Synthesis of N_β-Substituted α,β-Diamino Acids via Stereoselective *N*-Michael Additions to a Chiral Bicyclic Dehydroalanine

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Abstract

The highly diastereoselective 1,4-conjugate additions of several nitrogen nucleophiles to chiral bicyclic dehydroalanines have been assessed effectively at room temperature in good to excellent yields without needing any catalyst or additional base. This methodology is general, simple, oxygen and moisture tolerant, high-yielding, totally chemo- and stereoselective. Significantly, most of the reaction adducts were obtained in nearly quantitative yield ~~without column chromatography purification~~. This procedure offers an efficient and practical approach for the synthesis of N_β-substituted α,β-diamino acids, such as 1-isohistidine, τ-histidinoalanine, β-benzylaminoalanine, β-(piperidin-1-yl)alanine, β-(azepan-1-yl)alanine and fluorescent and ciprofloxacin-containing amino acid derivatives.

Introduction

N$_{\beta}$-Substituted α,β-diamino acids, also considered as 2,3-diaminopropionic acid (DAP) or β-aminoalanine derivatives, are an important type of amino acids due to both their biological activities and their use as precursors of chiral ligands for stereoselective synthesis.[1] This scaffold appears in many natural products,[1,2] such as some neurotoxins (eg. β-*N*-methylamino-L-alanine, BMAA) or peptidic antibiotics (e.g. duramycin), and has also been used for the synthesis of pharmaceutical compounds.[3-6] DAP is a biosynthetic precursor of numerous antibiotics and a siderophore produced by *Staphylococcus aureus*.[7] Many synthetic approaches have been developed to obtain both DAP enantiomers in a stereoselective manner.[8-10] Other important *N*$_{\beta}$-substituted α,β-diamino acids are some analogous of histidine, such as β-(1-pyrazolyl)alanine or β-(1-triazolyl)alanine, that involve the substitution of imidazole ring by pyrazole or triazole, respectively, and have been used for diabetes treatment.[11-13] These compounds have been accessed by *N*-nucleophilic displacement of β-haloalanine derivatives,[14] by ring-opening of aziridines,[15] lactones[16] or cyclic sulfamidates,[17] or by chemoenzymatic synthesis using *N*-Michael addition on dehydroalanines (Dha).[18] On the other hand, 1-isohistidine is another analog also bearing an imidazole ring but with a different substitution pattern. This compound has been synthesized in its racemic form by *N*-Michael addition on Dha derivatives,[19-21] and in its enantiomerically pure form through Ni-organometallic catalysis.[22] Additionally, 1-isohistidine derivatives bearing charged imidazolium moieties have been prepared as precursors of chiral *N*-heterocyclic carbenes (Figure 1).[23]

On the other hand, among the multitude of non-proteinogenic amino acids, some of the most relevant are bis-α-amino acids, which are natural products formed in the metabolism of proteins and have different functions in various organisms.[24] For instance, lysinoalanine (LAL) or lanthionine (LAN) appear in nature as part of small peptides with antibiotic activity, such as cinnamycin, duramycin, epidermin, nisin or subtilin among others.[25] Histidinoalanine (HAL), which is formed by the cross-linkage of an alanine residue at its β position and a N atom of an imidazole ring of a histidine, is particularly relevant in this context. Two HAL regioisomers, denoted as τ-HAL and π-HAL, are possible depending on the imidazole nitrogen atom cross-linked to the alanine residue. Both regioisomers have been identified and described in a multitude of biological media.[26,27] HAL is a member of the structural family

of theonellamides, which are isolated from marine sponges and have been used as biomarkers for the study of membrane structures. In addition, HAL is related to atherosclerosis, cataracts and, in general, cellular aging.[28-30] HAL derivatives synthesis has been attempted by nucleophilic substitutions with moderate yields[32-34] or *N*-Michael additions on Dha derivatives, yet without stereoselectivity.[35,36] *N*-Michael reaction on Dha derivatives is a powerful strategy for selective protein modification [37,38] and to access α,β–diamino acids due to its operational simplicity (i.e. it does not require external activation) and atom economy[39,40] (Figure 1).

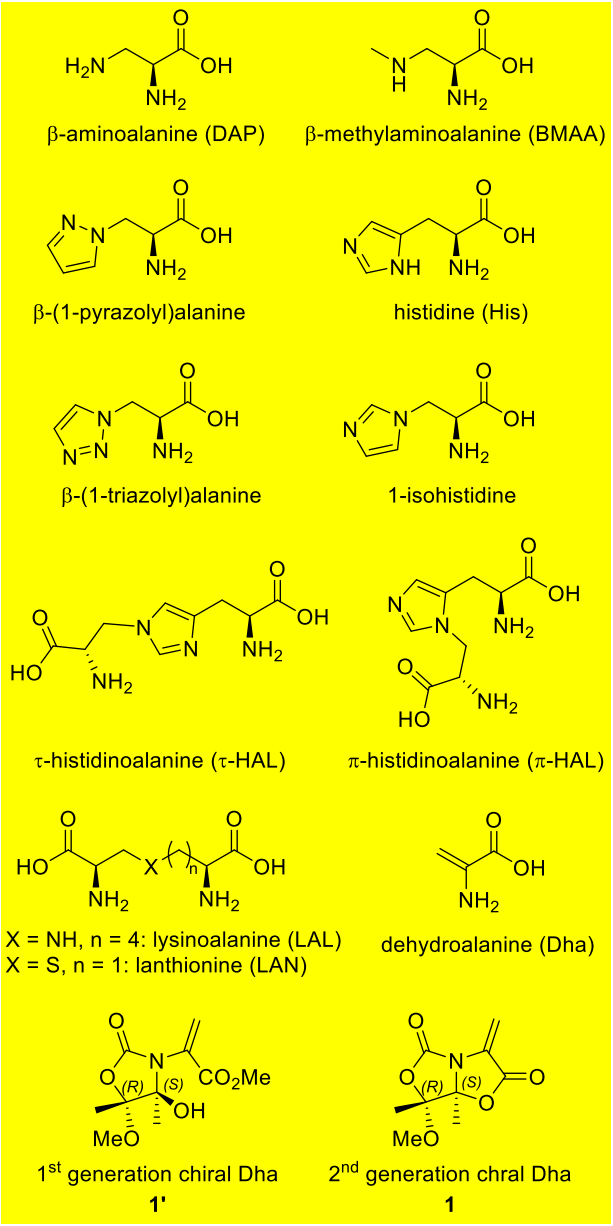


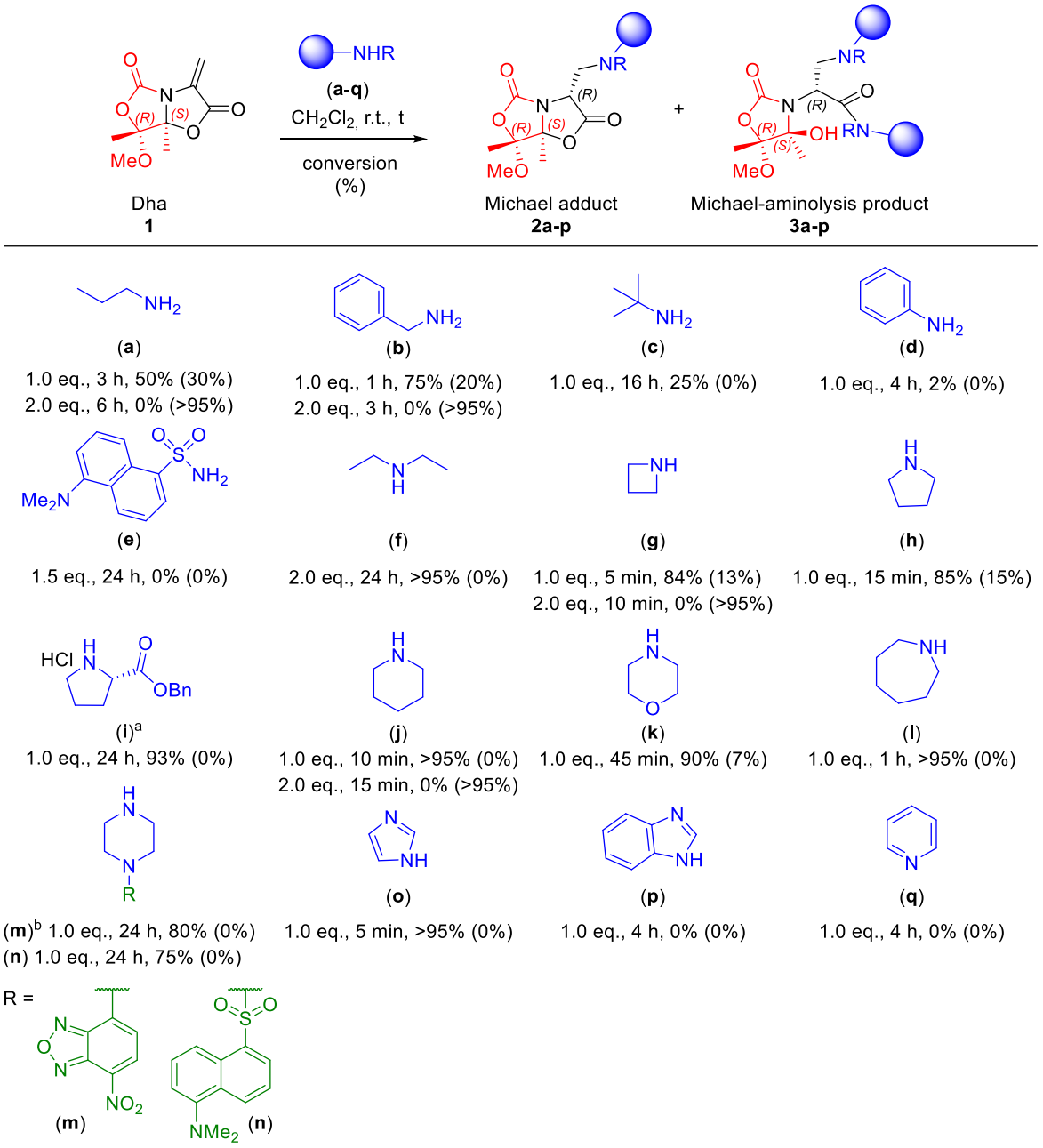
Figure 1. Some important N_{β}-substituted α,β-diamino acids, bis-α-amino acids and their precursor Dha, as well as chiral Dha **1'** and **1** used in the Michael additions.

Conventional Dha derivatives are normally poor Michael acceptors[41] so that strong bases or acids are needed to enhance their reactivity towards nucleophilic addition. We have recently described two different strategies to activate the Dha backbone by embedding it into achiral[42] and chiral cyclic scaffolds.[43] Initially, we reported the synthesis of a chiral Dha derivative **1'**, which was used in stereoselective *S*-Michael additions, showing excellent Michael acceptor properties, cleanly reacting with thiols using a base, under -78 ºC.[43] Furthermore, a second generation of chiral bicyclic Dha **1** was developed in our research group (Figure 1). Particularly, this chiral bicyclic Dha derivative **1**, available on a multigram scale from L-serine, has proved to constitute a useful platform for accessing cysteine and lanthionine derivatives in a highly stereoselective manner through base-assisted *S*-Michael addition reactions.[44,45] To further expand this methodology, we herein report a biomimetic approach to carry out the synthesis of N_{β}-substituted α,β-diamino acids and histidinoalanine derivatives in their enantio- and diastereomerically pure forms by stereoselectively installing a N atom linked to a β-carbon of an α-amino acid surrogate (chiral Dha **1**) through a base- and catalyst-free *N*-Michael addition.

