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Synthesis and Application of Oxidatively Modified Amino Acid Derivatives

Ph.D. Thesis

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DEKLARACE

Tato práce byla provedena na Ústavu Organické chemie a Biochemie, Akademii věd České republiky vedeným RnDr. Janem Konvalinkou, Csc., v laboratoři Dr. Ullricha Jahna v letech 2018-2024. Deklaruji, že jsem na dizertaci pracovala nezávisle s pomocí školitele a všechny užité zdroje jsou řádně citované. Prohlašuji, že tato práce nebo její podstatná část nebyly použity jako závěrečná práce pro získání jiného nebo podobného typu univerzitní kvalifikace.

DECLARATION

This work was carried out at the Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences directed by RNDr. Jan Konvalinka, CSc., in the laboratory of Dr. Ullrich Jahn in 2018-2024. I declare that I have worked on the Ph.D. thesis independently with the help of my supervisor and all used resources are properly cited. I confirm that the work or its substantial part has not been used as a thesis to obtain a different or similar type of university qualification.

V Praze

Dne 12/07/2024 Navyasree Venugopal

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ABSTRACT

Peptides and proteins are important targets for biochemical and pharmaceutical applications. Non-natural modifications to these biomolecules have gained significant interest in the field as these modifications can grant them novel properties. Strategic approaches are required to selectively modify a given peptide. A promising strategy for the chemical modification of peptides is the selective modification of the glycine unit within a peptide.

The first part of the project presents the novel methodology of enolate oxidation for the modification of glycine derivatives using the nitroxide radical 2,2,6,6-tetramethylpiperidine N-oxide (TEMPO) to generate glycine alkoxyamines. The methodology was extended to short peptides, revealing interesting selectivity and reactivity of amino acids. The alkoxyamines can be further modified by thermal homolysis or acid-mediated heterolysis to create a library of non-natural amino acids. Under physiological conditions, acid-mediated heterolysis was used to modify glycine-containing peptides, allowing access to cross-conjugated peptides.

The second part of the study involved synthesizing a series of hindered nitroxide radicals and applying them to the methodology of enolate oxidation of glycinates. The study focused on the reactivity of glycine enolate to different hindered nitroxides and thermal homolysis of newly generated glycine alkoxyamines.

Lastly, we demonstrate the application of the methodology to modify insulin by conjugating glycine alkoxyamines under physiological conditions *in vitro*. Additionally, we designed and synthesized a hydrophilic piperidine nitroxide with azide functionality at the 4^{th} position, which has the potential for nitroxide conjugation in various applications.

ABSTRACT (Czech)

Peptidy a proteiny jsou důležité látky pro biochemické a farmaceutické aplikace. Nepřírodní modifikace těchto biomolekul se dostaly do popředí vědeckého zájmu, protože jim mohou poskytnout nové vlastnosti. Selektivní modifikace daného peptidu vyžaduje strategický přístup. Slibnou strategií chemické modifikace peptidů je selektivní modifikace glycinové jednotky daného peptidu.

První část projektu představuje novou metodologii oxidace enolátů derivátů glycinu za použití nitroxidového radikálu 2,2,6,6-tetramethylpiperidin N-oxidu (TEMPO) poskytující alkoxyaminy glycinu. Tato metodologie byla rozšířena na krátké peptidy, což odhalilo zajímavou selektivitu a reaktivitu aminokyselin. Tyto alkoxyaminy mohou být dále modifikovány termální homolýzou nebo kysele katalyzovanou heterolýzou za vzniku knihovny nepřírodních aminokyselin. Kysele katalyzovaná heterolýza byla použita k modifikaci glycin-obsahujících peptidů za fyziologických podmínek, což umožnilo přístup ke konjugovaným peptidům.

Druhá část se zabývá syntézou bráněných nitroxidových radikálů a jejich aplikací v metodologii oxidací enolátů glycinátů. Tato studie se zaměřuje na reaktivitu enolátů glycintů s různými bráněnými nitroxidy a termální homolýzu nově generovaných alkoxyaminů glycinu.

Na závěr jsme demonstrovali aplikaci této metodologie na modifikaci insulinu konjugací alkoxyaminů glycinu za fyziologických podmínek *in vitro*. Navíc jsme navrhli a syntetizovali hydrofilní piperidin nitroxid s azidovou funkcí v pozici 4, který má potenciál v nitroxidové konjugaci v různých aplikacích.

LIST OF ABBREVIATIONS

AAs	amino acids
AcOH	acetic acid
Arg	arginine
Asp	aspartate
AZADO	2-Azaadamantane N-Oxyl
Boc	*tert*-butoxycarbonyl
Bn	benzyl
br	broad
Bz	benzoyl
cat.	catalytic
Cbz	benzyloxycarbonyl
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	N,N′-dicyclohexylcarbodiimide
DCM	dichloromethane
DIPEA	diisopropylethylamine
DLP	dilauroyl peroxide
DMAP	4-(dimethylamino)pyridine
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DTBP	di-*tert*-butyl peroxide
ECD	electronic circular dichroism
EI	electron ionization
EPR	electron paramagnetic resonance
ESI	electron spray ionization
equiv.	equivalent
$FeCp_2PF_6$	ferrocenium hexafluorophosphate
Fmoc	fluorenylmethyloxycarbonyl
Gly	glycine
HATU	1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate

HBTU	hexafluorophosphate-benzotriazole tetramethyl uronium
HMDS	hexamthyldisilazane
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
IOCB	Institute of Organic Chemistry and Biochemistry
KHMDS	potassium bis(trimethylsilayl)amide
LDA	lithium diisopropylamide
LHMDS	lithium bis(trimethylsilayl)amide
LRMS	low-resolution mass spectrometry
Lys	lysine
*m*CPBA	*meta*-chloroperbenzoic acid
m.p.	melting point
mRNA	messenger ribonucleic acid
MS	mass spectrometry
MW	microwave
NBS	*N*-bromosuccinimide
NHC	N-heterocyclic carbene
NMP	nitroxide mediated polymerization
NMR	nuclear magnetic resonance
Nor-AZADO	9-Azanoradamantane N-oxyl
PE	petroleum ether
PEG	polyethylene glycol
PG	protecting group
PHM	peptidylglycine monooxygenase
PRE	persistent radical effect
PTM	post translational modification
rac	racemic
R_f	retention factor (in chromatography)
ROESY	rotating frame Overhauser enhancement spectrometry
rt	room temperature
Sar	sarcosine
Ser	serine
SCE	standard calomel electrode
SET	single electron transfer

SG1	*N*-*tert*-butyl-*N*-[1-diethylphosphono-(2,2-dimethylpropyl)]nitroxide
*t*BuLi	*tert*-butyl lithium
TEMPO	(2,2,6,6- tetramethyl)piperidne-1-yl)oxyl radical
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPNO	2,2,5-trimethyl-4-phenyl-3-azahexane-3-nitroxide
TLC	thin layer chromatography
TMIO	1,1,3,3-tetramethylisoindolin-2-nitroxide
TMP	2,2,6,6- tetramethyl)piperidne-1-yl
TRIP	3,3′-Bis(2,4,6-triisopropylphenyl)-1,1′-binaphthyl-2,2′-diyl hydrogenphosphate
Trp	tryptophan
Tyr	tyrosine
UAA	unnatural amino acid
UV	ultraviolet
Val	valine
XRD	X-ray diffraction

COMMON LATIN ABBREVIATION

cf.	*confer/conferatur* – compare
de novo	a new, again from the beginning
et al.	*et alia* – and others
e.g.	*exempli gratia* – for example
i.e.	*id est* – that is
in situ	in its original place or position
in vitro	outside the living body, in an artificial environment
in vivo	inside the living body
infra	below
supra	above
vide	see, consult

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1. INTRODUCTION

1.1 Amino Acids, Peptides

1.1.1 Amino Acids, Peptides and Their Chemical Modifications

Nature employs a conserved set of 22 genetically encoded natural α-amino acids to yield an extensive array of protein structures designed for specific functionalities such as enzyme catalysis, transport, and mechanical support, among others. The global collection of proteins produced by organisms, known as the proteome, is complex and diverse. This diversity arises both at the mRNA level and through post-translational modifications.[1] The major class of post-translational modification (PTM) of a protein is the covalent addition of one or more groups, such as phosphoryl, acetyl or glycosyl, to one or more amino acid side chains. These modifications have functional consequences in proteins, such as conformational changes, functional enhancement or changes in function.[1]

