoccupied molecular orbitals (the HOMO and LUMO).[19b] The HOMO is logically viewed as nucleophilic or electron donating, while the LUMO is electrophilic and electron accepting. Furthermore, both chemical reactions and resonance can be explained by interactions (overlap) between a filled HOMO and an empty LUMO on one or more molecules.

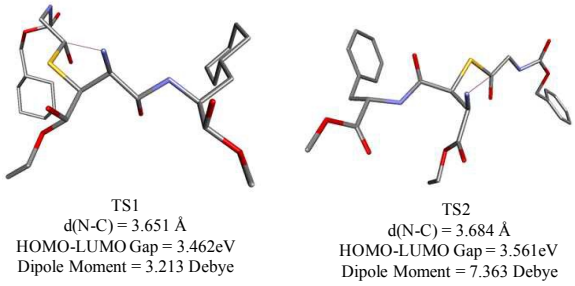


Figure 3. Preorganized transition states (TS1 and TS2)

Energy of FMOs (Frontier Molecular Orbitals - HOMO and LUMO) of a chemical species plays the key role in nucleophilic/electrophilic behaviour of a chemical species. Higher HOMO energy corresponds to the more reactive molecules towards electrophiles while lower LUMO energy is essential for nucleophilic attacks. Larger the HOMO-LUMO, energy gap the harder is the molecule. Based on the minimum HOMO-LUMO gap and lower bond distance of N-C including the lower dipole moment proves that TS1 is more favourable to yield α-Peptide (P1). Due to polar nature of sulfur atom, both nucleophilic and electrophilic attacks are possible at S center in organosulfur compounds. It is expected that replacement of H-atom of thiol group (-SH) enhances its nucleophilic nature that undergoes ring opening of aziridine ring at C-2 position linked with ester group as it would

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enhance the electrophilic nature at C-2 carbon more than C-3 position linked with amide group. We can also conclude that steric hindrance also plays a crucial role in this reaction. Stability of a species has been correlated with global hardness by maximum hardness principle; proposed by Parr and Pearson.[20] It states that maximum hardness leads to maximum stability including favourable chemical changes.

Conclusions

In conclusion, an amide-forming aziridine based ligation approach has been introduced that extended the scope of proposed ligation to aspartic acid junction. The strategy requires an aziridine based electrophilc handle to the *N*- side of an peptide fragment that ligate with various peptide thio acids to produce native peptides in good yields. This methodology adopts one pot ligation-desulfurization approach and could be extended to the other amino acid linkage sites by introducing properly designed aziridine handle to *N*- side of one of the peptide fragment. The proposed ligation reaction works smoothly and provides a new route to synthesize aspartic acid based peptides with highly regiospecfic manner. The present strategy has opened new doors for extending the scope of chemical ligation at asparatic acid junction for preparing long chain peptides of choice.

Experimental Section

General Experimental Methods. All chemicals were obtained from commercial suppliers and used without further purification. All reactions were carried out in oven-dried glassware with magnetic stirring. Purification of reaction products was carried out by flash column chromatography using silica gel 60 (100-120 mesh) and TLC visualization was accompanied by UV light, $KMnO_4$ and Phosphomolybidic acid stains. Nuclear Magnetic Resonance spectra were recorded on Bruker 400 spectrometer. All ^{1}H NMR (400MHz) and ^{13}C NMR (101 MHZ) spectra were recorded either in $CDCl_3$ or DMSO-d_6 with TMS as standard in δ units, parts per million (ppm). All coupling constants J were reported in Hz. The following abbreviations were used to describe peak splitting patterns when appropriate: s = singlet, d = doublet, t = triplet, dd = doublet of doublet, m = multiplet and brs = broad singlet. High resolution mass spectrometry (HRMS) was performed with an Agilent 6210 instrument using time-of-flight (TOF-MS) with electro spray ionization (ESI). Melting points were determined on a capillary point apparatus equipped with a digital thermometer and are uncorrected.

Computational Analysis. The full conformation search was performed by using the MMX force field, in PCMODEL v.9.3 software.[18a] The molecular structure rearrangement is an important factor to understand the best global minimum energy conformer.[18b] All of the reactant, product, and transition state geometry involved in the reactions were optimized using density functional theory (DFT) calculations (specifically, BP86 functional) by employment of the def-TZVP basis set in Turbomole software.[19a,21] All points were confirmed as minima or transition states by calculation of the harmonic vibrational frequencies, by use of analytical second derivatives. Vibrational frequency analysis was performed in all cases to ensure that the molecules possess- no imaginary frequency. To obtain corrections for the zero-point energies (ZPEs) and to ascertain that the computed transition states were first-order saddle points. In order to probe the quality of these B-P86/def-TZVP calculations, we also carried out optimizations at the MP2 level with the same basis set.[22] The pathway for this reaction was obtained by employment of the reaction modelling and energy profile. Single-point energy calculations were also carried out at the B-P86/def-TZVP level of theory to get more accurate results. The ZPE corrections were scaled by a factor of 0.98. Transition state reaction path sampling computations were done to confirm that the transition states connected the appropriate reactants and products for the radical cyclization reactions examined in our study.[23] Solvent effects on the reactions have also been considered by employing B-P86/def-TZVP optimizations of the gas-phase stationary points and using a relatively simple COSMO-RS method based on the continuum model of Andreas Klamt.[24] dialkyl (S,S)-aziridine-2,3-dicarboxylates[25] (**1a-b**) and amino and peptide thioacids[26] (**2/3**) were prepared by following literature procedure.