Results and Discussion

Substrate scope. We first investigated the potential of the first generation chiral Dha **1'** as an *N*-Michael acceptor with several amines as nitrogen nucleophiles but mixture of compounds were detected due to epimerization, in these basic conditions, of the carbon attached to the hydroxyl group of the chiral auxiliary (Figure 1 and Supporting Information). Next, we investigated the potential of Dha **1** as an *N*-Michael acceptor with several nitrogen nucleophiles (Scheme 1) with different hybridizations at the N atom in order to test the reaction scope. These reactions were carried out in dichloromethane at room temperature using stoichiometric amounts or a slight excess of the nucleophile. In most cases a single 1,4-conjugation adduct (labelled as **2**) was obtained, although in some cases a sequential double 1,4- and 1,2-ring opening addition (i.e. aminolysis) reaction was observed to give bis-adducts labelled as **3**. Of note, a unique diastereoisomer of either **2** or **3** adducts was obtained in all cases, indicating a complete diastereocontrolled addition. This facilitated the analysis of the

reaction mixtures by ^{1}H NMR and allowed us to use the products in subsequent reactions without any further purification. In detail, aliphatic primary amines (**a** and **b**) proceeded with high conversions and stereoselectivities towards *N*-Michael addition (**2**) in equimolar conditions. However, a by-product (**3**), corresponding to the subsequent ring-opening aminolysis of the lactone moiety of adduct **2** by a second amine molecule was also observed in both cases. These bis-adducts could be obtained quantitatively upon addition of two equivalents of amine. Less nucleophilic and/or sterically hindered primary amines (**c**-**e**) proved to be poorly, or even non-reactive under these conditions. On the other hand, secondary amines (**f**-**n**), particularly cyclic amines, were extremely reactive providing quantitative conversions within minutes in many cases. This was expected due to their marked nucleophilic character.[46] Moreover, the stability of the *N*-Michael adducts towards aminolysis was better controlled with these reagents than in the case of primary amines, especially with more hindered amines, such as diethylamine (**f**) or protected L-proline (**i**), which achieved complete productivity towards 1,4-addition even using more than one amine equivalent. Within this context, piperazine moieties, such as those present in fluorescent 4-nitrobenzofurazan-7-yl derivative **m** and dansyl derivative **n** (see Supporting Information for their spectroscopic properties), are widely used as handles in drug design and covalent binding[47] and have also proven to improve the anticancer activity of thiosemicarbazones.[48] Both derivatives showed lower nucleophilicity, needing nearly 24 h to achieve high conversion values; nevertheless, clean and high conversion fluorescent tagging was obtained in both cases (75-80%). Regarding *N*-sp^2-hybridized heterocyclic nucleophiles, imidazole (**o**) showed quantitative conversions in just 5 min, whereas benzimidazole (**p**) or pyridine (**q**) were totally unreactive under the same conditions after 4 h.



Scheme 1. Reaction between chiral bicyclic Dha **1** and a range of *N*-nucleophiles (**a-q**) of different electronic and steric nature. The percentage numbers indicate the conversion values towards *N*-Michael adducts **2**, and Michael–aminolysis products **3** (in parentheses) measured by ^{1}H NMR spectroscopy. [a]Triethylamine (1.0 equiv.) was added to neutralize hydrochloric acid. [b]The reaction was performed in DMF at 50 ºC due to poor solubility of the reagents in dichloromethane.

Chemoselectivity and adducts stability. The reaction between Dha **1** and a slight excess of benzylamine (**b**) was monitored by ^{1}H NMR spectroscopy (Figures **2** and **S1** in the Supporting Information). The *N*-Michael reaction was found to proceed faster, and adduct **2b** was subsequently transformed at a slightly slower rate into the corresponding derivative **3b** after ring-opening aminolysis of the oxazolidin-5-one ring by an additional equivalent of benzylamine. Such competitive aminolysis reaction *did not* proceed on the starting Dha **1**, but only right after the carbonyl-deactivating conjugated alkene was suppressed by the *N*-Michael addition reaction. This demonstrates the complete intrinsic chemoselectivity of the 1,4- *vs*. 1,2-conjugate addition reaction. Of note, the enolate intermediate generated after the conjugate addition of the amine to the double bond was not observed; instead, the protonated adduct **2b** was observed even at short reaction times, suggesting that the nucleophilic attack of the amine, not the subsequent enolate intermediate protonation, is the reaction rate-limiting step. The maximum abundance of *N*-Michael adduct **2b** (44%) was found to accumulate after 9 h of reaction, when the amount of unreacted Dha **1** was still significant (37%) and bis-adduct **3b** generated from **2b** had risen up to 19%. Passed this point, the amount of decreasing adduct **2b** and increasing bis-adduct **3b** were equal (38%) after 15 h of reaction time. The rate constants corresponding to both consecutive second–order reactions (i.e. *N*-Michael addition and aminolysis) were estimated following the protocol described by Frost and Schwemer:[49] $k_{Michael} = 1.3 \cdot 10^{-4}$ M^{-1} s^{-1}; $k_{Aminolysis} = 2.7 \cdot 10^{-5}$ M^{-1} s^{-1} (see Supporting Information). As described above, this Michael–aminolysis domino process is unavoidable for primary amines (**a**-**b**), but can be significantly controlled and even suppressed with secondary amines (**f**-**n**) and imidazole (**o**).

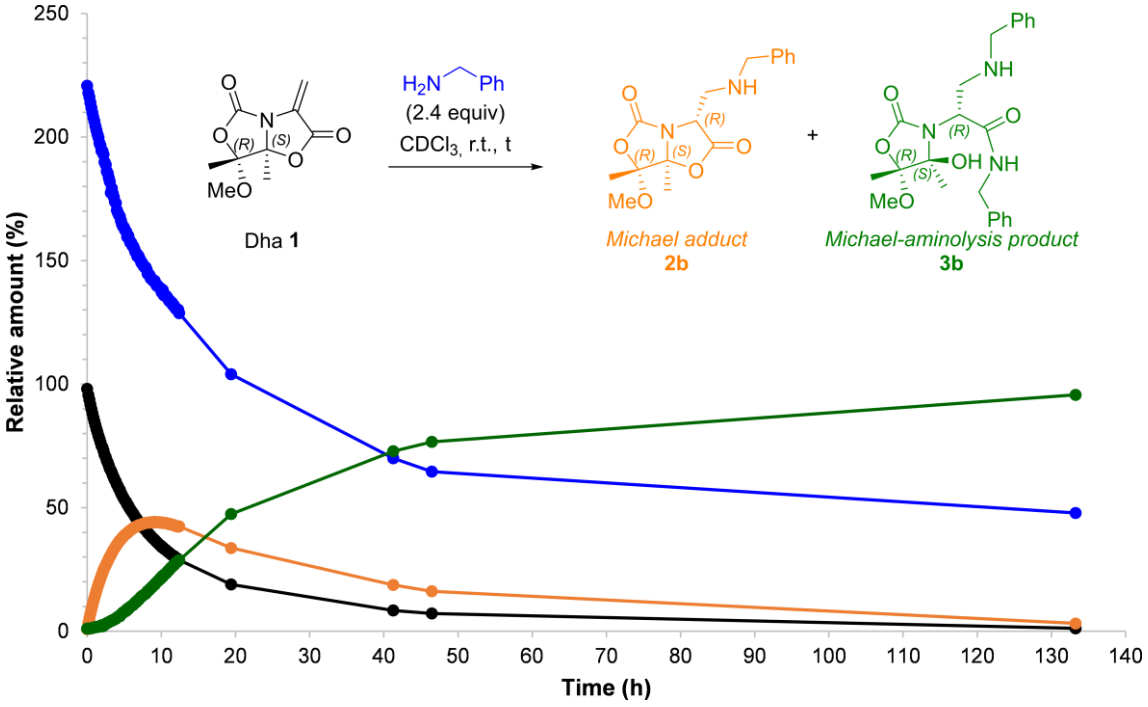


Figure 2. Monitoring of the reaction between Dha **1** (in black) and an excess of benzylamine (**b**, in blue) in $CDCl_3$ by ^1H NMR spectroscopy at 298 K. The corresponding *N*-Michael adduct **2b** and the subsequent aminolysis bis-adduct **3b** are shown in orange and green, respectively.

Stereoselectivity. The absolute configuration of the new stereocenter (C3) of compounds **2a-o** created upon the 1,4-conjugate addition was determined from NOESY-NMR experiments. The medium-size NOE cross-peaks between the methylene group linked to the nucleophile and the bridgehead (**C7a**) methyl group of the 2,5-dioxotetrahydro-5*H*-oxazolo[4,3-b]oxazole system confirmed that C3 displays an *R*-configuration in adducts **2a-o** (Figure **3A**). Monocrystals of compound **3b** suitable for X-ray diffraction analysis were obtained, thus confirming the (*R*) configuration at C2'' in compound **3b**, which corresponds to C3 in its precursor **2b** (Figure **3B**). These results indicate that the stereochemical outcome of the *N*-Michael addition reaction is identical to that observed for the addition of sulfur nucleophiles,[44,45] demonstrating the highly conserved stereoinduction mechanism for the protonation of the enolate adduct upon conjugate addition in Dha **1**.

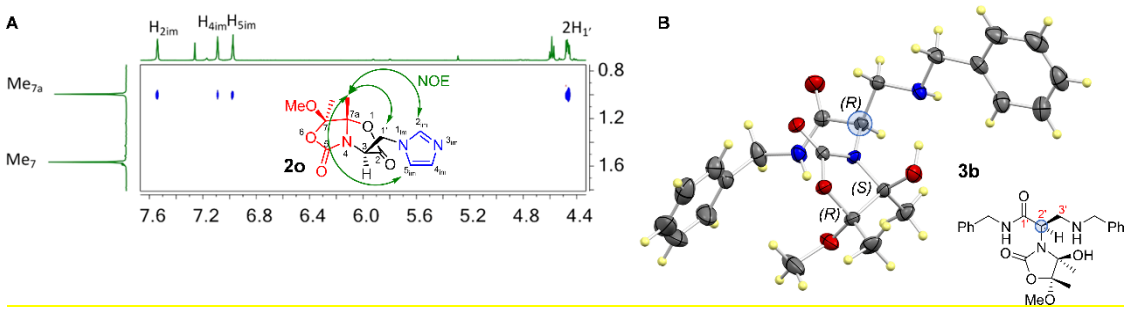


Figure 3. A) 2D NOESY NMR experiment for compound **2o** performed with 400 MHz equipment by using $CDCl_3$ as a solvent at 298 K. **B**) ORTEP3 diagram of compound **3b** obtained by X-ray diffraction analysis showing thermal ellipsoids at the 75% probability level.