Chemical modifications are important for creating engineered peptides and proteins that can elucidate essential structural determinants governing their functions or discover novel functional roles.[2-3] Recent progress in the modification of proteins confirms them as a strong tool for bioimaging, detailed functional analysis of PTM and construction of protein based biosensors.[4-6] Moreover, peptide-based structural frameworks have also emerged as target molecules in the field of pharmaceutical development.[7]

A number of methodologies for chemical modification of proteins have been reported[5, 8-11] and can be classified as 1) Incorporation of unnatural amino acids (UAAs) by genetic code expansion and their subsequent modification using biorthogonal reactions, 2) recognition driven chemical labelling, and 3) modification of proteins using the reactivity of existing amino acids.[4] The first approach of modification consists of incorporation of UAAs in a protein by genetic codon expansion techniques and the newly introduced UAAs, consisting of azide, alkyne, alkene, ketone or tetrazine functional groups, allow site selective modification of target proteins by biorthogonal reactions.[12-15] However, it is very challenging to incorporate UAAs site-specifically into endogenous proteins in live-cells. On the other hand, the recognition driven chemical modifications of proteins allow protein-specific modifications in live cells without any genetic manipulations. A specific interaction of the protein with a designed labelling reagent at the ligand binding site of a protein facilitates the selective modification of an amino acid side chain. However, this approach requires an appropriate ligand for selective

recognition in each protein, which makes it a complex process.[16-18] The method of modification of naturally occurring amino acids surpasses the alternative approaches due to its convenient strategy, obviating the requirement for specialized techniques such as artificial genetic manipulation or designing of specific peptide tags.[4] This strategy facilitates the synthesis of biomaterials in a time and cost-effective manner. However, it is noteworthy that the methodology is restricted to peptides or isolated proteins *in vitro*. Nonetheless, even within these limitations, the practical utility and applicability of this approach bestows significant value in chemical modification of peptides and proteins.

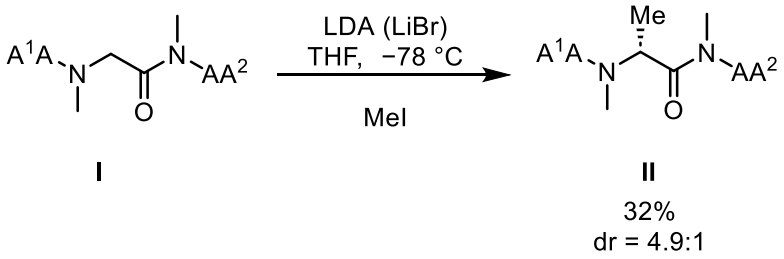
The method of modification of naturally occurring amino acids can be categorized into two fundamental strategies: 1) modifying the preexisting side chains of amino acids, and 2) introducing side chains by modification at the peptide backbone. Recent development in residue specific peptide modifications includes functionalization of aliphatic side chains, aromatic side chains and polar side chains. Lysine and cysteine act as the most popular target in residue-specific peptide modification as convenient nucleophilic handles and was showcased in diverse peptide modification strategies.[10, 19] However, achieving chemoselectivity to enable site-specific functionalization remains a significant challenge in the modification of peptide sidechains.[11]

On the other hand, backbone modification presents a promising strategy, as it allows for the selective introduction of diverse functionalities and leads to profound structural changes.[20] A few intriguing examples for selective backbone modifications such as non-ribosomal peptide synthetase inspired C-H oxidative modification facilitated by iron,[21] one-pot radical scission-oxidation of hydroxyproline to *N*-alkyl 4-oxohomoalanine,[22] histidine-directed amide *N*-functionalization mediated by copper[23] were reported in recent years utilizing the reactivity of a specific amino acid in the peptide. Moreover, the concept of memory of chirality has successfully applied to asymmetric α-alkylation of amino acids and extensively studied for the enantioselective synthesis of quaternary amino acids.[24-28] An effective and promising strategy towards the peptide backbone modification exploits the reactivity of a glycine unit in a peptide resulting a chemo selective transformation, thanks to the unique nature of glycine compared to other proteogenic amino acids.[29] (refer chapter 1.1.2)

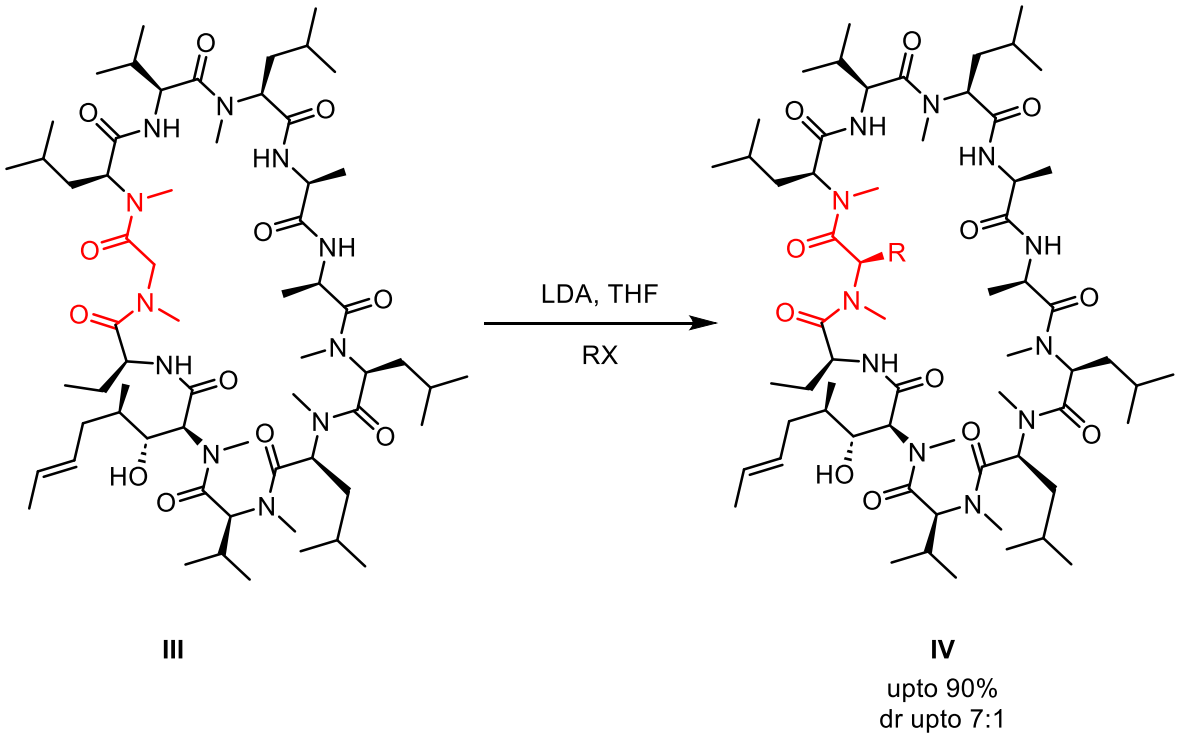
1.1.2 Modification of Glycine Derivatives

1.1.2.1 Chemical Modification of Sarcosine Units

N-methyl glycine, known as sarcosine, was well studied for modification by Seebach using enolate chemistry and extensively applied this technique to short peptides **I**, resulting modified peptides **II** through alkylation (Scheme 1).[30] Lithium enolates of oligopeptides were studied with different alkylating reagents and different diastereoselectivities were observed. A neighboring *N*-alkyl amino acid, in the C-terminal direction, to the sarcosine unit was necessary for successful alkylation. Furthermore, this methodology proved effective in the site-selective alkylation of the immunosuppressive cyclic undecapeptide, cyclosporine A **III**, at a sarcosine subunit (Scheme 2).[31] Interestingly, no epimerization of starting peptides were observed under strong basic reaction conditions.