General procedure for aziridine-2,3-dicarboxylate hydrolysis followed by amino acid/peptide coupling. To the solution of aziridine-2,3-dicarboxylate (1.0 equiv.) (**1a-b**) in MeOH at 0 ^{o}C $LiOH.2H_2O$ (0.9 equiv.) was added, and the reaction mixture was stirred for 12 h at room temperature. The progress of the reaction was monitored by TLC. After the complete consumption of **1**, the solvent was evaporated to dryness under reduced pressure and the crude mixture was washed by diethyl ether. The crude mass was then used as such for the next coupling step. To this stirred crude mass in dry DMF, DPPA (1.2 equiv) and L-Phe-OMe/ L-Phe-L-Ala-OMe (1.0 equiv) were added at 0 ^{o}C under nitrogen atmosphere. Thereafter, triethyl amine (2.5 equiv) was added drop-wise and the stirring continued for 16-18 h at room temperature. After desired time, the reaction was quenched with ice-cold water, and extracted with ethyl acetate. The organic layer was first washed with saturated solution of $NaHCO_3$ and then with citric acid solution. Thereafter, the organic layer was dried using anhydrous Na_2SO_4 and the solvent was dried under reduced pressure to yield crude product. Flash Column chromatography of this crude product yielded the pure aziridine based amino acid/peptide (**6a-c**) using ethyl acetate–hexanes eluents.

Ethyl (2*S*,3*S*)-3-(((*S*)-1-methoxy-1-oxo-3-phenylpropan-2-yl)carbamoyl)aziridine-2-carboxylate. (Azy(OEt)-L-Phe-OMe) (6a). (2S,3S)-diethyl-aziridine-2,3-dicarboxylate (0.50 g, 2.67 mmol), $LiOH.2H_2O$ (0.10 g, 2.40 mmol), L-Phe-OMe (0.478 g, 2.67 mmol), DPPA (0.88 g, 3.204 mmol), Et_3N (0.67 g, 6.67 mmol); R_f = 0.3 (silica gel, EtOAc/Hexanes, 2:3); Yield 62% (0.53 g); Pale yellow oil; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.31 – 7.25 (m, 3H), 7.08 (d, *J* = 7.2 Hz, 2H), 6.87 – 6.78 (m, 1H), 4.83 – 4.73 (m, 1H), 4.19 (q, *J* = 7.2 Hz, 2H), 3.76 (s, 3H), 3.22 – 3.13

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(m, 1H), 3.00 (dd, *J* = 14.0, 7.0 Hz, 1H), 2.79 (d, *J* = 9.0 Hz, 1H), 2.29 (d, *J* = 6.1 Hz, 1H)), 1.75 (t, *J* = 8.4 Hz, 1H), 1.25 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 171.2, 170.9, 167.5, 135.9, 129.5, 129.3, 128.7, 127.3, 61.8, 53.1, 52.5, 37.9, 37.7, 35.5, 14.2. HRMS (ESI) m/z, calcd for $C_{16}H_{20}N_2O_5$ $[M+H]^+$ 321.1445, found 321.1451.

Methyl (2*S*,3*S*)-3-(((S)-1-methoxy-1-oxo-3-phenylpropan-2-yl)carbamoyl)aziridine-2-carboxylate. (Azy(OMe)-L-Phe-OMe) (6b). (2S,3S)-dimethyl-aziridine-2,3-dicarboxylate (0.50 g, 3.14 mmol), $LiOH.2H_2O$ (0.12 g, 2.83 mmol), L-Phe-OMe (0.562 g, 3.14 mmol), DPPA (1.03 g, 3.768 mmol), Et_3N (0.79 g, 7.85 mmol); R_f = 0.2 (silica gel, EtOAc/Hexanes, 1:1); Yield 64% (0.61 g); Pale yellow gel. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.35 – 7.24 (m, 3H), 7.11 – 7.03 (m, 2H), 6.81 (d, *J* = 8.7 Hz, 1H), 4.85 – 4.76 (m, 1H), 3.77 (s, 3H), 3.75 (s, 3H), 3.18 (dd, *J* = 13.9, 5.6 Hz, 1H), 3.00 (dd, *J* = 13.9, 7.1 Hz, 1H), 2.80 (d, *J* = 9.1 Hz, 1H), 2.28 (d, *J* = 7.6 Hz, 1H), 1.75 (t, *J* = 8.5 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 171.7, 170.9, 167.5, 135.8, 129.4, 129.3, 128.8, 128.7, 127.4, 53.1, 52.6, 52.5, 37.9, 37.3, 35.6. HRMS (ESI) m/z, calcd for $C_{15}H_{19}N_2O_5$ $[M+H]^+$ 307.1288, found 307.1284.

Methyl (2*S*,3*S*)-3-(((*S*)-1-(((*S*)-1-methoxy-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamoyl)aziridine-2-carboxylate. (Azy(OMe)-L-Phe-L-Ala-OMe (6c). (2S,3S)-dimethyl-aziridine-2,3-dicarboxylate (0.20 g, 1.23 mmol), $LiOH.2H_2O$ (0.05 g, 1.13 mmol), L-Phe-L-Ala-OMe (0.3 g, 1.23 mmol), DPPA (0.41 g, 1.51 mmol), Et_3N (0.32 g, 3.14 mmol); R_f = 0.2 (silica gel, EtOAc/Hexanes, 7:3); Yield 68% (0.31 g); White solid, mp: 68 °C. ^{1}H NMR (400 MHz, Chloroform-d) *δ* 7.34 – 7.27 (m, 3H), 7.20 – 7.15 (m, 2H), 6.87 (d, *J* = 8.2 Hz, 1H), 6.42 (d, *J* = 7.2 Hz, 1H), 4.60 (q, *J* = 8.0, 1H), 4.50 (p, J = 7.2 Hz, 1H), 3.76 (s, 3H), 3.74 (s, 3H), 3.12 (dd, *J* = 14.0, 6.7 Hz, 1H), 3.03 – 2.94 (m, 1H), 2.78 (d, *J* = 2.3 Hz, 1H), 2.24 (d, *J* = 2.2 Hz, 1H), 1.90 (brs, 1H), 1.37 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.0, 170.2, 168.1, 136.4, 129.4, 128.8, 127.3, 53.8, 53.1, 52.7, 48.4, 38.2, 37.5, 35.5, 18.3. HRMS (ESI) m/z, calcd for $C_{18}H_{24}N_3O_6$ $[M+H]^+$ 378.166, found 378.1742.