Computational analysis. The mechanism of the *N*-Michael addition reaction on Dha **1** was examined computationally using the PCM/M06-2X/6-31+G(d,p) method in dichloromethane and methylamine as an abbreviated model for primary sp^3-hybridized amines[50] (see Computational details and Supporting Information). The calculated stepwise mechanism proposed in Figure 4, which involves two amine molecules –one reacting as a nucleophile and the other one acting as a 'catalytic' proton shuttle– is in line with the experimental observations: 1) the amine nucleophilic attack was calculated to be endergonic and rate-limiting, with a calculated activation barrier ($\Delta G^{\ddagger}_{calc}$ = 22.4 kcal mol^{-1} for transition state **1_TS$_{add\text{-}2}$_a'**) in good agreement with the experimentally determined one ($\Delta G^{\ddagger}_{exp}$ ~ 23 kcal mol^{-1}); and 2) the subsequent protonation step was calculated to be irreversible (ΔG_{calc} ~ -5 kcal mol^{-1}) and to proceed through the experimentally observed *Si* face of the bicyclic intermediate enolate with complete stereoselectivity ($\Delta\Delta G^{\ddagger}_{Si–Re}$ ~ 5 kcal mol^{-1} for transition states **1_TS$_{prot\text{-}Si\text{-}2}$_a'** and **1_TS$_{prot\text{-}Re\text{-}2}$_a'**). The large preference for the concave (*Si*) face arises mostly from the development of torsional (i.e. bond-eclipsing) strain in the five-membered oxazolidin-5-one upon protonation by the convex (*Re*) face, in agreement with the theoretical predictions for reactions on similar bicyclic substrates.[44,51,52]

The intrinsic reactivities of different nitrogen reagents (primary and secondary acyclic/cyclic amines, sulfonamides, aniline, pyridine and imidazole) towards the initial nucleophilic addition step, were also examined computationally. In order to facilitate comparison across nucleophiles with very different electronic and steric characteristics, the corresponding

transition structures involving just one nucleophile molecule were calculated, which allowed reproducing the experimental reactivity trends (Figure **S2**).

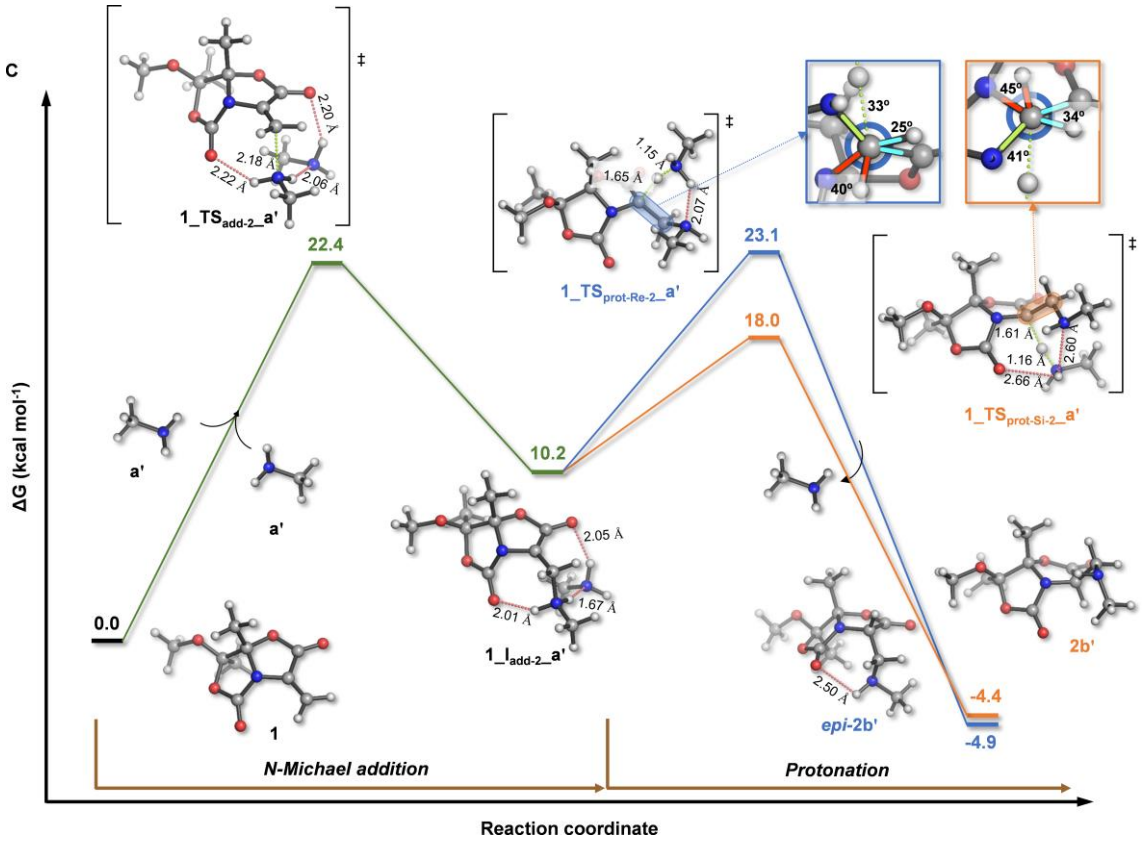
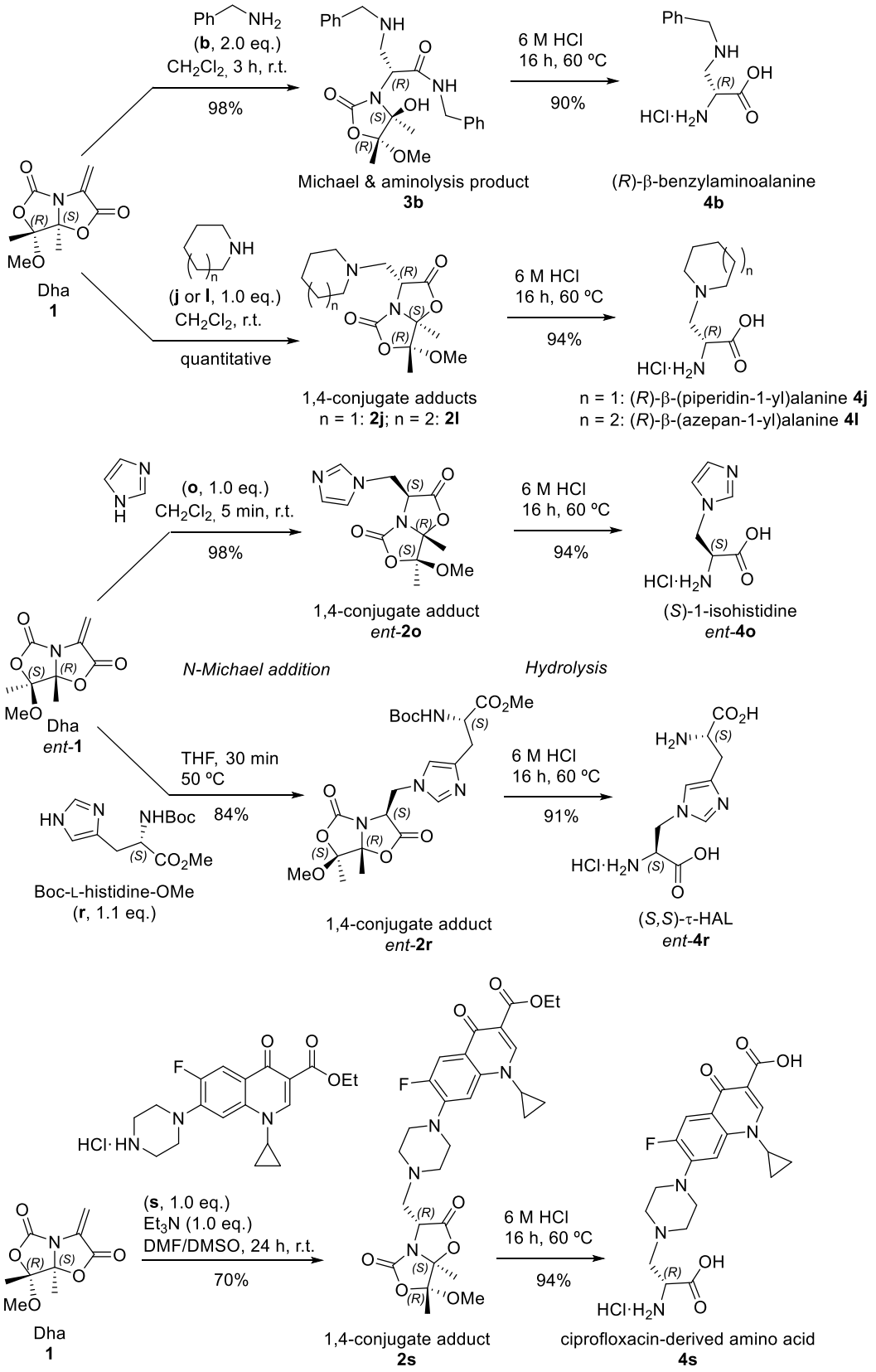


Figure 4. Minimum energy pathway for the reaction of Dha **1** with two molecules of model methylamine (**a'**) calculated with PCM_{CH2Cl2}/M06-2X/6-31+G(d,p). The rate-determining step is the Michael addition (green), whereas the selectivity arises from the protonation step, being the *Si* face approach (orange) much more favorable than the *Re* face approach (blue).

Application in amino acids synthesis. Representative *N*-Michael reaction products were subjected to acidic hydrolysis to obtain the corresponding sidechain-modified, unprotected amino acids (Scheme 2). For this application, either mono-adducts **2** or bis-adducts **3** can be used, since all protecting groups at the *N*α-amino and carboxyl groups are being removed. Also, when analogues of amino acids showing the same absolute configuration at Cα than

the naturally occurring ones were needed, their syntheses were started from the enantiomer of Dha **1** (labelled as *ent*-**1**) obtained from D-serine[44,45] following exactly the same methodology described above. We first hydrolyzed benzylamine derivative **3b** by treatment with aqueous 6 M HCl at 60 ºC for 16 h, leading to the N_{β}-benzyl-protected unnatural enantiomer of diaminopropanoic acid (D-DAP, **4b**) as a hydrochloride salt in nearly quantitative yield after solid-phase extraction purification. In addition, Michael adducts **2j** and **2l** were easily hydrolyzed following the same conditions above described to obtain β-(piperidin-1-yl) and β-(azepan-1-yl)alanine, as hydrochloride salts **4j** and **4l**, respectively, in quantitative yields. (*S*)-1-Isohistidine (*ent*-**4o**) was obtained as hydrochloride also in excellent yield from imidazole adduct *ent*-**2o** under the same hydrolysis conditions. Encouraged by these results, a natural isomer of histidinoalanine ((*S*,*S*)-τ-HAL, *ent*-**4r**) was synthetized in its diastereomerically pure form and good overall yield (76%) by reacting N_{α}-Boc-L-histidine methyl ester with *ent*-**1** in THF as a solvent and subsequently hydrolyzing the corresponding sole adduct *ent*-**2r**.