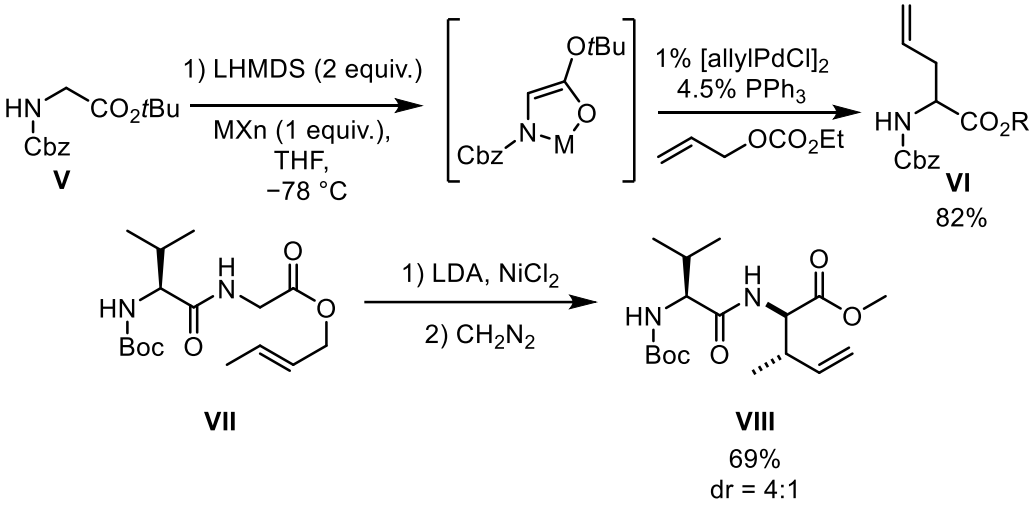
Scheme 1: Alkylation of sarcosine unit in a peptide



Scheme 2: Modification of Cyclosporin A

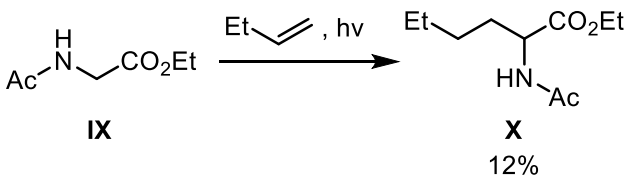
1.1.2.2 Chemical Modification of Glycine Units

Glycine, being the simplest amino acid, is amenable to modification through the introduction of an R group at the α-carbon position. Kazmaier and colleagues studied the alkylation reaction of enolates of glycine esters (Scheme 3). The protected glycine ester **V** on treatment with LHMDS generates the enolate and subsequent addition of metal salt MX_n resulted transmetallation. These chelated enolates act as nucleophiles for palladium catalyzed allylic alkylation reaction, resulting alkylated glycine derivative **VI** in good yield.[32] Continuous investigations of chelated glycine enolate led them to report novel methodology to stereoselectively modify glycine derivative **VII** via the enolate-Claisen rearrangement and modified peptide **VIII** was obtained (Scheme 3).[33-34] The stereoselectivity of the reaction depends on a number of factors such as the *N*-protecting group on the peptide chain, the chelating metal salt and the stereochemistry of the existing amino acid. The synthetic potential of glycine enolates was further explored, resulting in a series of modified peptides.[35-36] Importantly, a methodology of stereoselective Pd-catalyzed allylic alkylation was utilized to synthesize the natural product trapoxin A.[37]



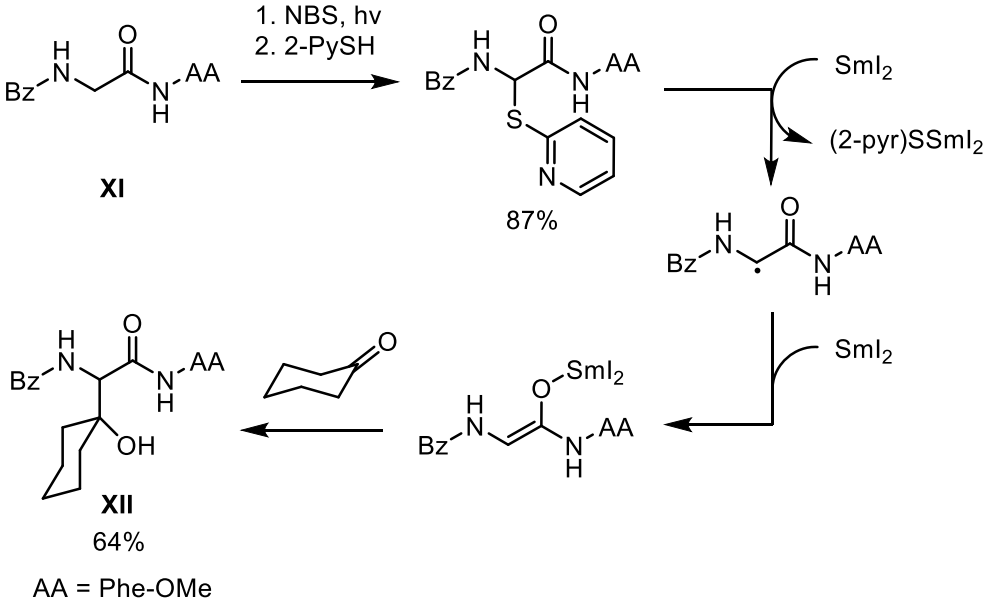
Scheme 3: Glycine enolate reactions

Compared to glycine enolates, radical-based methodology was less explored, but gained more importance recently.[29, 38-39] The UV light-mediated alkylation of *N*-acetylglycine ethyl ester **IX** with terminal alkenes to obtain norleucine derivatives **X** constituted one of the first methods for radical amino acid modification, but selectivities were moderate (Scheme 4).[40-41]



Scheme 4: Photoalkylation of glycine derivative

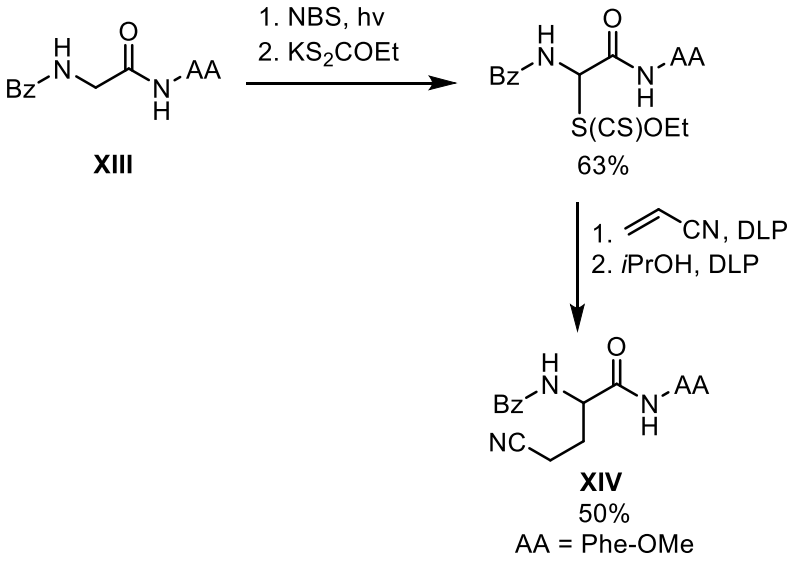
Easton and colleagues studied the relative stability of amino acid radicals and found that secondary glycyl radicals are more stable than tertiary radicals, formed from valine or alanine.[42] This is attributed to their particular geometry as well as the captodative effect.[43] Later, this selectivity for glycine derivatives was applied in photobromination reactions with *N*-bromosuccinimide (NBS) to obtain bromoglycine derivatives.[44] An important application of this method was reported by Skrydstrup *et al.* who employed glycine photobromination in peptides **XI** as a handle for nucleophilic substitution reactions and subsequent reductive transformations with samarium diiodide providing a glycine enolate, which served as a nucleophile toward carbonyl compounds providing β-hydroxy-α-amino acid-containing peptides **XII** (Scheme 5). Here, the pyridyl sulfide glycyl unit served as an appropriate precursor for the glycine enolate and the strategy enabled the synthesize of a library of non-natural amino acids.[45] However, the strategy demands the N-terminal nitrogen protecting group to be benzoyl. Extensive decomposition was observed with other common protecting groups, such as Boc and Cbz.



Scheme 5: Skrydstrup methodology of reductive functionalization in glycine dipeptide

Hiemstra/Speckamp[46] and Skrydstrup[47] groups used more stable glycinyl xanthates,[48] which serve as precursors for thermal radical addition reactions. Glycine derivatives **XIII** were

first subjected to a radical bromination step with NBS. The unstable glycyl bromide intermediate was directly treated with potassium salt of *O*-ethyl dithiocarbonate to obtain stable xanthates. Subsequent thermal radical addition of xanthate to olefins at refluxing condition for 48 h and desulfurization by reductive cleavage of C-S bond provided diverse substituted amino acids or dipeptides **XIV** (Scheme 6). However, the efficiencies drop significantly for tripeptides.