General procedure for Ligation strategy. To a solution of aziridine-2,3-dicarboxylate (or aziridine based phenylalanine derivative) (**1**/**6**) (1.0 equiv) in DMF (0.1 mmol/mL), thioacid (**2**/**3**) (1.1 equiv) was added and the mixture was stirred for 4-6 h at 25 °C until TLC shows the complete consumption of **1**/**6**. After completion of reaction, the reaction mixture was quenched with ice-cold water, and extracted with ethyl acetate (20 mL x 2) to give the crude intermediate that was used as such for next step without any purification.

General procedure for thiol group reduction. The crude ligated reaction mass was dissolved in MeOH (0.03 M) and temperature of reaction mass was lowered to -10 °C (using ice-salt bath). At this temperature, $NiCl_2.6H_2O$ (3 equiv.) and $NaBH_4$ (9 equiv) was added and reaction mixture was stirred at the same temperature for 15 min. After 15 min stirring, the reaction mixture was filtered through celite pad and filtrate was concentrated to give crude peptide product that was further purified by flash chromatography using gradients of ethyl acetate-hexane as eluent.

Diethyl ((*S*)-3-(4-(benzyloxy)phenyl)-2-((tert-butoxycarbonyl)amino)propanoyl)-*L*-aspartate (Boc-L-Tyr(OBz)-L-Asp(OEt)-OEt) (5c). Compound **1** (0.10 g, 0.53 mmol), Boc-L-Tyr(OBz)-SH (0.24 g, 0.64 mmol); R_f = 0.2 (silica gel, EtOAc/Hexanes, 3:7); Yield 78% (0.21 g); Gel. ^{1}H NMR (400 MHz, Chloroform-*d*) *δ* 7.44 – 7.31 (m, 5H), 7.14 – 7.09 (m, 2H), 6.90 (dd, *J* = 8.7, 2.3 Hz, 2H), 6.85 (d, *J* = 7.9 Hz, 1H), 5.03 (s, 2H), 4.98-4.89 (m, 1H), 4.83-4.74 (m, 1H), 4.39-4.31 (m, 1H), 4.19 (q, *J* = 7.2 Hz, 2H), 4.14 – 4.06 (m, 2H), 3.06-2.98 (m, 2H), 2.95 (dd, *J* = 16.3, 4.3 Hz, 1H), 2.80 (dd, *J* = 17.2, 4.7 Hz, 1H),1.41 (s, 9H), 1.25 – 1.19 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 171.3, 170.9, 170.4, 158.1, 155.5, 137.2, 130.6, 130.5, 128.8, 128.2, 127.7, 115.2, 80.4, 70.2, 62.1, 61.2, 48.9, 18.5, 36.5, 36.4, 29.9, 28.5, 14.3. HRMS (ESI) m/z, calcd for $C_{29}H_{39}N_2O_8$ $[M+H]^+$ 543.2701, found 543.2702.

Diethyl ((*S*)-5-(benzyloxy)-2-((tert-butoxycarbonyl)amino)-5-oxopentanoyl)-*L*-aspartate (Boc-L-Glu(OBz)-L-Asp(OEt)-OEt) (5d). Compound **1** (0.10 g, 0.53 mmol), Boc-L-Glu(OBz)-SH (0.22 g, 0.64 mmol); R_f = 0.2 (silica gel, EtOAc/Hexanes, 3:7); Yield 72% (0.19 g); Oil. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.40 – 7.30 (m, 5H), 7.12 (dd, J = 31.7, 8.4 Hz, 1H), 5.30 (dd, J = 18.7, 7.4 Hz, 1H), 5.14 (s, 2H), 4.86 – 4.77 (m, 1H), 4.26 – 4.07 (m, 5H), 3.05-2.96 (m, 1H), 2.80 (dt, J = 17.1, 4.7 Hz, 1H), 2.58 – 2.40 (m, 2H), 2.22 – 2.11 (m, 1H), 1.97 (dd, J = 14.5, 7.3 Hz, 1H), 1.43 (s, 9H), 1.33 – 1.19 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 173.2, 171.5, 171.0, 170.5, 155.7, 135.9, 128.8, 28.5, 80.3, 66.8, 62.1, 61.3, 54.1, 53.8, 48.8, 36.4, 30.5, 29.9, 28.5, 14.3. HRMS (ESI) m/z, calcd for $C_{25}H_{35}N_2O_{10}$ $[M+H]^+$, found.

Diethyl ((benzyloxy)carbonyl)-*L*-phenylalanyl-*L*-aspartate (Cbz-L-Phe-L-Asp(OEt)-OEt) (5e). Compound **1** (0.10 g, 0.53 mmol), Cbz-L-Phe-SH (0.20 g, 0.64 mmol); R_f = 0.3 (silica gel, EtOAc/Hexanes, 3:7); Yield 82% (0.20 g); White solid, mp: 123-124 °C. ^{1}H NMR (400 MHz, Chloroform-d) *δ* 7.38 – 7.27 (m, 6H), 7.25 – 7.15 (m, 4H), 6.79 (d, J = 8.0 Hz, 1H), 5.25 (d, J = 8.2 Hz, 1H), 5.08 (s, 2H), 4.77 (dt, J = 8.3, 4.4 Hz, 1H), 4.50 – 4.44 (m, 1H), 4.19 (q, J = 7.1 Hz, 2H), 4.15 – 4.06 (m, 2H), 3.11 (d, J = 6.1 Hz, 2H), 2.99 (dd, J = 17.2, 4.4 Hz, 1H), 2.79 (dd, J = 17.3, 4.6 Hz, 1H), 1.28-1.19 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 170.9, 170.8, 170.4, 156.0, 136.2, 129.5, 128.7, 128.4, 128.2, 127.2, 67.2, 62.1, 61.3, 56.1, 48.9, 38.6, 36.4, 14.3, 14.3. HRMS (ESI) m/z, calcd for $C_{25}H_{30}N_2O_7$ $[M+H]^+$ 471.2126, found 471.2120.