Finally, an enantiomerically pure α-amino acid sidechain-modified with ciprofloxacin was synthetized in good overall yield (70%), by reacting Dha **1** with the previously synthesized hydrochloride salt of piperazine-containing ciprofloxacin ethyl ester[53] in DMF/DMSO as a solvent and in the presence of triethylamine to neutralize HCl. Hydrolysis of adduct **2s** yielded the enantiopure drug-amino acid conjugate **4s** in good overall yield (94%). Ciprofloxacin belongs to the second generation of fluoroquinolone antibiotics and displays high antimicrobial activity, outstanding pharmacokinetic properties, and limited side effects. Despite its importance in the clinical treatment of infections, the widespread antimicrobial drug-resistance makes ciprofloxacin increasingly ineffective and has stimulated the development of new derivatives with increased antibacterial potency.[54,55] Since incorporating N^4-carboxymethyl groups has shown to boost up the antibacterial activity of ciprofloxacin,[56] and that conjugates of this drug in which the piperazine ring is attached through a peptide bond are less active,[57,58] we applied our methodology to easily obtain a sidechain-conjugated amino acid-ciprofloxacin hybrid. Such building block could be further incorporated into cell-penetrating cargo[59] or antibiotic[60,61] peptides.



Scheme 2. Synthesis of β-*N*-substituted-α-amino acids **4b**, **4j**, **4l**, *ent*-**4o**, e*nt*-**4r** and **4s**.

Conclusions

In summary, an efficient approach for the synthesis of N_β-substituted α,β-diamino acids from a chiral dehydroalanine derivative readily accessible from serine has been developed. This reaction takes place at room temperature without needing any catalyst or additional base, and tolerates a broad range of nitrogen nucleophiles. The method is general, operationally simple, high-yielding, highly regio- and stereoselective, and oxygen and moisture tolerant. In addition, in the cases where the amine being used is volatile, the most often-used purification methods such as column chromatography and recrystallization are not necessary, notably increasing the easiness and productivity of the process.

Experimental Section

General and Experimental Methods

Commercial reagents were used without further purification. Analytical thin layer chromatography (TLC) was performed on Macherey-Nagel precoated aluminum sheets with a 0.20 mm thickness of silica gel 60 with fluorescent indicator UV254. TLC plates were visualized with UV light and by staining with a potassium permanganate solution (0.75 g $KMnO_4$, 5 g K_2CO_3, and 0.63 mL 10% NaOH in 100 mL water) or a ninhydrin solution (1.5 g ninhydrin in 100 mL of *n*-butanol and 3.0 mL acetic acid). Column chromatography was performed on silica gel (230−400 mesh) or on alumina (neutral, 50−200 μm, 60 Å). ^{1}H and ^{13}C{1H} NMR spectra were measured with a 300 or 400 MHz spectrometer with TMS as the internal standard. Multiplicities are quoted as singlet (s), broad singlet (br s), doublet (d), doublet of doublets (dd), triplet (t), or multiplet (m). Spectra were assigned using COSY and HSQC experiments. All NMR chemical shifts (δ) were recorded in ppm, and coupling constants (J) were reported in hertz (Hz). The results of these experiments were processed with MestreNova software. High resolution electrospray mass (ESI) spectra were recorded on a microTOF spectrometer; accurate mass measurements were achieved by using sodium formate as an external reference.

2D NMR Experiments

Spectra were assigned using COSY and HSQC experiments. NOESY experiments were recorded on a 400 MHz spectrometer at 298 K. The experiments were conducted by using

phase-sensitive ge-2D NOESY spectra. The number of scans used was 16, and the mixing time was 800 ms.

_N_-Michael adducts (2 and 3)

(3*R*,7*R*,7a*S*)-7-Methoxy-7,7a-dimethyl-3-((propylamino)methyl)dihydro-5*H*-oxazolo[4,3-*b*]oxazole-2,5(3*H*)-dione (2a)

N-Propylamine (9 µL, 0.11 mmol) was added to a CH_2Cl_2 solution (1.1 mL) of dehydroalanine **1** (23 mg, 0.11 mmol). The reaction mixture was stirred at room temperature for 3 h and the solvent was then removed under vacuum to afford a mixture of **2a** (50%), **3a** (30%), and **1** (20%) as judged by ^{1}H NMR spectrum. HRMS ESI+ (m/z): 273.1446 $[M+H]^+$; calcd for $C_{12}H_{21}N_2O_5^+$: 273.1445 extracted from the reaction mixture. ^{1}H NMR data of the major compound **2a** extracted from the mixture: (400 MHz, $CDCl_3$) δ (ppm) 0.88-0.97 (m, 3H, $H^{3''}$), 1.48–1.56 (m, 2H, $H^{2''}$), 1.62 (s, 3H, Me^7), 1.69 (s, 3H, Me^{7a}), 2.58–2.76 (m, 2H, $H^{1''}$), 3.05 (dd, *J* = 13.3, 8.1 Hz, 1H, $H^{1'}$), 3.16 (dd, *J* = 13.2, 4.8 Hz, 1H, $H^{1'}$), 3.51 (s, 3H, OMe), 4.50 (dd, *J* = 8.1, 4.8 Hz, 1H, H^3).

(*R*)-2-((4*S*,5*R*)-4-Hydroxy-5-methoxy-4,5-dimethyl-2-oxooxazolidin-3-yl)-*N*-propyl-3-propylaminopropanamide (3a)

N-Propylamine (17 µL, 0.21 mmol) was added to a CH_2Cl_2 solution (1.0 mL) of dehydroalanine **1** (22 mg, 0.10 mmol). The reaction mixture was stirred at room temperature for 6 h and the solvent was then removed under vacuum to afford **3a** (34 mg, 0.10 mmol, quant.) as a yellow oil. $[\alpha]^{20}_D$ -71.4 (c 1.0, $CHCl_3$). HRMS ESI+ (m/z): 332.2180 $[M+H]^+$; calcd for $C_{15}H_{30}N_3O_5^+$: 332.2185. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.83–0.93 (m, 6H, $H^{3''}$, $H^{6''}$), 1.36 (s, 3H, Me^4), 1.43–1.55 (m, 4H, $H^{2''}$, $H^{5''}$), 1.58 (s, 3H, Me^5), 2.51–2.68 (m, 2H, $H^{1''}$), 3.11–3.38 (m, 3H, $H^{4''}$, $H^{3'}$), 3.42 (s, 3H, OMe), 3.86 (dd, *J* = 10.5, 4.4 Hz, 1H, $H^{2'}$), 6.87 (t, *J* = 5.9 Hz, 1H, PrN*H*CO). ^{13}C{1H} NMR (100 MHz, $CDCl_3$) δ (ppm) 11.3 ($C^{6''}$), 11.6 ($C^{3''}$), 14.4 (Me^5), 20.5 (Me^4), 22.6 ($C^{5''}$), 22.6 ($C^{2''}$), 41.3 ($C^{4''}$), 45.5 ($C^{3'}$), 51.0 (OMe), 51.2 ($C^{1''}$), 53.7 ($C^{4'}$), 56.9 ($C^{2'}$), 89.3 (C^4), 108.9 (C^5), 156.2 (C^2), 168.6 ($C^{1'}$).

(3*R*,7*R*,7a*S*)-3-((Benzylamino)methyl)-7-methoxy-7,7a-dimethyldihydro-5*H*-oxazolo[4,3-*b*]oxazole-2,5(3*H*)-dione (2b)

Benzylamine (11 µL, 0.10 mmol) was added to a CH_2Cl_2 solution (1.0 mL) of dehydroalanine **1** (21 mg, 0.10 mmol). The reaction mixture was stirred at room temperature for 1 h and the solvent was then removed under vacuum to afford a mixture of **2b** (75%), **3b** (20%), and **1** (5%) as judged by ^{1}H NMR spectrum. HRMS ESI+ (m/z): 321.1449 $[M+H]^+$; calcd for $C_{16}H_{21}N_2O_5^+$: 321.1445 extracted from the reaction mixture. ^{1}H NMR data of the major compound **2b** extracted from the mixture: (400 MHz, $CDCl_3$) δ (ppm) 1.61 (s, 3H, Me^7), 1.63 (s, 3H, Me^{7a}), 3.02 (dd, *J* = 13.2, 8.4 Hz, 1H, $H^{1'}$), 3.16 (dd, *J* = 13.2, 4.8 Hz, 1H, $H^{1'}$), 3.50 (s, 3H, OMe), 3.80–3.97 (m, 2H, $H^{2'}$), 4.55 (dd, *J* = 8.4, 4.8 Hz, 1H, H^3), 7.28–7.37 (m, 5H, arom.).

(*R*)-*N*-Benzyl-3-(benzylamino)-2-((4*S*,5*R*)-4-hydroxy-5-methoxy-4,5-dimethyl-2-oxooxazolidin-3-yl)propanamide (3b)

Benzylamine (24 µL, 0.22 mmol) was added to a CH_2Cl_2 solution (1.1 mL) of dehydroalanine **1** (23 mg, 0.11 mmol). The reaction mixture was stirred at room temperature for 3 h and the solvent was then removed under vacuum to afford **3b** (47 mg, 0.11 mmol, quant.) as a white solid. Mp: 124 – 127 ºC. $[\alpha]^{20}_D$ -20.1 (c 1.0, $CHCl_3$). HRMS ESI+ (m/z): 428.2172 $[M+H]^+$; calcd for $C_{23}H_{30}N_3O_5^+$: 428.2185. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.37 (s, 3H, Me^4), 1.59 (s, 3H, Me^5), 3.34 (s, 3H, OMe), 3.37–3.48 (m, 2H, $H^{3'}$), 3.76–3.85 (m, 2H, $H^{4'}$), 4.01 (dd, *J* = 9.9, 5.0 Hz, 1H, $H^{2'}$), 4.37–4.54 (m, 2H, $H^{5'}$), 7.21–7.36 (m, 11H, NH, arom.). ^{13}C{1H} NMR (100 MHz, $CDCl_3$) δ (ppm) 14.4 (Me^5), 20.5 (Me^4), 43.7 ($C^{5'}$), 45.7 ($C^{3'}$), 51.0 (OMe), 53.7 ($C^{4'}$), 56.8 ($C^{2'}$), 89.5 (C^4), 108.9 (C^5), 127.0, 127.2, 127.4, 127.6, 128.0, 128.6, 128.7, 128.9, 129.0, 137.7, 137.8 (arom.), 156.2 (C^2), 168.8 ($C^{1'}$).