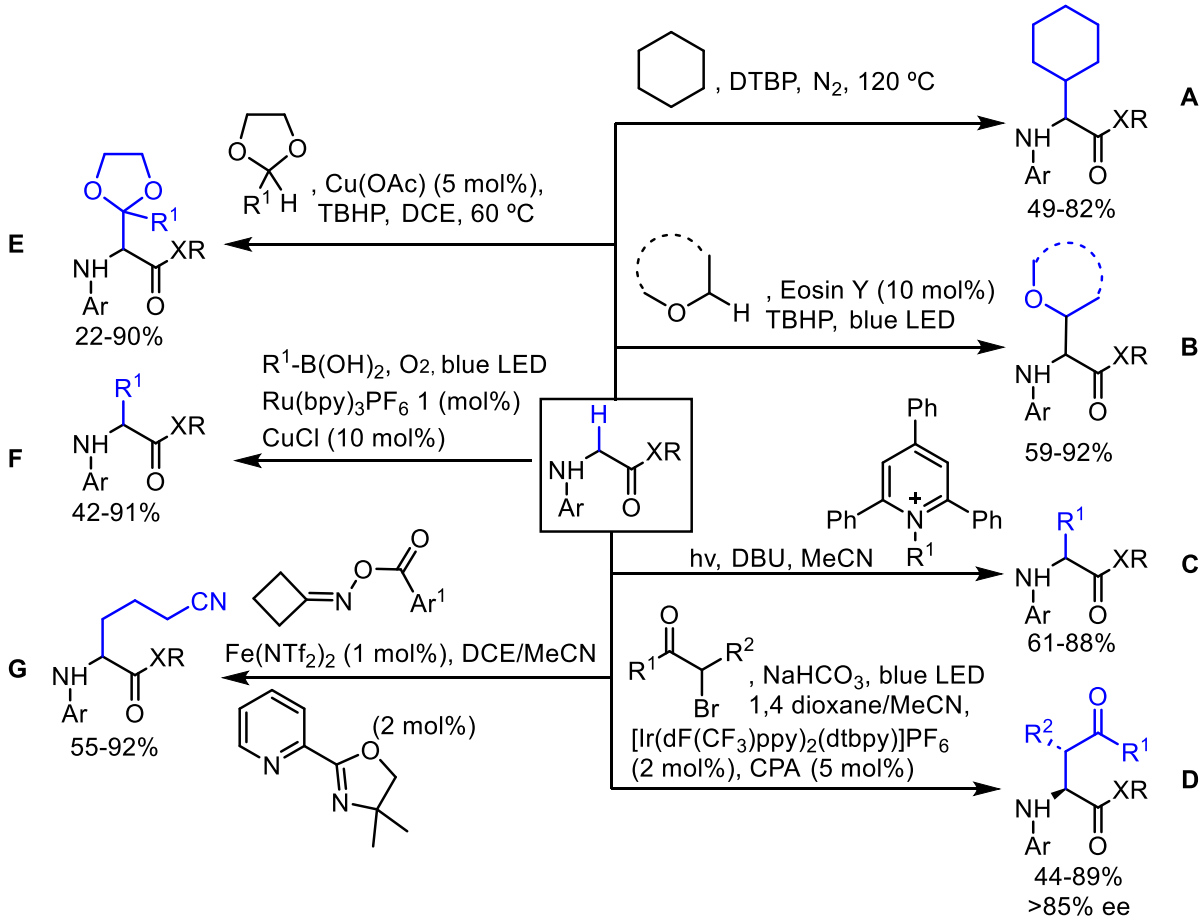


Scheme 6: Glycine functionalization via xanthates

Sim and coworker summarized recent developments in radical mediated C-H alkylation of glycine derivatives to provide unnatural α-amino acids.[49-50] A few interesting methodologies to modify glycine units are summarized in Scheme 7.

A di-*tert*-butyl peroxide (DTBP) promoted α-alkylation of α-amino carbonyl compounds by simple alkanes has been reported. The strategy features the metal-free cleavage of a sp^3 C-H bond by *tert*-butoxyl radical and addition of the alkyl radical to glycine imine intermediate (Scheme 7, A).[51] A visible light-induced oxidative α-alkylation of glycine derivatives with ethers provides unnatural amino acids in good yield (Scheme 7, B).[52] The methodology provides a metal-free approach, utilizing Eosin Y as a photocatalyst, to modify glycine esters and the reaction tolerates cyclic and acyclic ethers. Visible light-promoted alkylation of glycine derivatives using alkyl pyridinium salts (Katritzky salts) has been developed to obtain modified unnatural amino acids. The strategy also allows site-selective alkylation at the *N*-terminal glycine moiety in short peptides (Scheme 7, C).[53] A stereoselective unnatural amino acid synthesis was reported by cross-coupling glycine esters and racemic α-bromo ketones

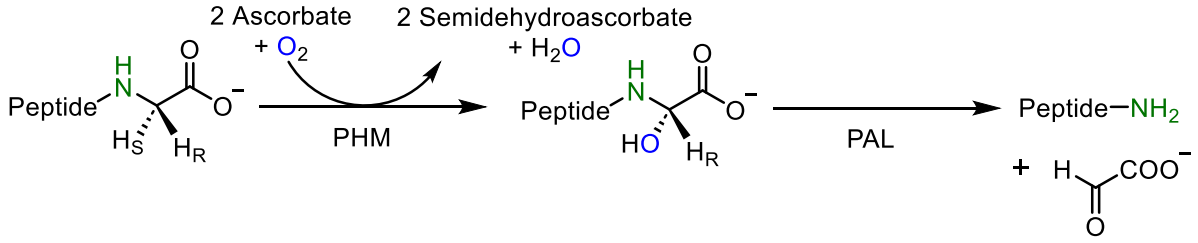
catalyzed by synergistic Bronsted acid/photoredox catalysts (Scheme 7, D). The photoredox cycle of the Ir(III) photocatalyst generates the α-keto radical and the glycine iminium ion, while the chiral phosphoric acid (CPA) acts as a bifunctional catalyst to both assemble the reactive species via H-bonding interactions and facilitate asymmetric radical addition to the iminium complex,[54] providing enantioenriched unnatural amino acids.[55] Site-selective copper catalyzed oxidative alkylation of glycine derivatives via glycine iminium complex was reported using 2-substituted 1,3 dioxolanes as alkylating agents (Scheme 7, E).[56] The strategy also extends to glycine containing peptides and selective alkylation was achieved. An interesting approach of aerobic oxidative alkylation of glycine derivatives with alkyl boronic acids using Ru/Cu metal complex system under visible light irradiation also allows modification of amino acids (Scheme 7, F).[57] The iron-catalyzed cyanoalkylation of glycine derivatives promoted by pyridine-oxazoline ligands allows the incorporation of a cyano group into amino acids and peptides, which can serve as a handle for further functionalization (Scheme 7, G).[58]



Scheme 7: Recently developed methodologies for modification of glycine units.

Most of these methodologies (Scheme 7) either give rise to simple alkylated glycinates which might have limited scopes or use transition metals, which might limit them to use in complex biological systems. Furthermore, there is a continuous interest in the development of novel glycine modification methodologies that hold promising potential for application in biological systems.

In contrast to all these chemical modifications of glycine units by alkylation, nature modifies the glycine unit in a peptide by C-alpha hydroxylation using a copper-dependent enzyme peptidylglycine monooxygenase (PHM). This process acts as a critical post-translational step in peptide hormone processing to achieve carboxamide C-terminal of many mammalian bioactive peptide hormones.[59] The cleavage step is catalyzed by the enzyme peptidyl-α-hydroxyglycine-α-amidating lyase (PAL) to produce amidated peptide and glyoxylate (Scheme 8). PHM catalyzes stereospecific oxygenation of the glycine α-carbon of peptidylglycine substrates, and the structural studies strongly suggests that the oxygenation reaction proceeds via activation of α-C-H bond of glycine unit by a copper bound oxygen species of PHM and subsequent radical coupling.[60] A similar, α-oxygenation reaction of glycine derivatives will be an interesting reaction that can be developed to achieve chemical modification of glycine-containing peptides.