Diethyl N^2,N^6-bis(((benzyloxy)carbonyl)-*L*-lysyl-*L*-aspartate (Cbz-L-Lys(Cbz)-L-Asp(OEt)-OEt) (5f). Compound **1** (0.10 g, 0.53 mmol), Cbz-L-Lys(Cbz)-SH (0.27 g, 0.64 mmol); R_f = 0.2 (silica gel, EtOAc/Hexanes, 3:7); Yield 75% (0.23 g); White solid, mp: 105-106 °C.^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.29 – 7.20 (m, 10H), 6.93 – 6.85 (m, 1H), 5.53 (d, *J* = 8.0 Hz, 1H), 5.05 – 4.93 (m, 5H), 4.79 – 4.69 (m, 1H), 4.20 – 3.98 (m, 5H), 3.16 – 3.06 (m, 2H), 2.93 (dd, *J* = 17.1, 4.6 Hz, 1H), 2.71 (dd, *J* = 17.1, 4.6 Hz, 1H), 1.88 – 1.71 (m, 2H), 1.67 – 1.54 (m, 1H), 1.53 – 1.39 (m, 1H), 1.35 – 1.30 (m, 2H), 1.21 – 1.10 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 171.7, 171.1, 170.6, 156.8, 156.3, 136.8, 136.4, 128.7, 128.7, 128.2, 67.2, 66.8, 62.2, 61.4, 54.8, 48.7, 40.5,

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36.3, 29.9, 29.5, 22.2, 14.3, 14.2. HRMS (ESI) m/z, calcd for $C_{30}H_{39}N_3O_9$ $[M+H]^+$ 586.2759, found 586.2764.

Diethyl ((*S*)-2-((*S*)-2-(((benzyloxy)carbonyl)amino) propanamido)-5-methoxy-5-oxopentanaoyl)-*L*-aspartate (Cbz-L-Ala-L-Glu(OMe)-L-Asp(OEt)-OEt) (5g). Compound **1** (0.10 g, 0.53 mmol), Cbz-L-Ala-L-Glu(OMe)-SH (0.25 g, 0.64 mmol); R_f = 0.2 (silica gel, EtOAc/Hexanes, 2:3); Yield 70% (0.19 g); Light brown solid, mp: 123-124 °C.^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.43 (d, *J* = 8.5 Hz, 1H), 7.38 – 7.28 (m, 5H), 7.26 (d, *J* = 6.2 Hz, 1H), 5.72 (d, *J* = 7.4 Hz, 1H), 5.12 (d, AB pattern, *J* = 11.9 Hz, 1H), 5.08 (d, AB pattern, *J* = 12.2 Hz, 1H), 4.87 – 4.78 (m, 1H), 4.64 – 4.54 (m, 1H), 4.33 (t, *J* = 7.1 Hz, 1H), 4.19 (qd, *J* = 8.7, 7.9, 2.5 Hz, 2H), 4.16 – 4.07 (m, 2H), 3.66 (s, 3H), 2.98 (dd, *J* = 17.1, 4.9 Hz, 1H), 2.79 (dd, *J* = 17.8, 4.2 Hz, 1H), 2.53 – 2.42 (m, 2H), 2.16 (dd, *J* = 14.2, 6.9 Hz, 1H), 2.04 – 1.96 (m, 1H), 1.37 (d, *J* = 7.0 Hz, 3H), 1.27 – 1.17 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 174.0, 172.7, 170.9, 170.5, 156.1, 136.4, 128.6, 128.3, 128.2, 67.1, 62.0, 61.3, 52.4, 52.0, 50.7, 48.8, 36.3, 30.2, 28.0, 19.0, 14.2, 14.2. HRMS (ESI) m/z, calcd for $C_{25}H_{35}N_3O_8$ $[M+H]^+$ 538.2395, found 538.2396.

Methyl (8*S*,11*S*)-11-benzyl-8-(2-methoxy-2-oxoethyl)-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triazadodecan-12-oate. (Cbz-L-Gly-L-Asp(OMe)-L-Phe-OMe) (7a). Compound **6b** (0.10 g, 0.33 mmol), Cbz-L-Gly–SH (0.10 g, 0.39 mmol); R_f = 0.6 (silica gel, EtOAc/Hexanes, 7:3); Yield 75% (0.12 g); White solid, mp: 128-130 °C. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.30 (d, *J* = 4.5 Hz, 5H), 7.24 – 7.20 (m, 2H), 7.17 – 7.12 (m, 1H), 7.06 (d, *J* = 7.6 Hz, 2H), 6.93 (d, *J* = 6.9 Hz, 1H), 5.27 – 5.14 (m, 1H), 5.06 (s, 2H), 4.78 – 4.64 (m, 2H), 4.45 – 4.31 (m, 1H), 3.74 (d, *J* = 5.6 Hz, 2H), 3.64 (s, 3H), 3.62 (s, 3H), 3.09 (dd, *J* = 13.9, 5.6 Hz, 1H), 2.97 (dd, *J* = 14.0, 7.0 Hz, 1H), 2.93 – 2.85 (m, 1H), 2.53 (dd, *J* = 17.1, 6.8 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 172.7, 171.7, 169.9, 169.2, 156.7, 136.2, 136.0, 129.4, 128.8, 128.5, 128.4, 127.3, 67.6, 53.7, 52.6, 52.4, 49.1, 44.8, 37.7, 35.3. HRMS (ESI) m/z, calcd for $C_{25}H_{30}N_3O_8$ $[M+H]^+$ 500.2027, found 500.2000.