(3*R*,7*R*,7a*S*)-3-((*tert*-Butylamino)methyl)-7-methoxy-7,7a-dimethyldihydro-5*H*-oxazolo[4,3-*b*]oxazole-2,5(3*H*)-dione (2c)

tert-Butylamine (10 µL, 0.10 mmol) was added to a CH_2Cl_2 solution (1.0 mL) of dehydroalanine **1** (22 mg, 0.10 mmol). The reaction mixture was stirred at room temperature for 16 h and the solvent was then removed under vacuum to afford a mixture of **2c** (25%) and **1** (75%) as judged by ^{1}H NMR spectrum. HRMS ESI+ (m/z): 287.1603 $[M+H]^+$; calcd for $C_{13}H_{23}N_2O_5^+$: 287.1601 extracted from the reaction mixture. ^{1}H NMR data of the minor compound **2c** extracted from the mixture: (400 MHz, $CDCl_3$) δ (ppm) 1.10 (s, 9H, $(CH_3)_3C$),

1.60 (s, 3H, Me^{7}), 1.71 (s, 3H, Me^{7a}), 3.02 (dd, *J* = 12.6, 6.9 Hz, 1H, $H^{1'}$), 3.09 (dd, *J* = 12.5, 4.6 Hz, 1H, $H^{1'}$), 3.50 (s, 3H, OMe), 4.43 (dd, *J* = 6.9, 4.6 Hz, 1H, H^{3}).

(3*R*,7*R*,7a*S*)-3-((Diethylamino)methyl)-7-methoxy-7,7a-dimethyldihydro-5*H*-oxazolo[4,3-*b*]oxazole-2,5(3*H*)-dione (2f)

Diethylamine (21 µL, 0.20 mmol) was added to a CH_2Cl_2 solution (0.9 mL) of dehydroalanine **1** (20 mg, 0.09 mmol). The reaction mixture was stirred at room temperature for 24 h and the solvent was then removed under vacuum to afford **2f** (24 mg, 0.09 mmol, quant.) as a colorless oil. $[\alpha]^{20}_{D}$ -50.9 (c 1.0, $CHCl_3$). HRMS ESI+ (m/z): 287.1610 $[M+H]^+$; calcd for $C_{13}H_{23}N_2O_5^+$: 287.1607. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.00–1.07 (m, 6H, $H^{2''}$), 1.60 (s, 3H, Me^{7}), 1.68 (s, 3H, Me^{7a}), 2.61–2.75 (m, 4H, $H^{1''}$), 2.97 (dd, *J* = 14.5, 7.4 Hz, 1H, $H^{1'}$), 3.06 (dd, *J* = 14.4, 3.8 Hz, 1H, $H^{1'}$), 3.49 (s, 3H, OMe), 4.44 (dd, *J* = 7.4, 3.8 Hz, 1H, H^{3}). ^{13}C{1H} NMR (100 MHz, $CDCl_3$) δ (ppm) 11.9 ($C^{2''}$), 16.8 (Me^{7}), 22.1 (Me^{7a}), 47.1 ($C^{1''}$), 51.7 (OMe), 53.1 ($C^{1'}$), 60.2 (C^{3}), 101.7 (C^{7a}), 108.2 (C^{7}), 159.3 (C^{5}), 168.6 (C^{2}).

(3*R*,7*R*,7a*S*)-3-(Azetidin-1-ylmethyl)-7-methoxy-7,7a-dimethyldihydro-5*H*-oxazolo[4,3-*b*]oxazole-2,5(3*H*)-dione (2g)

Azetidine (4 µL, 0.06 mmol) was added to a CH_2Cl_2 solution (0.6 mL) of dehydroalanine **1** (13 mg, 0.06 mmol). The reaction mixture was stirred at room temperature for 5 min and the solvent was then removed under vacuum to afford a mixture of **2g** (84%), **3g** (13%) and **1** (3%) as judged by ^{1}H NMR spectrum. HRMS ESI+ (m/z): 271.1292 $[M+H]^+$; calcd for $C_{12}H_{19}N_2O_5^+$: 271.1288 extracted from the reaction mixture. NMR data of the major compound **2g** extracted from the mixture: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.63 (s, 3H, Me^{7}), 1.74 (s, 3H, Me^{7a}), 2.07–2.23 (m, 2H, $H^{2''}$), 2.85 (dd, *J* = 12.8, 8.9 Hz, 1H, $H^{1'}$), 2.96 (dd, *J* = 12.8, 3.9 Hz, 1H, $H^{1'}$), 3.30–3.44 (m, 4H, $H^{1''}$), 3.52 (s, 3H, OMe), 4.48 (dd, *J* = 8.9, 3.9 Hz, 1H, H^{3}).

(4*S*,5*R*)-3-((*R*)-1,3-Di(azetidin-1-yl)-1-oxopropan-2-yl)-4-hydroxy-5-methoxy-4,5-dimethyloxazolidin-2-one (3g)

Azetidine (14 µL, 0.20 mmol) was added to a CH_2Cl_2 solution (1.0 mL) of dehydroalanine **1** (21 mg, 0.10 mmol). The reaction mixture was stirred at room temperature for 10 min and the solvent was then removed under vacuum to afford **3g** (33 mg, 0.10 mmol, quant.) as a

yellow oil. $[\alpha]^{20}_D$ -8.2 (c 1.0, $CHCl_3$). HRMS ESI+ (m/z): 328.1882 $[M+H]^+$; calcd for $C_{15}H_{26}N_3O_5^+$: 328.1867. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.39 (s, 3H, Me^4), 1.58 (s, 3H, Me^5) 2.06–2.29 (m, 4H, $H^{2''}$, $H^{4''}$), 3.02 (dd, *J* = 13.3, 10.9 Hz, 1H, $H^{3'}$), 3.15 (dd, *J* = 13.3, 2.8 Hz, 1H, $H^{3'}$), 3.24–3.32 (m, 2H, $H^{1''}$), 3.37–3.47 (m, 5H, OMe, $H^{1''}$), 3.59 (dd, *J* = 10.9, 2.8 Hz, 1H, $H^{2'}$), 3.99–4.31 (m, 4H, $H^{3''}$). ^{13}C{1H} NMR (100 MHz, $CDCl_3$) δ (ppm) 14.2 (Me^5), 16.0 ($C^{2''}$), 17.1 ($C^{4''}$), 20.3 (Me^4), 49.0 ($C^{3''}$), 46.5 ($C^{4''}$), 50.6 (OMe), 51.8 ($C^{3''}$), 51.8 ($C^{2'}$), 54.8 ($C^{1''}$), 56.8 ($C^{3'}$), 89.1 (C^4), 108.3 (C^5), 155.4 (C^2), 166.7 ($C^{1'}$).

(3*R*,7*R*,7a*S*)-7-Methoxy-7,7a-dimethyl-3-(pyrrolidin-1-ylmethyl)dihydro-5*H*-oxazolo[4,3-*b*]oxazole-2,5(3*H*)-dione (2h)

Pyrrolidine (10 µL, 0.12 mmol) was added to a CH_2Cl_2 solution (0.5 mL) of dehydroalanine **1** (25 mg, 0.12 mmol). The reaction mixture was stirred at room temperature for 15 min and the solvent was then removed under vacuum to afford a mixture of **2h** (85%) and **3h** (15%) as judged by ^{1}H NMR spectrum. $[\alpha]^{20}_D$ -71.0 (c 1.0, $CHCl_3$) corresponding to the mixture. HRMS ESI+ (m/z): 285.1446 $[M+H]^+$; calcd for $C_{13}H_{21}N_2O_5^+$: 285.1445 extracted from the reaction mixture. NMR data of the major compound **2h** extracted from the mixture: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.61 (s, 3H, Me^7), 1.68 (s, 3H, Me^{7a}), 1.78–1.83 (m, 4H, $H^{2''}$), 2.63–2.73 (m, 4H, $H^{1''}$), 2.92 (dd, *J* = 13.2, 8.9 Hz, 1H, $H^{1'}$), 3.05 (dd, *J* = 13.2, 3.7 Hz, 1H, $H^{1'}$), 3.49 (s, 3H, OMe), 4.48 (dd, *J* = 8.8, 3.6 Hz, 1H, H^3). ^{13}C{1H} NMR (100 MHz, $CDCl_3$) δ (ppm) 16.8 (Me^7), 22.0 (Me^{7a}), 23.7 ($C^{2''}$), 51.7 (OMe), 54.5 ($C^{1''}$), 56.4 ($C^{1'}$), 61.5 (C^3), 101.9 (C^{7a}), 108.3 (C^7), 159.4 (C^5), 171.5 (C^2).

(*S*)-Benzyl 1-(((3*R*,7*R*,7a*S*)-7-methoxy-7,7a-dimethyl-2,5-dioxotetrahydro-5*H*-oxazolo[4,3-*b*]oxazol-3-yl)methyl)pyrrolidine-2-carboxylate (2i)

HCl·L-Pro-OBn (24 mg, 0.10 mmol) and triethylamine (14 µL, 0.10 mmol) were added to a CH_2Cl_2 solution (1.0 mL) of dehydroalanine **1** (21 mg, 0.10 mmol). The reaction mixture was stirred at room temperature for 24 h and the solvent was then removed under vacuum to afford **2i** (40 mg, 0.10 mmol, 93%) as a white sticky foam. $[\alpha]^{20}_D$ -80.8 (c 1.0, $CHCl_3$). HRMS ESI+ (m/z): 419.1814 $[M+H]^+$; calcd for $C_{21}H_{27}N_2O_7^+$: 419.1813. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.57 (s, 3H, Me^7), 1.60 (s, 3H, Me^{7a}), 1.85–2.22 (m, 4H, $H^{3''}$, $H^{4''}$), 2.78–2.88 (m, 1H, $H^{5''}$), 3.13–3.17 (m, 1H, $H^{1'}$), 3.18–3.25 (m, 1H, $H^{5''}$), 3.35–3.40 (m, 1H, H1'), 3.46 (s, 3H, OMe), 3.71 (dd, *J* = 8.7, 4.6 Hz, 1H, $H^{2''}$), 4.54 (dd, *J* = 8.8, 3.5 Hz, 1H, H^3),

5.08–5.20 (m, 2H, PhCH_2), 7.31–7.38 (m, 5H, arom.). ^{13}C{1H} NMR (75 MHz, $CDCl_3$) δ (ppm) 16.5 (Me^7), 21.8 (Me^{7a}), 23.1 ($C^{4''}$), 29.2 ($C^{3''}$), 51.5 (OMe), 52.3 ($C^{1'}$), 52.7 ($C^{5''}$), 60.8 (C^3), 63.8 ($C^{2''}$), 66.3 (PhCH$_2$), 101.6 (C^{7a}), 108.0 (C^7), 128.1, 128.2, 128.4, 128.5, 128.6, 135.7 (arom.), 159.0 (C^5), 171.0 (C^2), 173.3 (CO$_2$Bn).