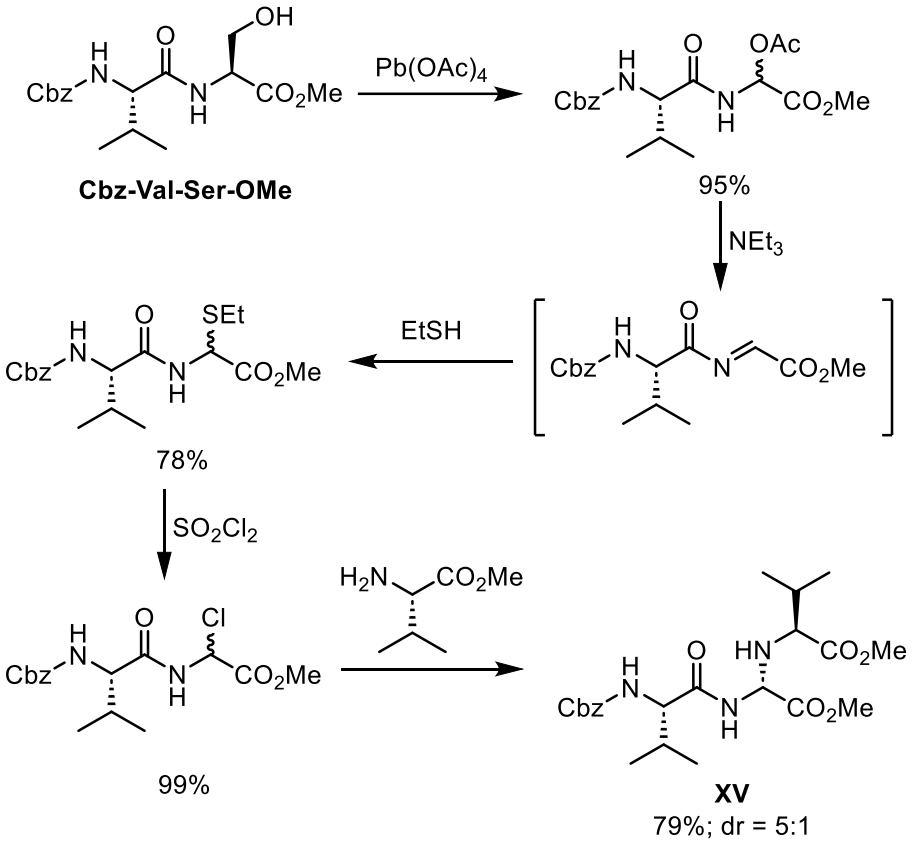
Scheme 8: Glycine modification in nature catalyzed by enzyme.

1.1.3 Peptide Modifications by Conjugation

The emergence of unique methodologies coupled with various conjugation techniques enabled the accessibility of diverse non-natural peptides and modified proteins.

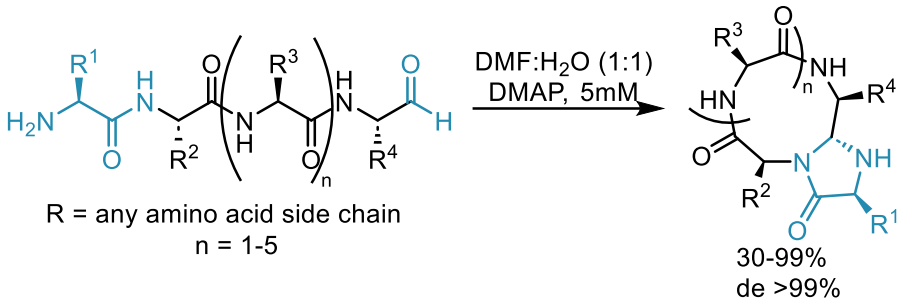
Steglich and coworkers have developed an interesting strategy to generate electrophilic glycine equivalents within oligopeptides and were then reacted with nucleophiles to obtain modified oligopeptides (Scheme 9). Seryl or threonyl peptides were reacted with lead tetraacetate to obtain α-acetoxyglycine derivatives. Dehydroglycine derivatives were *in situ* formed by treating the α-acetoxyglycine with tertiary amines and readily reacted with powerful

nucleophiles such as thiols and organometallics. However, in the case of weaker nucleophiles, it was necessary to convert α-thiolate to more reactive chloroglycyl derivative by treating them with sulfuryl chlorides. Subsequent, nucleophilic reaction on chloroglycyl peptides with amino esters provided interesting aminal derivatives of peptides **XV**.[61] The strategy has been employed to conjugate an antibiotic, norfloxacin, to a peptide sequence and an efficient prodrug has been synthesized.[62] However, the longer and stepwise reaction sequence may limit the synthesis of these peptide-based cross-conjugated products.



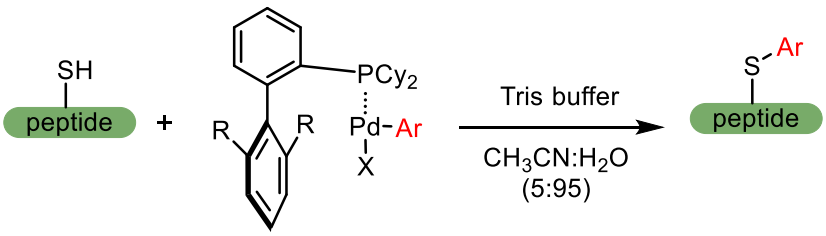
Scheme 9: Reactivity of electrophilic glycine derivatives

Recently, "CyClick" strategy was exploited to construct peptide macrocycles via intramolecular hydrogen bonding and is highly chemoselective for N-terminus of the peptide with C-terminal aldehyde. The methodology of intramolecular coupling of an aldehyde and amine, featuring aminal unit in the peptide, was explored to synthesize 12 to 23 membered ring peptide macrocycles (Scheme 10).[63-64]



Scheme 10: Peptide macrocycle formation via intramolecular hydrogen bonding

Another interesting approach for peptide modification by conjugation was reported utilizing selective arylation of thiol containing peptides (Scheme 11). Aryl palladium reagents react with free thiol moieties in the peptide and the thiol conjugates were found to be stable towards acids, bases, and external thiol nucleophiles. The strategy has also been utilized to access a promising class of antibody-drug conjugates by coupling drug loaded palladium complex to the antibody Trastuzumab.[65]



Scheme 11: Bioconjugation of cysteine containing peptides with aryl palladium complex

Insulin has been a primary focus of peptide chemists since its isolation and subsequent breakthrough in the treatment of diabetes mellitus (Figure 1). The chemical modification of insulin was investigated with the objective of developing insulin analogues that would act either rapidly or over a longer period of time, in order to mimic insulin secretion in healthy individuals. Insulin analogues modified with the addition of Arg-Arg unit at the C terminus of β-chain known as insulin glargine (Lantus),[66] addition of fatty acids,[67] acylated analogue insulin detemir[68] are a few of early analogues used in clinical trials. The chemical modification of insulin is primarily achieved through site selective acylation, reductive cleavage of disulfide bonds, reductive alkylation, use of protecting groups, conjugation of carbohydrates, lipids and ligands.[69]

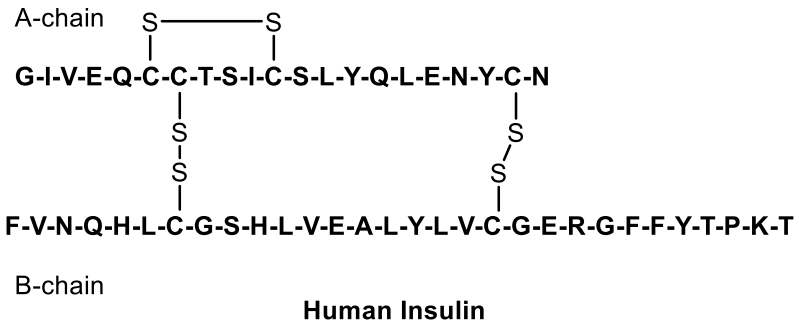
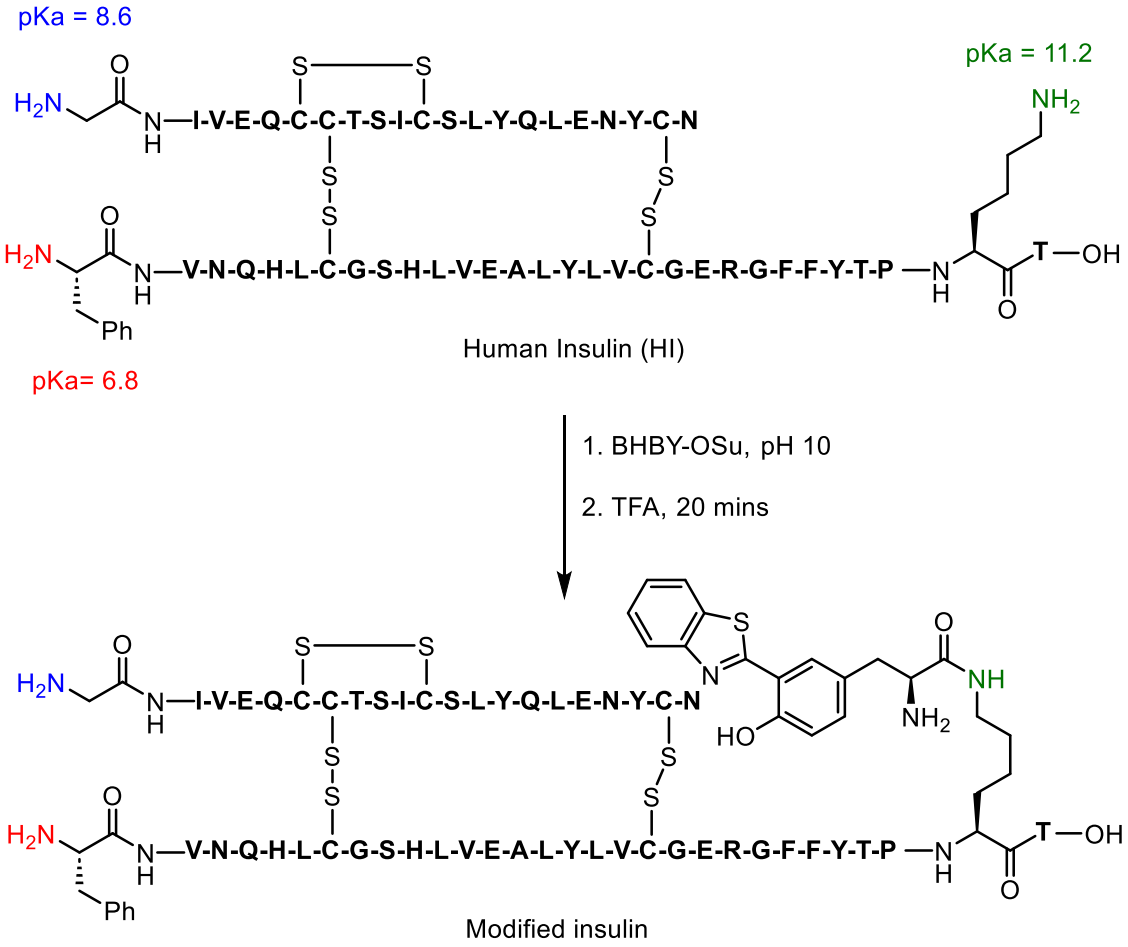