Methyl (6*S*,9*S*,12*S*)-12-benzyl-6-isopropyl-9-(2-methoxy-2-oxoethyl)-2,2-dimethyl-4,7,10-trioxo-3-oxa-5,8,11-triazatridecan-13-oate. (Boc-L-Val-L-Asp(OMe)-L-Phe-OMe) (7b). Compound **6b** (0.10 g, 0.33 mmol), Boc-L-Val-SH (0.09 g, 0.39 mmol); R_f = 0.2 (silica gel, EtOAc/Hexanes, 2:3); Yield 80% (0.13 g); White solid, mp: °C.^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.28 – 7.20 (m, 2H), 7.19 – 7.15 (m, 1H), 7.12 – 7.02 (m, 3H), 6.93 (d, *J* = 7.7 Hz, 1H), 4.92 (d, *J* = 8.2 Hz, 1H), 4.76 – 4.65 (m, 2H), 3.88 (t, *J* = 7.1 Hz, 1H), 3.62 (s, 3H), 3.61 (s, 3H), 3.03 (d, *J* = 6.1 Hz, 2H), 2.96 (dd, *J* = 17.3, 3.8 Hz, 1H), 2.54 (dd, *J* = 17.3, 6.7 Hz, 1H), 1.80 – 1.70 (m, 1H), 1.37 (s, 9H), 0.81 – 0.74 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 172.6, 171.8, 171.5, 169.9, 156.0, 135.9, 129.5, 128.8, 127.4, 80.4, 59.6, 53.9, 52.5, 52.4, 49.1, 38.0, 37.4, 35.7, 28.5, 24.8, 15.8, 11.6. HRMS (ESI) m/z, calcd for $C_{25}H_{37}N_3O_8Na$ $[M+Na]^+$ 530.2473, found 530.2593.

Methyl (9*S*,12*S*,15*S*)-15-benzyl-9-(((benzyloxy)carbony)amino)-12-(2-ethoxy-2-oxoethyl)-3,10,13-trioxo-1-phenyl-2-oxa-4,11,14-triazahexadecan-16-oate. (Cbz-L-Lys(Cbz)-L-Asp(OEt)-L-Phe-OMe) (7c). Compound **6a** (0.10 g, 0.31 mmol), Cbz-L-Lys(Cbz)-SH (0.16 g, 0.38 mmol); R_f = 0.2 (silica gel, EtOAc/Hexanes, 2:3); Yield 68% (0.14 g); White solid, mp: 120–121°C. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.40 – 7.27 (m, 13H), 7.25 – 7.19 (m, 2H), 7.16 – 7.12 (m, 2H), 6.97 (d, *J* = 7.7 Hz, 1H), 5.55 (d, *J* = 7.1 Hz, 1H), 5.12 (d, *J* = 5.4 Hz, 1H), 5.08 (s, 2H), 5.05 (d, *J* = 4.1 Hz, 3H), 4.80– 4.69 (m, 2H), 4.11 (q, *J* = 6.5, 5.5 Hz, 2H), 3.69 (s, 3H), 3.20 – 2.97 (m, 5H), 2.62 (dd, *J* = 17.2, 6.5 Hz, 1H), 1.85 – 1.58 (m, 4H), 1.57 – 1.48 (m, 1H), 1.47 – 1.36 (m, 1H), 1.31 – 1.22 (m, 1H), 1.18 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 172.5, 171.9,171.3, 169.8, 156.9, 136.7, 136.6, 136.3, 132.2, 129.6, 128.7, 128.7, 128.4, 128.3, 128.3, 128.2, 127.2, 67.3, 66.9, 65.2, 61.8, 53.8, 53.1, 52.3, 49.3, 40.4, 37.8, 35.5, 29.9, 29.5, 22.8, 14.2. HRMS (ESI) m/z, calcd for $C_{38}H_{47}N_4O_{10}$ $[M+H]^+$ 719.3287, found 719.3288.

Methyl (5*S*,8*S*,11*S*,14*S*)-14-benzyl-11-(2-ethoxy-2-oxoethyl)-8-(3-methoxy-3-oxopropyl)-5-methyl-3,6,9,12-tetraoxo-1-phenyl-2-oxa-4,7,10,13-tetraazapentadecan-15-oate. (Cbz-L-Ala-L-Glu(OMe)-L-Asp(OEt)-L-Phe-OMe) (7d). Compound **6a** (0.10 g, 0.31 mmol), Cbz-L-Ala-L-Glu(OMe)-SH (0.14 g, 0.38 mmol); R_f = 0.1 (silica gel, EtOAc/Hexanes, 1:1); Yield 78% (0.16 g); White solid, mp: 138–139°C. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.58 (d, *J* = 7.3 Hz, 1H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.41 – 7.29 (m, 5H), 7.25 – 7.14 (m, 6H), 5.42 (d, *J* = 5.7 Hz, 1H), 5.16 (d, AB pattern, *J* = 12.2 Hz, 1H), 5.07 (d, AB pattern, *J* = 12.3 Hz, 1H), 4.84 (q, *J* = 6.7 Hz, 1H), 4.75 (q, *J* = 7.2 Hz, 1H), 4.42 (q, *J* = 6.4 Hz, 1H), 4.20 – 4.06 (m, 3H), 3.65 (s, 3H), 3.64 (s, 3H), 3.15 (dd, *J* = 14.0, 5.9 Hz, 1H), 3.07 (dd, *J* = 13.8, 7.4 Hz, 1H), 2.85 (dd, *J* = 16.9, 6.5 Hz, 1H), 2.78 (dd, *J* = 15.1, 7.3 Hz, 1H), 2.44 (q, *J* = 7.0, 6.4 Hz, 2H), 2.06 (p, *J* = 7.9, 7.1 Hz, 2H), 1.39 (d, *J* = 7.2 Hz, 3H), 1.19 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 174.7, 173.1, 171.8, 171.1, 170.6, 169.7, 156.4, 136.3, 136.0, 129.3, 128.6, 128.5, 128.4, 128.3, 128.0, 126.8, 67.2, 61.4, 53.7, 53.4, 52.1, 52.0, 51.4, 49.5, 37.7, 35.6, 30.1, 26.2, 18.2, 14.0. HRMS (ESI) m/z, calcd for $C_{33}H_{43}N_4O_{11}$ $[M+H]^+$ 671.2923, found 671.2927.