(3*R*,7*R*,7a*S*)-7-Methoxy-7,7a-dimethyl-3-(piperidin-1-ylmethyl)dihydro-5*H*-oxazolo[4,3-*b*]oxazole-2,5(3*H*)-dione (2j)

Piperidine (11 µL, 0.10 mmol) was added to a CH_2Cl_2 solution (0.9 mL) of dehydroalanine **1** (21 mg, 0.10 mmol). The reaction mixture was stirred at room temperature for 10 min and the solvent was then removed under vacuum to afford **2j** (31 mg, 0.10 mmol, quant.) as a colorless oil. $[\alpha]^{20}_D$ -81.3 (c 1.0, $CHCl_3$). HRMS ESI+ (m/z): 299.1609 $[M+H]^+$; calcd for $C_{14}H_{23}N_2O_5^+$: 299.1601. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.38–1.46 (m, 2H, $H^{3''}$), 1.52–1.62 (m, 7H, Me^7, $H^{2''}$), 1.70 (s, 3H, Me^{7a}), 2.52–2.62 (m, 4H, $H^{1''}$), 2.84 (dd, *J* = 14.1, 7.3 Hz, 1H, $H^{1'}$), 2.93 (dd, *J* = 14.0, 3.5 Hz, 1H, $H^{1'}$), 3.49 (s, 3H, OMe), 4.49 (dd, *J* = 7.3, 3.5 Hz, 1H, H^3). ^{13}C{1H} NMR (100 MHz, $CDCl_3$) δ (ppm) 16.8 (Me^7), 22.0 (Me^{7a}), 24.0 ($C^{3''}$), 25.9 ($C^{2''}$), 51.7 (OMe), 54.7 ($C^{1''}$), 59.2 ($C^{1'}$), 60.4 (C^3), 101.8 (C^{7a}), 108.3 (C^7), 159.2 (C^5), 171.9 (C^2).

(4*S*,5*R*)-4-Hydroxy-5-methoxy-4,5-dimethyl-3-((*R*)-1-oxo-1,3-di(piperidin-1-yl)propan-2-yl)oxazolidin-2-one (3j)

Piperidine (22 µL, 0.22 mmol) was added to a CH_2Cl_2 solution (1.1 mL) of dehydroalanine **1** (23 mg, 0.11 mmol). The reaction mixture was stirred at room temperature for 15 min and the solvent was then removed under vacuum to afford **3j** (42 mg, 0.11 mmol, quant.) as a white solid. Mp: 140 – 142 ºC. $[\alpha]^{20}_D$ +3.9 (c 1.0, $CHCl_3$). HRMS ESI+ (m/z): 384.2491 $[M+H]^+$; calcd for $C_{19}H_{34}N_3O_5^+$: 384.2493. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.33–1.74 (m, 18H, Me^4, Me^5, $H^{2''}$, $H^{3''}$, $H^{5''}$, $H^{6''}$), 2.22–2.77 (m, 4H, $H^{1''}$), 2.87 (dd, *J* = 14.1, 2.8 Hz, 1H, $H^{3'}$), 2.98 (dd, *J* = 14.1, 10.7 Hz, 1H, $H^{3'}$), 3.0–3.17 (m, 2H, $H^{4''}$), 3.37 (s, 3H, OMe), 3.49–3.61 (m, 1H, $H^{4''}$), 3.96–4.06 (m, 1H, $H^{4''}$), 4.09 (dd, *J* = 10.6, 2.8 Hz, 1H, $H^{2'}$). ^{13}C{1H} NMR (100 MHz, $CDCl_3$) δ (ppm) 14.2 (Me^5), 19.9 (Me^4), 23.8 ($C^{3''}$), 24.7 ($C^{6''}$), 25.1 ($C^{2''}$), 25.5 ($C^{5''}$), 26.4 ($C^{5''}$), 43.9 ($C^{4''}$), 46.5 ($C^{4''}$), 50.4 ($C^{2'}$), 50.6 (OMe), 53.5 ($C^{1''}$), 54.7 ($C^{1''}$), 58.6 ($C^{3'}$), 89.5 (C^4), 107.5 (C^5), 155.0 (C^2), 165.5 ($C^{1'}$).

(3*R*,7*R*,7a*S*)-7-Methoxy-7,7a-dimethyl-3-(morpholinomethyl)dihydro-5*H*-oxazolo[4,3-*b*]oxazole-2,5(3*H*)-dione (2k)

Morpholine (12 µL, 0.14 mmol) was added to a CH_2Cl_2 solution (1.4 mL) of dehydroalanine **1** (30 mg, 0.14 mmol). The reaction mixture was stirred at room temperature for 45 min and the solvent was then removed under vacuum to afford a mixture of **2k** (90%), **3k** (7%) and **1** (3%) as judged by ^{1}H NMR spectrum. $[\alpha]^{20}_D$ -79.2 (c 1.0, $CHCl_3$) corresponding to the mixture. HRMS ESI+ (m/z): 301.1391 $[M+H]^+$; calcd for $C_{13}H_{21}N_2O_6^+$: 301.1394 extracted from the reaction mixture. NMR data of the major compound **2k** extracted from the mixture: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.61 (s, 3H, Me^7), 1.69 (s, 3H, Me^{7a}), 2.58–2.65 (m, 4H, $H^{1''}$), 2.79 (dd, *J* = 13.8, 7.9 Hz, 1H, $H^{1'}$), 2.94 (dd, *J* = 13.8, 3.5 Hz, 1H, $H^{1'}$), 3.50 (s, 3H, OMe), 3.67–3.76 (m, 4H, $H^{2''}$), 4.50 (dd, *J* = 7.9, 3.5 Hz, 1H, H^3). ^{13}C{1H} NMR (100 MHz, $CDCl_3$) δ (ppm) 16.8 (Me^7), 22.2 (Me^{7a}), 51.8 (OMe), 53.9 ($C^{1''}$), 58.9 ($C^{1'}$), 60.5 (C^3), 66.9 ($C^{2''}$), 101.8 (C^{7a}), 108.3 (C^7), 159.2 (C^5), 171.5 (C^2).

(3*R*,7*R*,7a*S*)-3-(Azepan-1-ylmethyl)-7-methoxy-7,7a-dimethyldihydro-5*H*-oxazolo[4,3-*b*]oxazole-2,5(3*H*)-dione (2l)

Azepane (11 µL, 0.10 mmol) was added to a CH_2Cl_2 solution (1.0 mL) of dehydroalanine **1** (21 mg, 0.10 mmol). The reaction mixture was stirred at room temperature for 60 min and the solvent was then removed under vacuum to afford **2l** (31 mg, 0.10 mmol, quant.) as a yellow oil. $[\alpha]^{20}_D$ -82.9 (c 1.0, $CHCl_3$). HRMS ESI+ (m/z): 313.1754 $[M+H]^+$; calcd for $C_{15}H_{25}N_2O_5^+$: 313.1758. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.48–1.72 (m, 14H, Me^7, Me^{7a}, $H^{2''}$, $H^{3''}$), 2.77–2.89 (m, 4H, $H^{1''}$), 3.04 (dd, *J* = 14.2, 7.8 Hz, 1H, $H^{1'}$), 3.16 (dd, *J* = 14.2, 3.8 Hz, 1H, $H^{1'}$), 3.49 (s, 3H, OMe), 4.47 (dd, *J* = 7.8, 3.7 Hz, 1H, H^3). ^{13}C{1H} NMR (100 MHz, $CDCl_3$) δ (ppm) 16.8 (Me^7), 22.0 (Me^{7a}), 27.2 ($C^{3''}$), 28.2 ($C^{2''}$), 51.7 (OMe), 55.3 ($C^{1''}$), 58.0 ($C^{1'}$), 60.7 (C^3), 101.7 (C^{7a}), 108.2 (C^7), 159.2 (C^5), 171.9 (C^2).

(3*R*,7*R*,7a*S*)-7-methoxy-7,7a-dimethyl-3-((4-(7-nitrobenzo[*c*][1,2,5]oxadiazol-4-yl)piperazin-1-yl)methyl)dihydro-5*H*-oxazolo[4,3-*b*]oxazole-2,5(3*H*)-dione (2m)

4-Nitro-7-(piperazin-1-yl)-benzo[*c*][1,2,5]oxadiazole[62] (25 mg, 0.11 mmol) was added to a DMF solution (0.7 mL) of dehydroalanine **1** (15 mg, 0.07 mmol). The reaction mixture was stirred at 50 ºC for 24 h and the solvent was then removed under vacuum giving a mixture of

2m (80%) and **1** (20%). The crude mixture was purified by column chromatography on alumina (Hexane/AcOEt, 2:8) to afford **2m** (12 mg, 0.03 mmol, 37%) as a brown solid. Mp: 85–88 ºC. $[\alpha]^{20}_{D}$ -10.2 (c 1.0, $CHCl_3$). HRMS ESI+ (m/z): 463.1566 $[M+H]^+$; calcd for $C_{19}H_{23}N_6O_8^+$: 463.1577. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.60 (m, 3H, Me^7), 1.70 (s, 3H, Me^{7a}), 2.82–2.98 (m, 5H, $H^{1'}$, $H^{1''}$), 3.11 (dd, *J* = 13.7, 3.4 Hz, 1H, $H^{1'}$), 3.53 (s, 3H, OMe), 4.09–4.18 (m, 4H, $H^{2''}$), 4.57 (dd, *J* = 8.4, 3.4 Hz, 1H, H^3), 6.31 (d, *J* = 9.0, 1H, H^{2B}), 8.43 (d, *J* = 8.9, 1H, H^{3B}). ^{13}C{1H} NMR (75 MHz, $CDCl_3$) δ (ppm) 16.6 (Me^7), 22.2 (Me^{7a}), 49.2 ($C^{2''}$), 51.7 (OMe), 52.5 ($C^{1''}$), 58.1 ($C^{1'}$), 60.2 (C^3), 101.7 (C^{7a}), 102.7 (C^{2B}), 108.3 (C^7), 124.0 (C^{4B}), 135.0 (C^{3B}), 144.8 (C^{1B}), 144.8 (C^{6B}), 145.5 (C^{5B}), 159.1 (C^5), 171.0 (C^2).

(3*R*,7*R*,7a*S*)-3-((4-((5-(Dimethylamino)naphthalen-1-yl)sulfonyl)piperazin-1-yl)methyl)-7-methoxy-7,7a-dimethyldihydro-5*H*-oxazolo[4,3-b]oxazole-2,5(3*H*)-dione (2n)

1-Dansylpiperazine[62] (46 mg, 0.15 mmol) was added to a CH_2Cl_2 solution (1.0 mL) of dehydroalanine **1** (22 mg, 0.10 mmol). The reaction mixture was stirred at room temperature for 24 h and the solvent was then removed under vacuum to afford a mixture of **2n** (75%) and **1** (25%) as judged by ^{1}H NMR spectrum. $[\alpha]^{20}_{D}$ -53.4 (c 1.0, $CHCl_3$) corresponding to the mixture. HRMS ESI+ (m/z): 533.2066 $[M+H]^+$; calcd for $C_{25}H_{33}N_4O_7S^+$: 533.2070 extracted from the reaction mixture. NMR data of the major compound **2n** extracted from the mixture: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.57 (m, 3H, Me^7), 1.59 (s, 3H, Me^{7a}), 2.62–2.69 (m, 4H, $H^{1''}$), 2.72 (dd, *J* = 13.8, 8.4 Hz, 1H, $H^{1'}$), 2.88 (s, 6H, Me^D), 2.90 (dd, *J* = 13.8, 3.4 Hz, 1H, $H^{1'}$), 3.17–3.25 (m, 4H, $H^{2''}$), 3.45 (s, 3H, OMe), 4.39 (dd, *J* = 8.4, 3.4 Hz, 1H, H^3), 7.17 (d, *J* = 7.6, 1H, H^{6D}), 7.48–7.58 (m, 2H, H^{3D}, H^{7D}), 8.17 (d, *J* = 7.3, 1H, H^{4D}), 8.40 (d, *J* = 9.0, 1H, H^{2D}), 8.55 (d, *J* = 8.6, 1H, H^{8D}). ^{13}C{1H} NMR (75 MHz, $CDCl_3$) δ (ppm) 16.6 (Me^7), 22.1 (Me^{7a}), 45.5 (Me^D), 45.5 ($C^{2''}$), 51.7 (OMe), 52.7 ($C^{1''}$), 58.2 ($C^{1'}$), 60.4 (C^3), 101.7 (C^{7a}), 108.3 (C^7), 115.4 (C^{6D}), 119.8 (C^{2D}), 123.2 (C^{3D}), 128.2 (C^{7D}), 130.1 (C^{4aD}), 130.6 (C^{8aD}), 130.7 (C^{4D}), 130.8 (C^{8D}), 132.6 (C^{1D}), 151.8 (C^{5D}), 159.1 (C^5), 171.1 (C^2).