Figure 1: Human Insulin structure

A recent development of a chemically modified human insulin derivative by a conjugation of 3-(2-benzothiazolyl)tyrosine to the ε-amino group of the Lys-29 residue of the human insulin β chain enables visual monitoring of insulin aggregation (Scheme 12).[70]



Scheme 12: 3-(2-Benzothiazolyl)tyrosine conjugation to human insulin

Furthermore, the biological activity of different peptides and proteins were enhanced through modification by conjugation of lipids, carbohydrates, multimerization, arylation, fluorescent probes, and other novel bio-orthogonal chemistry.[20, 71-74]

Development of novel methodologies for chemical modification of peptides has created numerous opportunities for peptide and protein research, drug development, and various applications within the realms of biological and chemical sciences. This avenue in peptide drug development continues to exhibit applications, prompting substantial encouragement for numerous studies within the field. However, there is still a demand for the development of novel peptide structures with fruitful applications, which requires the use of new and interesting methodologies for synthesizing unnatural peptides and peptide conjugations.[11, 75]

1.2 Nitroxides

1.2.1 Nitroxide Structure

Nitroxyl radicals, commonly referred to as nitroxides, constitute a class of organic compounds featuring a nitrogen-oxygen (N-O) bond, where a delocalized unpaired electron is shared between the nitrogen and oxygen atoms. The resonance structures A and B (Figure 2) illustrate the phenomenon of electron delocalization associated with these radicals.

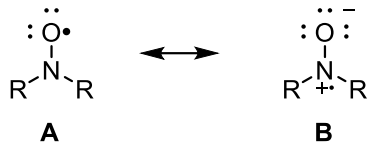


Figure 2: Resonance structures of nitroxide

The initial report on a nitroxide compound dates back to 1845, describing an inorganic nitroxide known as Fremy's salt ($(KO_3S)_2NO^{\bullet}$).[76-77] The field of nitroxides witnessed significant advancements following the discovery of 4-oxo-2,2,6,6-tetramethyl pipepridine-*N*-oxyl radical (4-oxo-TEMPO) **XVI** (Figure 3) by Lebedev and Kazarnovsky in 1959.[78] Nitroxide chemistry has greatly evolved and many nitroxides have been successfully developed and studied (Figure 3). Piperidine nitroxides are a major class of nitroxides, consisting of 2,2,6,6-tetramethyl piperidine (TEMPO), which is one of the most common and commercially available nitroxides used in organic chemistry. AZADO and Nor-AZADO are also used as efficient catalysts for alcohol oxidation to corresponding carbonyl compounds.[79] Development of new nitroxides, particularly acyclic nitroxides such as TIPNO[80] and SG1[81] made it possible to control the nitroxide mediated polymerization of acrylate derivatives, acrylamides and dienes.[82-83] Pyrrolidine and pyrroline nitroxides are among the most commonly used nitroxides *in vivo* because of their resistance to reduction and used as spin labels.[84-85] These milestone nitroxide and others have paved the way for the exploration of various applications and opportunities in the field of chemistry.[86]

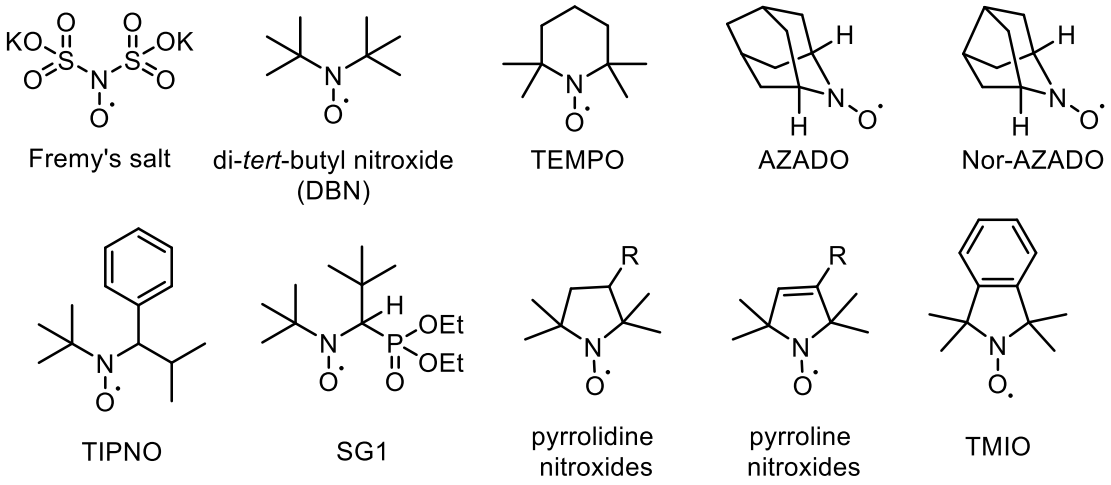


Figure 3: A few important nitroxides

TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) is among the most widely used and readily available nitroxides employed in organic synthesis. Over the years, researchers have synthesized and investigated functionalized derivatives of TEMPO.[87] A series of analogous nitroxides **XIX-XXIV** featuring diverse functionalities can be accessed from 4-oxo-TEMPO **XVI** (Figure 4). This transformation takes advantage of the reactivity of the carbonyl group while leaving the core nitroxide functionality unaltered. The functional group present at the 4-position offers the unique ability to incorporate the nitroxide into various systems without direct involvement of the nitroxide group itself.[88-90] This strategic approach enables the use of the nitroxide for spin labelling, a technique widely employed in studying free radicals and reactive intermediates in various biological and chemical processes.[91-92]