Methyl (8*S*,11*S*,14*S*)-14-benzyl-8-isopropyl-11-(2-methoxy-2-oxoethyl)- 3,6,9,12-tetraoxo-1-phenyl-2-oxa-4,7,10,13-tetraazapentadecan-15-oate. (Cbz-Gly-L-Val-L-Asp(OMe)-L-Phe-OMe) (7e). Compound **6b** (0.10 g, 0.33 mmol), Cbz-Gly-L-Val-SH (0.13 g, 0.39 mmol); R_f = 0.3 (silica gel, EtOAc); Yield 84% (0.16 g); White solid, mp: 135–136°C. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.30 – 7.25 (m, 5H), 7.24 – 7.20 (m, 2H), 7.20 – 7.10 (m, 2H), 7.09 – 6.98 (m, 3H), 6.71 (d, *J* = 8.2 Hz, 1H), 5.54 – 5.44 (m, 1H), 5.05 (s, 2H), 4.81 – 4.67 (m, 2H), 4.21 (t, *J* = 7.2 Hz, 1H), 3.84 (dd, *J* = 17.0, 5.6 Hz, 1H), 3.76 (dd, *J* = 17.8, 6.6 Hz, 1H), 3.61 (s, 3H), 3.59 (s, 3H), 3.10 – 2.95 (m, 2H), 2.88 (dd, *J* = 17.0, 5.0 Hz, 1H), 2.58 (dd, *J* = 17.1, 5.1 Hz, 1H), 2.06 – 1.88 (m, 1H), 0.80 (d, *J* = 6.8 Hz, 3H), 0.75 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 172.4, 171.6, 171.0, 169.9, 156.9, 136.3, 136.0, 129.5, 128.8, 128.5, 128.3, 127.3, 67.5, 58.8, 53.8, 52.5, 52.4, 49.4, 44.9, 37.8, 35.9, 31.0, 19.4, 17.9. HRMS (ESI) m/z, calcd for $C_{30}H_{39}N_4O_9$ $[M+H]^+$ 599.2712, found 599.2718.

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DOI: 10.1039/C8OB00676H

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Methyl (5*S*,8*S*,11*S*,14*S*)-8,14-dibenzyl-11-(2-methoxy-2-oxoethyl)-5-methyl-3,6,9,12-tetraoxo-1-phenyl-2-oxa-4,7,10,13-tetraazapentadecan-15-oate. (Cbz-L-Ala-L-Phe-L-Asp(OMe)-L-Phe-OMe) (7f). Compound **6b** (0.10 g, 0.33 mmol), Cbz-L-Ala-L-Phe-SH (0.15 g, 0.39 mmol); R_f = 0.2 (silica gel, EtOAc/Hexanes, 7:3); Yield 82% (0.18 g); White solid, mp: 168–170°C.^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.33 – 7.21 (m, 6H), 7.18 – 7.10 (m, 6H), 7.08 – 7.01 (m, 5H), 6.67 (d, *J* = 7.3 Hz, 1H), 5.25 (d, *J* = 6.4 Hz, 1H), 5.01 (d, *J* = 12.0 Hz, 1H), 4.91 (d, *J* = 12.3 Hz, 1H), 4.78 – 4.70 (m, 1H), 4.69 – 4.63 (m, 1H), 4.56 (q, *J* = 6.9 Hz, 1H), 4.05 (q, *J* = 6.8 Hz, 1H), 3.60 (s, 3H), 3.55 (s, 3H), 3.05 (dd, *J* = 14.0, 5.9 Hz, 1H), 3.00 – 2.91 (m, 3H), 2.74 (dd, *J* = 17.0, 5.6 Hz, 1H), 2.59 (dd, *J* = 16.7, 6.3 Hz, 1H), 1.20 (d, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 172.8, 172.0, 171.7, 170.8, 169.9, 156.4, 136.3, 136.2, 136.1, 129.4, 129.4, 129.3, 128.9, 128.8, 128.7, 128.5, 128.3, 127.3, 127.2, 67.5, 54.5, 53.9, 52.5, 52.2, 51.2, 49.5, 37.8, 37.5, 35.8, 18.2. HRMS (ESI) m/z, calcd for $C_{35}H_{41}N_4O_9$ $[M+H]^+$ 661.2868, found 661.2828.

Methyl (5*S*, 11*S*,14*S*)-5,14-dibenzyl-11-(2-methoxy-2-oxoethyl)-3,6,9,12-tetraoxo-1-phenyl-2-oxa-4,7,10,13-tetraazapentadecan-15-oate. (Cbz-L-Phe-Gly-L-Asp(OMe)-L-Phe-OMe) (7g). Compound **6b** (0.10 g, 0.33 mmol), Cbz-L-Phe-Gly-SH (0.15 g, 0.39 mmol); R_f = 0.3 (silica gel, EtOAc/Hexanes, 4:1); Yield 74% (0.15 g); White solid, mp: 124–126°C.^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.51 (d, *J* = 8.6 Hz, 1H), 7.36 – 7.30 (m, 5H), 7.28 – 7.23 (m, 7H), 7.21 – 7.17 (m, 4H), 7.15 – 7.11 (m, 1H), 5.84 (t, *J* = 9.0 Hz, 1H), 5.07 (d, *J* = 12.3 Hz, 1H), 5.00 (d, *J* = 12.3 Hz, 1H), 4.90 – 4.83 (m, 1H), 4.79 (td, *J* = 7.4, 3.9 Hz, 1H), 4.54 – 4.41 (m, 1H), 3.94 (dd, *J* = 16.8, 5.8 Hz, 1H), 3.74 (dd, *J* = 16.7, 5.2 Hz, 1H), 3.65 (s, 3H), 3.63 (s, 3H), 3.33 – 3.25 (m, 1H), 3.19 – 3.10 (m, 1H), 3.09 – 2.97 (m, 2H), 2.84 (dd, *J* = 17.0, 5.7 Hz, 1H), 2.77 – 2.67 (m, 1H).). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 172.2, 172.0, 171.9, 170.2, 169.0, 156.5, 136.7, 136.5, 136.2, 129.5, 129.4, 128.8, 128.7, 128.7, 128.3, 128.1, 128.1, 127.2, 127.1, 67.3, 61.6, 56.5, 53.8, 52.5, 52.3, 43.3, 39.1, 37.7, 35.7. HRMS (ESI) m/z, calcd for $C_{34}H_{39}N_4O_9$ $[M+H]^+$ 647.2712, found 647.2682.