(3*S*,7*S*,7a*R*)-3-((1*H*-Imidazol-1-yl)methyl)-7-methoxy-7,7a-dimethyldihydro-5*H*-oxazolo[4,3-*b*]oxazole-2,5(3*H*)-dione (*ent*-2o)

Imidazole (7 mg, 0.10 mmol) was added to a CH_2Cl_2 solution (1.0 mL) of dehydroalanine *ent*-**1** (22 mg, 0.10 mmol). The reaction mixture was stirred at room temperature for 5 min

and the solvent was then removed under vacuum to afford *ent*-**2o** (28 mg, 0.10 mmol, quant.) as a yellowish oil. $[\alpha]^{20}_D$ +44.5 (c 1.0, $CHCl_3$). HRMS ESI+ (m/z): 282.1089 $[M+H]^+$; calcd for $C_{12}H_{16}N_3O_5^+$: 282.1084. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.00 (s, 3H, Me^{7a}), 1.57 (s, 3H, Me^7), 3.48 (s, 3H, OMe), 4.39–4.54 (m, 2H, $H^{1'}$), 4.59 ('t', *J* = 4.6 Hz, 1H, H^3), 6.96–6.99 (m, 1H, H^{4im}), 7.07–7.11 (m, 1H, H^{5im}), 7.52–7.56 (m, 1H, H^{2im}). ^{13}C{1H} NMR (75 MHz, $CDCl_3$) δ (ppm) 17.1 (Me^7), 21.2 (Me^{7a}), 46.5 ($C^{1'}$), 52.1 (OMe), 61.9 (C^3), 101.8 (C^{7a}), 109.4 (C^7), 119.8 (C^{4im}), 130.6 (C^{5im}), 137.8 (C^{2im}), 158.8 (C^5), 169.7 (C^2).

(3*R*,7*R*,7a*S*)-3-((1*H*-Imidazol-1-yl)methyl)-7-methoxy-7,7a-dimethyldihydro-5*H*-oxazolo[4,3-*b*]oxazole-2,5(3*H*)-dione (2o)

Imidazole (8 mg, 0.12 mmol) was added to a CH_2Cl_2 solution (1.2 mL) of dehydroalanine **1** (25 mg, 0.12 mmol). The reaction mixture was stirred at room temperature for 5 min and the solvent was then removed under vacuum to afford **2o** (32 mg, 0.11 mmol, quant.) as a yellowish oil. $[\alpha]^{20}_D$ -44.1 (c 1.0, $CHCl_3$). HRMS ESI+ (m/z): 282.1082 $[M+H]^+$; calcd for $C_{12}H_{16}N_3O_5^+$: 282.1084. ^{1}H and ^{13}C{1H} NMR spectra are identical to its enantiomer *ent*-**2o**.

(*S*)-Methyl 2-((*tert*-butoxycarbonyl)amino)-3-(1-(((3*S*,7*S*,7a*R*)-7-methoxy-7,7a-dimethyl-2,5-dioxotetrahydro-2*H*-oxazolo[4,3-*b*]oxazol-3-yl)methyl)-1*H*-imidazol-4-yl)propanoate (*ent*-2r)

Boc-L-His-OMe (140 mg, 0.52 mmol) was added to a THF solution (1.0 mL) of dehydroalanine *ent*-**1** (95 mg, 0.45 mmol). The reaction mixture was stirred at 50 ºC for 30 min and the solvent was then removed under vacuum. The crude mixture was purified by column chromatography on silicagel (CH_2Cl_2/MeOH, 95:5) to afford *ent*-**2r** (178 mg, 0.37 mmol, 84%) as a yellow oil. $[\alpha]^{20}_D$ +54.7 (c 1.0, $CHCl_3$). HRMS ESI+ (m/z): 483.2071 $[M+H]^+$; calcd for $C_{21}H_{31}N_4O_9^+$: 483.2086. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.02 (s, 3H, Me^{7a}), 1.43 (s, 9H, $C(CH_3)_3$), 1.57 (s, 3H, Me^7), 2.93–3.12 (m, 2H, H^β), 3.48 (s, 3H, OMe), 3.70 (s, 3H, CO_2Me), 4.33–4.46 (m, 2H, $H^{1'}$), 4.45–4.60 (m, 2H, H^α, H^3), 5.78 (d, *J* = 8.2 Hz, 1H, N*H*Boc), 6.75 (s, 1H, H^{5im}), 7.46 (s, 1H, H^{2im}). ^{13}C{1H} NMR (75 MHz, $CDCl_3$) δ (ppm) 17.1 (Me^7), 21.4 (Me^{7a}), 28.4 ($C(CH_3)_3$), 30.1 (C^β), 46.6 ($C^{1'}$), 52.1 (OMe), 52.4 (CO_2CH_3), 53.5 (C^α), 61.8 (C^3), 79.8 ($C(CH_3)_3$), 101.8 (C^{7a}), 109.4 (C^7), 117.4 (C^{5im}), 137.7 (C^{2im}), 138.9 (C^{4im}), 155.6 ($NHCO_2{}^t$Bu), 158.7 (C^5), 169.6 (C^2), 172.5 (CO_2Me).

Ethyl 1-cyclopropyl-6-fluoro-7-(4-(((3*R*,7*R*,7a*S*)-7-methoxy-7,7a-dimethyl-2,5-dioxotetrahydro-5*H*-oxazolo[4,3-*b*]oxazol-3-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (2s)

Ciprofloxacin ethyl ester hydrochloride (35 mg, 0.10 mmol) and triethylamine (14 µL, 0.10 mmol) were added to a 1:1 DMF/DMSO solution (1.0 mL) of dehydroalanine **1** (21 mg, 0.10 mmol). The reaction mixture was stirred at room temperature for 24 h and the solvent was then removed under vacuum to afford a mixture of **2s** (70%) and **1** (30%). Mp: 111–114 ºC corresponding to the mixture. $[\alpha]^{20}_{D}$ -10.2 (c 1.0, $CHCl_3$) corresponding to the mixture. HRMS ESI+ (m/z): 573.2373 $[M+H]^+$; calcd for $C_{28}H_{34}FN_4O_8^+$: 573.2355 extracted from the reaction mixture. NMR data of the major compound **2s** extracted from the mixture: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.99–1.04 (m, 2H, H^{2Cp}), 1.23–1.34 (m, 5H, H^{2Cp}, H^{2Et}), 1.53 (s, 3H, Me^7), 1.61 (s, 3H, Me^{7a}), 2.71–2.83 (m, 5H, $H^{1'}$, $H^{1''}$), 2.93–2.97 (m, 1H, $H^{1'}$), 3.18–3.24 (m, 4H, $H^{2''}$), 3.37–3.42 (m, 4H, OMe, H^{1Cp}), 4.24 (q, 2H, *J* = 7.1 Hz, H^{1Et}), 4.46 (dd, 1H, *J* = 8.2, 3.2 Hz, H^3), 7.18 (d, 1H, *J* = 6.9 Hz, H^{8CF}), 7.84 (d, 1H, *J* = 13.2, H^{5CF}), 8.40 (s, 1H, H^{2CF}). ^{13}C{1H} NMR (100 MHz, $CDCl_3$) δ (ppm) 8.1 (C^{2Cp}), 14.3 (C^{2Et}), 16.5 (Me^7), 22.0 (Me^{7a}), 34.5 (C^{1Cp}), 49.6 ($C^{2''}$), 51.6 (OMe), 52.8 ($C^{1''}$), 58.2 ($C^{1'}$), 60.2 (C^3), 60.6 (C^{1Et}), 101.6 (C^{7a}), 104.9 (C^{8CF}), 108.1 (C^7), 109.9 (C^{3CF}), 112.9 (d, *J* = 23.0 Hz, C^{5CF}), 130.0 (C^{4aCF}), 137.9 (C^{8aCF}), 144.3 (d, *J* = 10.6 Hz, C^{7CF}), 148.0 (C^{2CF}), 153.2 (d, *J* = 248.5 Hz, C^{6CF}), 159.0 (C^5), 165.2 (CO_2Et), 171.1 (C^2), 173.1 (C^{4CF}).

Amino acids (4)

(*R*)-β-Benzylaminoalanine hydrochloride (4b)

Compound **3b** (47 mg, 0.11 mmol) was suspended in a 6 M HCl aqueous solution (10.0 mL) and the reaction mixture was stirred at 60 ºC for 16 h. The solvent was then removed under vacuum, the crude mixture was dissolved in water (5 mL) and washed with ethyl acetate (5 mL) to afford amino acid hydrochloride **4b** and benzylamine hydrochloride after solid phase extraction in a C18 cartridge. Mp: 220–230 (dec.). $[\alpha]^{20}_{D}$ -22.2 (c 10.0, 6 M HCl). HRMS ESI+ (m/z): 195.1130 $[M+H]^+$; calcd for $C_{10}H_{15}N_2O_2^+$: 195.1128. ^{1}H NMR (300 MHz, D_2O) δ (ppm) 3.50–3.55 (m, 2H, H^{β}), 4.09 (dd, *J* = 8.2, 6.6 Hz, 1H, H^{α}), 4.31–4.46 (m, 2H, $PhCH_2$), 7.52–7.55 (m, 5H, arom.). ^{13}C{1H} NMR (75 MHz, D_2O) δ (ppm) 45.3 (C^{β}), 49.0 (C^{α}), 51.4 (Ph*C*H_2), 128.8, 129.2, 129.4, 129.8, 129.9, 132.6 (arom.), 170.7 (CO_2H).