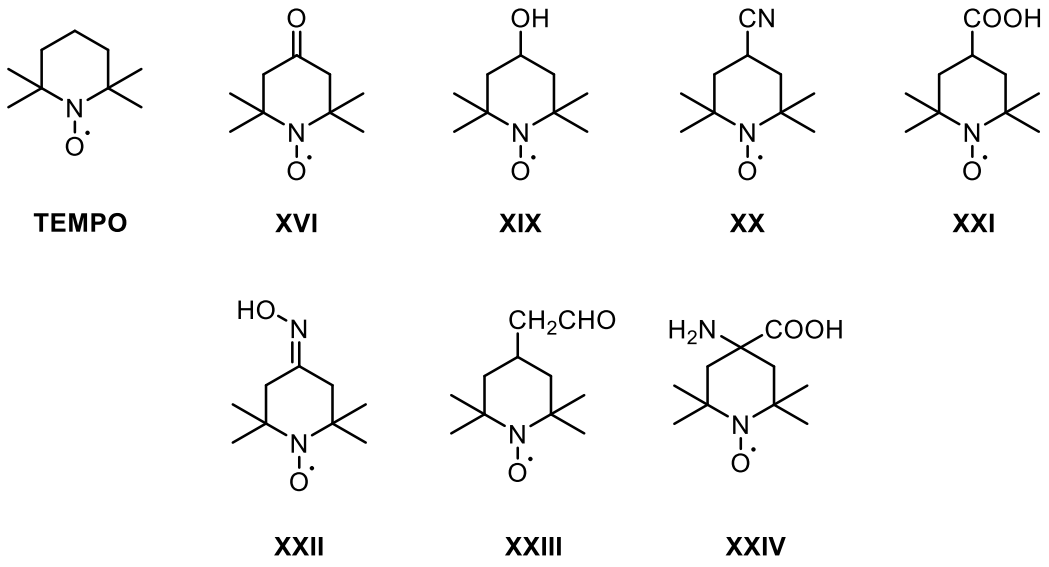
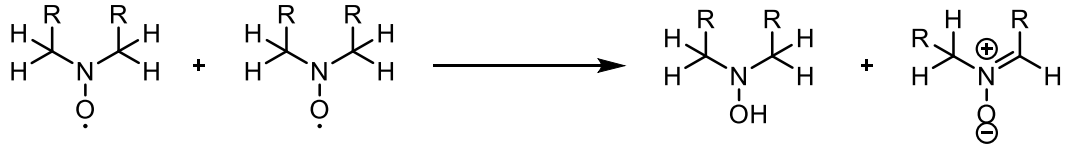


Figure 4: TEMPO analogues

1.2.2 Physical and Chemical Properties

Nitroxides are classified as paramagnetic compounds due to the presence of the unpaired electron, which makes them amenable to analysis by Electron Spin Resonance (ESR or EPR) spectroscopy. EPR spectra provide valuable information, including the nitrogen coupling constant α^N, which offers insights into the distribution of spin density at the nitrogen atom.[91]

Nitroxides can be functionalized with various alkyl groups or aryl groups[93] on the nitrogen atom. In general, nitroxides bearing primary and secondary alkyl groups are typically less stable compared to those with tertiary alkyl groups. AZADO, Nor-AZADO, TIPNO, SG1 are a few sable nitroxide radicals bearing hydrogen atom(s) on the α-carbon. The instability of the primary and secondary alkyl-substituted nitroxides is primarily attributed to the higher likelihood of undergoing disproportionation reactions (Scheme 13), which limits their utility in certain applications, whereas nitroxides with tertiary alkyl groups are generally more robust and preferred for various purposes.[94]



Scheme 13: Disproportionation reaction of two nitroxides.

Nitroxides are also susceptible to oxidation to an oxammonium ion or reduction to a hydroxylamine. The stability of nitroxides towards these processes depends on 1) size of the nitroxide bearing ring, 2) substitution on the α-carbon of the nitroxide. Six membered piperidinyl nitroxides such as TEMPO are more prone to reduction than the five membered nitroxides pyrrolinyl radicals and saturated pyrrolidinyl radicals (Figure 5).[92] The effect of substituents on the 2,6- positions of the piperidine ring on the stability of the nitroxides has been studied and reported that electron-withdrawing substituents make the nitroxide more reduction-prone, while electron-donating groups have the opposite effect. This indicates that the electron-withdrawing substituents on the 2- and 6- positions of the piperidine destabilizes the nitroxides whereas the electron donating group stabilizes.[95] In addition to electronic effects, steric hindrance also affects the stability of nitroxides towards reduction. It has been observed that nitroxides with four bulky substituents adjacent to the N-O group exhibit a high level of resistance to reduction. For example, replacing the methyl groups in TEMPO with ethyl groups increases the stability of the nitroxide towards reduction by ascorbic acid due to more effective steric hindrance (Figure 5).[92, 95]

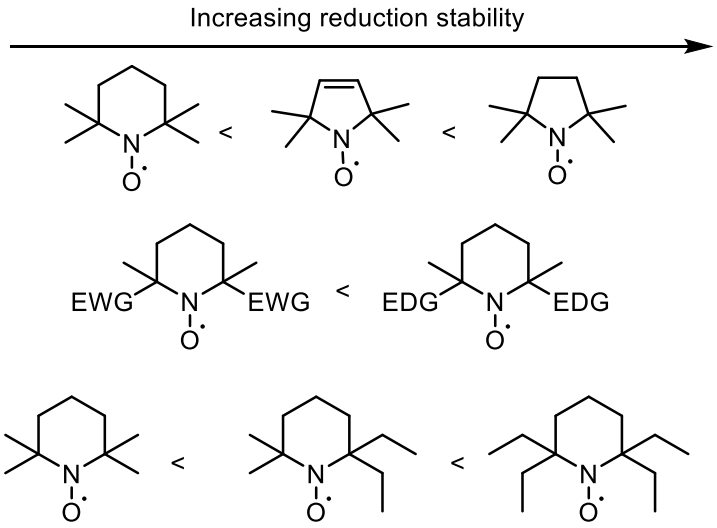


Figure 5: Comparison of reduction stability of TEMPO and its derivatives

Nonetheless, new stability studies towards reduction of highly strained pyrrolidine nitroxides with ethyl and *tert*-butyl groups at α-carbons of the nitroxide compound demonstrated unexpected fast reduction reaction with ascorbate, compared to 3-monosubstituted 2,2,5,5-tetraethylpyrrolidin-1-oxyls (Figure 6). The observation was explained by destabilization of the planar nitroxide moiety due to repulsion between these two *tert*-butyl groups *cis* to each other. Thus, the study demonstrates the limit of steric hindrance loading for higher resistance to reduction.[96]

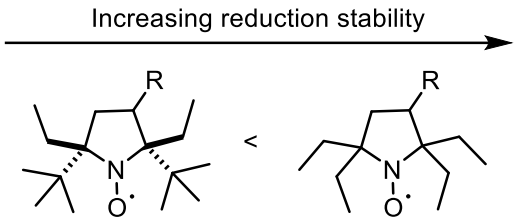


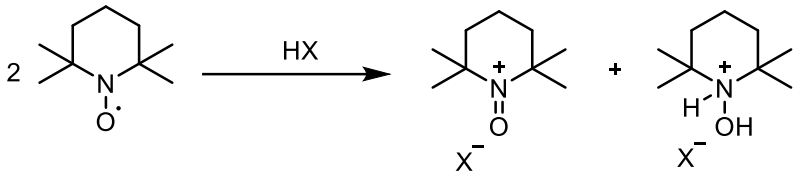
Figure 6: Comparison of reduction stability strained pyrrolidine nitroxides

Variable stabilities and reactivities of nitroxides are desired for specific applications, so it is important to design the nitroxide for the given objectives.

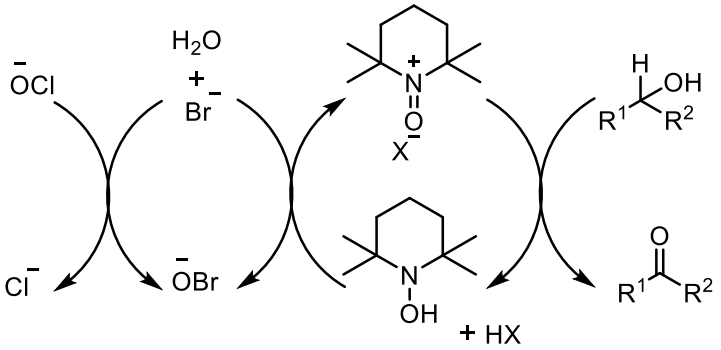
1.2.3 Applications in Organic Chemistry

1.2.3.1 Oxidation

Nitroxides have been used for oxidation of primary and secondary alcohols to the corresponding aldehydes, ketones and acids. However, the active oxidant is not the nitroxide, but the oxoammonium salt. The commonly used nitroxide TEMPO has an oxidation potential of 0.64 V (vs. standard calomel electrode (SCE)) implying it as a very mild oxidant.[97] However, the oxidative form of TEMPO, oxoammonium salt is a strong oxidant and oxidizes alcohols into aldehydes and ketones. Treatment of nitroxide with an acid, HX gives stable oxoammonium salt which can be isolated and used as stoichiometric oxidant in synthesis (Scheme 14).