Methyl (5*S*,8*S*,11*S*,14*S*,17*S*)-8,14-dibenzyl-11-(2-methoxy-2-oxoethyl)-5,17-dimethyl-3,6,9,12,15-pentaoxo-1-phenyl-2-oxa-4,7,10,13,16-pentaazaoctadecan-18-oate. (Cbz-L-Ala-L-Phe-L-Asp(OMe)-L-Phe-L-Ala-OMe) (8). Compound **6c** (0.08 g, 0.21 mmol), Cbz-L-Ala-L-Phe-SH (0.08 g, 0.233 mmol); R_f = 0.1 (silica gel, EtOAc); Yield 60% (0.09 g); White solid, mp: 204°C.^{1}H NMR (400 MHz, DMSO-d_6) δ 8.36 (d, *J* = 7.0 Hz, 1H), 8.23 (d, *J* = 7.9 Hz, 1H), 7.93 (t, *J* = 8.6 Hz, 1H), 7.41 (d, *J* = 7.5 Hz, 1H), 7.38 – 7.30 (m, 5H), 7.26 – 7.11 (m, 11H), 5.02 (d, *J* = 12.5 Hz, AB pattern, 1H), 4.97 (d, *J* = 12.4 Hz, AB pattern, 1H), 4.62 – 4.54 (m, 1H), 4.50 (dt, *J* = 8.5, 4.4 Hz, 1H), 4.45 – 4.42 (m, 1H), 4.27 (p, *J* = 7.1 Hz, 1H), 3.98 (p, *J* = 7.2 Hz, 1H), 3.61 (s, 3H), 3.56 (s, 3H), 3.02 (dd, *J* = 13.8, 4.4 Hz, 1H), 2.95 (dd, *J* = 13.9, 4.1 Hz, 1H), 2.85 – 2.66 (m, 4H), 1.29 (d, *J* = 7.3 Hz, 3H), 1.09 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) *δ* 172.8, 172.3, 171.0, 170.6, 169.8, 155.7, 137.6, 137.5, 136.9, 129.2, 129.2, 128.4, 128.1, 128.0, 127.8, 126.3, 65.5, 53.6, 53.6, 51.9, 51.6, 50.2, 49.4, 47.7, 37.6, 37.3, 36.0, 18.1, 16.9. HRMS (ESI) m/z, calcd for $C_{38}H_{46}N_5O_{10}$ $[M+H]^+$ 732.3239, found 732.3282.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This project was funded by Department of Science and Technology India. Thus authors acknowledge with thanks DST for financial support. Dr G. G. Pillai thanks COSMOlogic GmbH for permitting to use the Turbomole software.

Notes and references

1. a) P. E. Dawson, T. W. Muir, I. Clark-Lewis and S. B. H. Kent, *Science,* 1994, **266**, 776.
b) P. E. Dawson and S. B. Kent, *Annu. Rev. Biochem.*, 2000, **69**, 923.
2. a) L. Wang and M. N. Amin, *Chem. Biol*., 2014, **21**, 51.
b) S. S. Kulkarni, J. Sayers, B. Premdjee and R. J. Payne, *Nat. Rev. Chem.*, 2018, 0122.
c) A. M. Levinson, J. H. McGee, A.G. Roberts, G. S. Creech, T. Wang, M. T. Peterson, R. C. Hendrickson, G. L. Verdine and S. J. Danishefsky, *J. Am. Chem. Soc.*, 2017, **139**, 7632.
d) S. Tang, L. Liang, Y. Si, S. Gao, J. Wang, J. Liang, Z. Mei, J. Zheng and L. Liu, *Angew. Chem. Int. Ed.* 2017, **56**, 13333.
e) Z. Wang, W. Xu, L. Liu and T. F. Zhu, *Nat Chem.* 2016, **8**, 698.
f) M. Jbara, S. K. Maity, M. Morgan, C. Wolberger and A. Brik, *Angew Chem Int Ed Engl.* 2016, **55**, 4972.
g) P. Wang, S. Dong, J. H. Shieh, E. Peguero, R. Hendrickson, M. A. S. Moore and S. J. Danishefsky, *Science.* 2013, **13**, 342, 1357.
h) A. A. Vinogradov, E. D. Evans and B. L. Pentelute, *Chem. Sci.*, 2015, **6**, 2997.
i) S. Tang, Y. Si, Z. Wang, K. Mei, X. Chen, J. Cheng, J. Zheng and L. Liu, Angew. Chem. Int. Ed. 2015, **54**, 5713.
3 L. Z. Yan, and P. E. Dawson, *J. Am. Chem. Soc.* 2001*,* **123**, 526.
4. a) Z. Harpaz, P. Siman, K. S. A. Kumar and A. Brik, *Chem Bio Chem* 2010, **11**(9), 1232.
b) Z. Tan, S. Shang and S. J. Danishefsky, *Angew.Chem., Int. Ed.* 2010, **49**, 9500.
5. a) J. Chen, Q. Wan, Y. Yuan, J. Zhu and S. J. Danishefsky, *Angew. Chem., Int. Ed.* 2008, **47**, 8521.
b) C. Haase, H. Rohde and O. Seitz, *Angew. Chem., Int. Ed.* 2008, **47**, 6807.
6. a) D. Crich and A. Banerjee, *J. Am. Chem. Soc.* 2007, **129**, 10064.
b) L. R. Malin, A. M. Giltrap, L. J. Dowman and R. J. Payne, *Org. Lett.* 2015, **17**, 2070.
7. K. M. Cergol, R.E. Thompson, L.R. Malins, P. Turner and R. J. Payne, *Org. Lett.* 2014, **16**, 290.
8. R. Yang, K. K. Pasunooti, F. Li, X. Liu and C. Liu, *J. Am. Chem. Soc.* 2009, **131** (38), 13592.
9. a) S. D. Townsend, Z. Tan, S. Dong, S. Shang, J. A. Brailsford and S. J. Danishefsky, *J. Am. Chem. Soc.* 2012, 134, 3912.
b) S. Shang, Z. Tan, S. Dong and S. J. Danishefsky, *J. Am. Chem. Soc.* 2011, **133**, 10784.
c) H. Ding, A. Shigenaga, K. Sato, K. Morishita and A. Otaka, *Org. Lett.* 2011, **13**, 5588.
10. L. R. Malins, K. M. Cergol and R. J. Payne. *Chem. Sci.*, 2014, **5**, 260–266.