(*R*)-β-(Piperidin-1-yl)alanine hydrochloride (4j)

Compound **2j** (182 mg, 0.61 mmol) was suspended in a 6 M HCl aqueous solution (10.0 mL) and the reaction mixture was stirred at 60 ºC for 16 h. The solvent was then removed under vacuum, the crude mixture was dissolved in water (7 mL), washed with ethyl acetate (7 mL) and purified by solid phase extraction in a C18 cartridge to afford **4j** (102 mg, 0.59 mmol, 97%). Mp: 230–240 (dec.). $[\alpha]^{20}_{D}$ -14.7 (c 1.0, H_2O). HRMS ESI+ (m/z): 173.1283 $[M+H]^+$; calcd for $C_8H_{17}N_2O_2^+$: 173.1285 ^{1}H NMR (400 MHz, D_2O) δ (ppm) 1.44–1.55 (m, 1H, H^3), 1.69–1.84 (m, 3H, $2H^2$, H^3), 1.89–2.00 (m, 2H, H^2), 3.03–3.14 (m, 2H, H^1), 3.56 (dd, *J* = 14.0, 5.7 Hz, 1H, H^β), 3.60–3.70 (m, 2H, H^1), 3.71 (dd, *J* = 14.0, 7.6 Hz, 1H, H^β), 4.51 (dd, *J* = 7.6, 5.7 Hz, 1H, H^α). ^{13}C{1H} NMR (100 MHz, D_2O) δ (ppm) 20.8 (C^3), 22.7 ($2C^2$), 47.7 (C^α), 53.9 (C^1), 54.8 (C^1), 55.2 (C^β), 169.2 (CO_2H).

(*R*)-β-(Azepan-1-yl)alanine hydrochloride (4l)

Compound **2l** (167 mg, 0.53 mmol) was suspended in a 6 M HCl aqueous solution (10.0 mL) and the reaction mixture was stirred at 60 ºC for 16 h. The solvent was then removed under vacuum, the crude mixture was dissolved in water (7 mL), washed with ethyl acetate (7 mL) and purified by solid phase extraction in a C18 cartridge to afford **4l** (94 mg, 0.50 mmol, 94%). Mp: 230–240 (dec.). $[\alpha]^{20}_{D}$ -7.1 (c 1.0, H_2O). HRMS ESI+ (m/z): 187.1447 $[M+H]^+$; calcd for $C_9H_{19}N_2O_2^+$: 187.1441. ^{1}H NMR (400 MHz, D_2O) δ (ppm) 1.62–1.75 (m, 4H, H^3), 1.81–1.99 (m, 4H, H^2), 3.26–3.45 (m, 2H, H^1), 3.53–3.66 (m, 2H, H^1), 3.64 (dd, *J* = 13.7, 5.3 Hz, 1H, H^β), 3.72 (dd, *J* = 13.7, 8.5 Hz, 1H, H^β), 4.46 (dd, *J* = 8.5, 5.3 Hz, 1H, H^α). ^{13}C{1H} NMR (100 MHz, D_2O) δ (ppm) 23.3 ($2C^3$), 25.8 ($2C^2$), 47.8 (C^α), 55.1 (C^1), 55.4 (C^β), 56.9 (C^1), 169.2 (CO_2H).

L-Isohistidine hydrochloride (*ent*-4o)

Compound *ent*-**2o** (20 mg, 0.07 mmol) was suspended in a 6 M HCl aqueous solution (5.0 mL) and the reaction mixture was stirred at 60 ºC for 16 h. The solvent was then removed under vacuum, the crude mixture was dissolved in water (5 mL), washed with ethyl acetate (5 mL) and purified by solid phase extraction in a C18 cartridge to afford *ent*-**4o** (13 mg, 0.07 mmol, 94%) as a white solid. Mp: 220–230 (dec.). $[\alpha]^{20}_{D}$ -6.2 (c 1.0, H_2O). HRMS ESI+ (m/z): 156.0774 $[M+H]^+$; calcd for $C_6H_{10}N_3O_2^+$: 156.0768. ^{1}H NMR (400 MHz, D_2O) δ

(ppm) 4.54 (t, J = 5.5 Hz, 1H, H^{α}), 4.88 (d, J = 5.5 Hz, 2H, H^{β}), 7.44–7.72 (m, 2H, H^{4im}, H^{5im}), 8.90 (s, 1H, H^{2im}). $^{13}C\{1H\}$ NMR (100 MHz, D_2O) δ (ppm) 48.1 (C^{β}), 53.1 (C^{α}), 120.6 (C^{4im}), 122.3 (C^{5im}), 135.9 (C^{2im}), 169.0 (CO_2H). These physical data are in agreement with those reported in the literature.[22]

L,L-Nτ-histidinoalanine hydrochloride or (*S*,*S*)-τ-HAL (*ent*-4r)

Compound *ent*-**2r** (26 mg, 0.05 mmol) was suspended in a 6 M HCl aqueous solution (5.0 mL) and the reaction mixture was stirred at 60 ºC for 16 h. The solvent was then removed under vacuum, the crude mixture was dissolved in water (5 mL), washed with ethyl acetate (5 mL) and purified by solid phase extraction in a C18 cartridge to afford *ent*-**4r** (15 mg, 0.05 mmol, 91%) as a white solid. Mp: 230–240 (dec.). $[\alpha]^{20}_D$ +5.9 (c 1.0, H_2O). HRMS ESI+ (m/z): 243.1084 $[M+H]^+$; calcd for $C_9H_{15}N_4O_4^+$: 243.1088. ^1H NMR (400 MHz, D_2O) δ (ppm) 3.32 (d, J = 6.6 Hz, 2H, $H^{\beta'}$), 4.04 (t, J = 6.6 Hz, 1H, $H^{\alpha'}$), 4.26 (t, J = 5.1 Hz, 1H, H^{α}), 4.72–4.76 (m, 2H, H^{β}), 7.48 (s, 1H, H^{5im}), 8.80 (s, 1H, H^{2im}). $^{13}C\{1H\}$ NMR (75 MHz, D_2O) δ (ppm) 25.5 ($C^{\beta'}$), 48.6 (C^{β}), 53.6 ($C^{\alpha'}$), 59.2 (C^{α}), 121.0 (C^{5im}), 128.8 (C^{4im}), 136.4 (C^{2im}), 169.9 (CO_2H), 171.5 (CO_2H).

(*R*)-7-(4-(2-amino-2-carboxyethyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride (4s)

Compound **2s** (63 mg, 0.11 mmol) was suspended in a 6 M HCl aqueous solution (10.0 mL) and the reaction mixture was stirred at 60 ºC for 16 h. The solvent was then removed under vacuum, the crude mixture was dissolved in water (5 mL), washed with ethyl acetate (5 mL) and purified by solid phase extraction in a C18 cartridge to afford **4s** (47 mg, 0.10 mmol, 94%). Mp: 230–240 (dec.). $[\alpha]^{20}_D$ -2.0 (c 1.0, H_2O). HRMS ESI+ (m/z): 419.1715 $[M+H]^+$; calcd for $C_{20}H_{24}FN_4O_5^+$: 419.1725. ^1H NMR (300 MHz, D_2O) δ (ppm) 0.76–0.92 (m, 2H, H^{2Cp}), 1.06–1.22 (m, 2H, H^{2Cp}), 2.43–2.73 (m, 4H, H^2, H^{β}), 2.80–2.89 (m, 2H, H^2), 2.93–3.02 (m, 2H, H^1), 3.06–3.14 (m, 2H, H^1), 3.23–3.33 (m, 1H, H^{1Cp}), 3.39 (dd, J = 8.3, 4.5 Hz, 1H, H^{α}), 7.12–7.20 (m, 1H, H^{8CF}), 7.44–7.54 (m, 1H, H^{5CF}), 8.28 (s, 1H, H^{2CF}). $^{13}C\{1H\}$ NMR (75 MHz, D_2O) δ (ppm) 7.3 (C^{2CP}), 34.6 (C^{1Cp}), 44.2 (C^1), 49.2 (C^2), 50.4 (C^2), 52.3 (C^1), 53.6 (C^{α}), 62.6 (C^{β}), 105.9 (C^{8CF}), 111.2 (d, J = 22.6 Hz, C^{5CF}), 116.0 (C^{3CF}), 121.7 (C^{4aCF}), 138.1 (C^{8aCF}), 144.0 (d, J = 30.0 Hz, C^{7CF}), 147.1 (C^{2CF}), 152.8 (d, J = 247.5 Hz, C^{6CF}), 168.4 (CO_2H^{CF}), 172.3 (CO_2H), 175.3 (C^{4CF}).

X-ray Diffraction Analysis

CIF file for compound **3b** is presented in the Supporting Information. The SHELXL97 program[63] was used for the refinement of crystal structures, and hydrogen atoms were fitted at theoretical positions.

Quantum Mechanical Calculations

Full geometry optimizations were carried out with Gaussian 16[64] using the M06-2X hybrid functional[65] and 6-31+G(d,p) basis set in combination with ultrafine integration grids. Bulk solvent effects in dichloromethane were considered implicitly through the IEF-PCM polarizable continuum model.[66] The possibility of different conformations was taken into account. Frequency analyses were carried out at the same level used in the geometry optimizations, and the nature of the stationary points was determined in each case according to the appropriate number of negative eigenvalues of the Hessian matrix. The quasiharmonic approximation reported by Truhlar et al. was used to replace the harmonic oscillator approximation for the calculation of the vibrational contribution to enthalpy and entropy.[67] Scaled frequencies were not considered. Mass-weighted intrinsic reaction coordinate (IRC) calculations were carried out by using the Gonzalez and Schlegel scheme[68,69] -in order to ensure that the TSs indeed connected the appropriate reactants and products. Gibbs free energies (ΔG) were used for the discussion on the relative stabilities of the considered structures. The lowest energy conformer for each calculated stationary point was considered in the discussion; all the computed structures can be obtained from authors upon request. Cartesian coordinates, electronic energies, entropies, enthalpies, Gibbs free energies, and lowest frequencies of the calculated structures are available in the Supporting Information.

Supporting Information

Supporting information for this article is given via a link at the end of the document.

Additional experimental details, X–ray data (CIF), computational data and copies of NMR spectra for all new compounds (PDF).

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Notes

The authors declare no competing financial interest.

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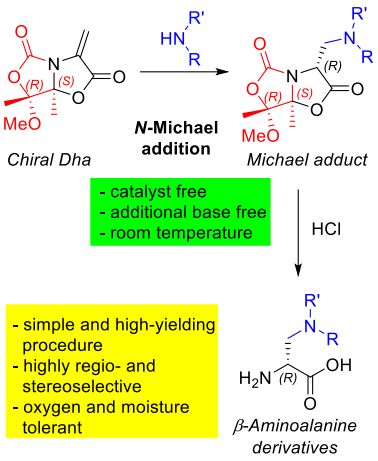
TOC Graphic



Short abstract

This work describes the efficient synthesis of enantiopure β-aminoalanine derivatives, using a highly regio- and stereoselective *N*-Michael addition of nitrogen-nucleophiles on a chiral bicyclic dehydroalanine. This key reaction takes place at room temperature without needing any catalyst or additional base, allowing the synthesis of 1-isohistidine, τ-histidinoalanine, β-alkylaminoalanines, and fluorescent and ciprofloxacin-containing amino acids.