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11. a) M. Villain, H. Gaertner and P. Botti, *Eur. J. Org. Chem.* 2003, **2003**, 3267.
b) X. Guan, M. R. Drake and Z. Tan, *Org. lett.* 2013, **15**, 6128.
c) R.E. Thompson, B. Chan, L. Radom, K. A. Jolliffe and R.J. Payne, *Angew. Chemie. Int. Ed.* 2003, **52**, 9723.
12. a) E. Saxon, J. I. Armstrong and C. R. Bertozzi, *Org. lett.* 2000, 2, 2141.
b) M. Köhn and R. Breinbauer, *Angew. Chemie. Int. Ed.* 2004, **43**, 3106.
c) M. Köhn, R. Wacker, C. Peters, H. Schröder, L. Soulère, R. Breinbauer, C.M. Niemeyer and H. Waldmann, *Angew. Chemie. Int. Ed.* **2003**, 42, 5830.
d) K.L. Kiick, E. Saxon, D.A. Tirrell and C.R. Bertozzi,. *Proc. Natl. Acad. Sci.* 2002, **99**, 19.
13. a) J. W. Bode, R. M. Fox and K. D. Baucom, *Angew. Chemie.* 2006, **118**, 1270.
b) V. R. Pattabiraman, A. O. Ogunkoya and J. W. Bode, *Angew. Chemie. Int. Ed.* 2012, **51**, 5114.
14. a) D. P. Galonić, W. A. van der Donk and D. Y. Gin, *J. Am. Chem. Soc.*2004, **126**, 12712.
b) D. P. Galonić, N. D. Ide, W. A. van der Donk and D. Y. Gin, *J. Am. Chem. Soc.*2005, **127**, 7359.
c). D. A. Ryan and D. Y. Gin, *J. Am. Chem. Soc.* 2008, **130**, 15228.
d) F. Crestey, M. Witt, K. Frydenvang, D. Staerk, J. W. Jaroszewski and H. Franzyk *J. Org. Chem.*2008, 73, 3566.
15. J. P. Tam, Y. A. Lu, C. F. Liu and J. Shao *PNAS*, 1995, **92**, 12485.
16. a) F. B. Dyer, C. M. Park, R. Joseph and P. Garner, *J. Am. Chem. Soc.* 2011, ***133***, 20033-20035
b) R. Joseph, F. B. Dyer and P. Garner, *Org. Lett.* 2013, **15**, 732.
17. K. Bajaj and R. Sakhuja, *Chem. Asian J.* 2017, **12**, 1869.
18. a) K. E. Gilbert, PC Model 9.3. PC Model 9.3, Serena Software: Bloomington, IN. 2011.
b) B. E. D. M. El-Gendy, E. H. Ghazvini Zadeh, A. C. Sotuyo, G. G. Pillai, A. R. Katritzky, *Chem. Biol. Drug Des.* 2013, **81**, 577–582.
19. a). A. D. Becke, *Phys. Rev. A* 1988, **38**, 3098–3100.
b) B. Schürmann, *Zeitschrift für Phys. Chemie* 1996, ***196***, 157–157.
20. R. G. Parr, R. G. Pearson, *J. Am. Chem. Soc.* 1983, **105**, 7512–7516.
21. R. Ahlrichs, M. Bär, M. Häser, H. Horn, C. Kölmel, *Chem. Phys. Lett.* 1989, **162**, 165–169.
22. F. Weigend, M. Häser, H. Patzelt, R. Ahlrichs, *Chem. Phys. Lett.* 1998, **294**, 143–152.
23. T. Helgaker, *Chem. Phys. Lett.* 1991, **182**, 503–510.
24. A. Klamt, G. Schüürmann, *J. Chem. Soc., Perkin Trans. 2* **1993**, No. 5, 799–805.
25. R. Hili, V. Rai and A. K. Yudin, *J. Am. Chem. Soc.*2010, **132**, 2889.
26. R. Khaybullin, S. S. Panda, A. O. Al-Youbi, A. R. Katritzky, *Synlett* **2014**, *25*, 247.

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Aziridine Based Electrophilic Handle for Asparatic Acid Ligation

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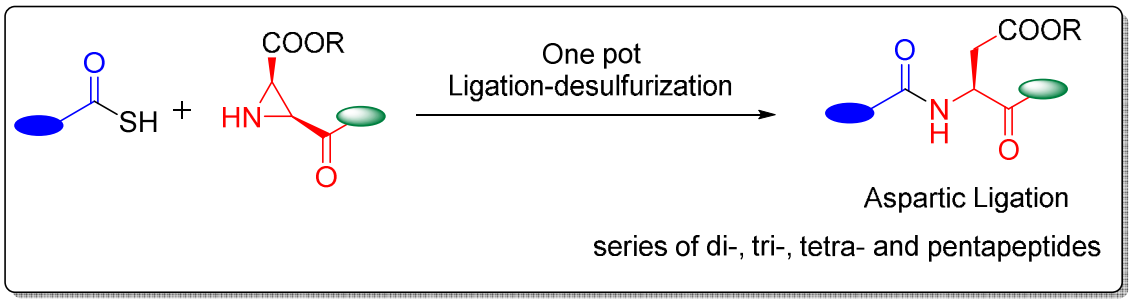

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