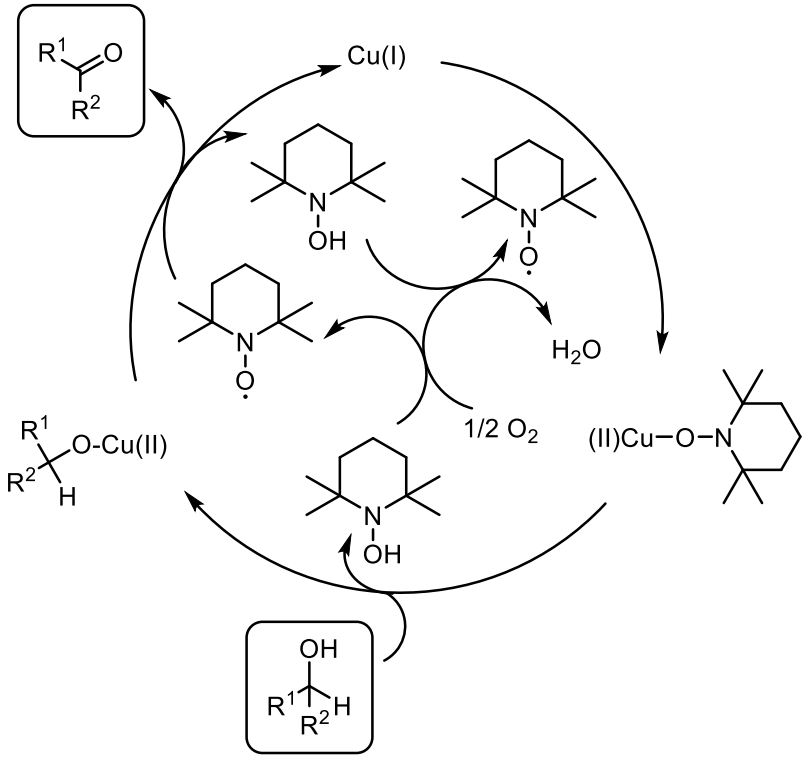
Scheme 14: Disproportionation of TEMPO by treatment with excess of HX



Scheme 15: Nitroxide catalyzed oxidation of alcohol mediated by sodium hypochlorite

Compared to stoichiometric oxidation of alcohols to ketones by an oxoammonium species[98] oxidation reactions by catalytic methods, in which the oxoammonium species is regenerated by a stoichiometric secondary oxidant has gained much interest. The most commonly used co-oxidant in nitroxide catalyzed alcohol oxidation is sodium hypochlorite (Anelli-Montanari protocol) and bromide anions act as co-catalysts. Hypochlorite (OCl^-) oxidizes bromide to hypobromite, which is the active oxidant for regeneration of the oxoammonium salt (Scheme 15).[87]

More interestingly, nitroxide-copper complex-catalyzed aerobic oxidation of alcohols can provide ketones (Scheme 16).[99-100] A Cu(I) complex is oxidized by TEMPO to the corresponding Cu(II)–TEMPO adduct. Ligand exchange with the alcohol gives a Cu(II)-alcohol complex and TEMPOH. The subsequent intermolecular hydrogen transfer reaction from Cu(II)-alcohol complex to TEMPO gives the corresponding aldehyde or ketone and the starting Cu(I) complex. TEMPO is regenerated from TEMPOH by air oxidation, a process which is heavily influenced by the pH value of the reaction medium. These processes have been mainly conducted with copper complexes[99] but other transition metals such as ruthenium,[101] molybdenum[102] and iron[103] have also been employed.



Scheme 16: Nitroxide-catalyzed aerobic oxidation of alcohol mediated by a copper complex.

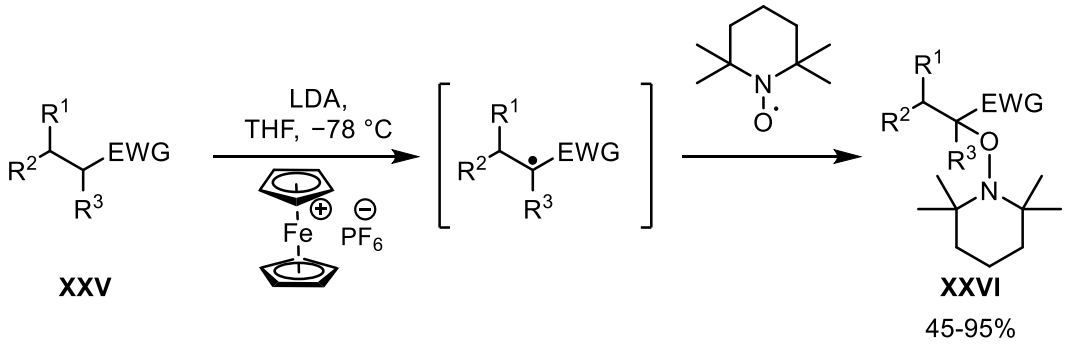
1.2.3.2 Trapping of C-centered Radicals

Nitroxides have been used to trap C-centered, transient radicals to form alkoxyamines. This process is controlled by the persistent radical effect (PRE), which is a kinetic phenomenon explaining the high cross-selectivity of radical-radical coupling. It states that in a process, where two radicals with different lifetimes, a transient radical (short-lived) and a persistent radical (longer-lived), were generated at equal rates, the cross-coupling between these two radicals takes place. The initial build up in the concentration of the persistent radical, caused

by the self-termination of the transient radical, steers the reaction to follow the cross coupling path.[104-105]

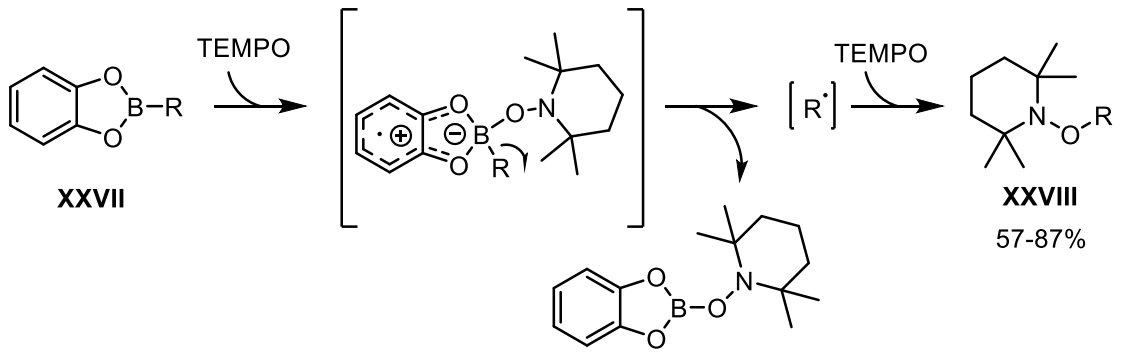
The C-O bond forming reaction is very fast as the reaction is between a transient radical and a persistent radical and the rate depends on the structure of the C-centered radical as well as the nitroxides. Smaller rate constants were observed for a C-centered radical coupling with more sterically hindered nitroxides.[106] The coupling products, alkoxyamines, were exploited in synthetic organic chemistry for the preparation of various class of compounds.[87, 107-109]

In 1998, Jahn reported an efficient methodology of enolate oxidation of esters **XXV** using $FeCp_2PF_6$ as a single electron oxidant. The enolate is oxidized and the transient radical couples to a persistent radical, TEMPO, present in the system (Scheme 17).[110] The methodology was extended to major classes of carbonyl compounds and the chemoselectivity of the reaction was explored.[111] The reaction yields the α-oxygenated product **XXVI** and the methodology is comparable to widely used oxygenation methods using MoOPH, Davis's oxaziridines or peroxydicarbonates. The methodology has been applied to various substrates and its potential has been explored.[112-114] Interestingly, the strategy has recently combined with oxy-cope rearrangement and used for the total synthesis of the natural product class of applanatumols.[115-116]



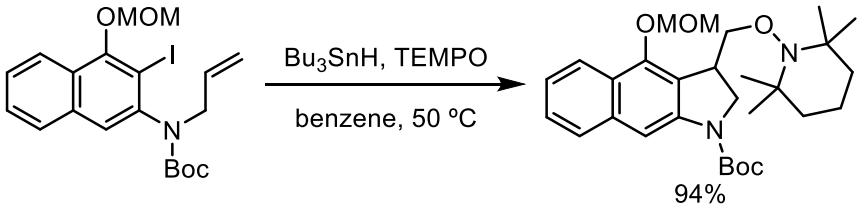
Scheme 17: α-oxygenation of carbonyl compounds using $FeCp_2PF_6$

Alkyl catcholboranes **XXVII**, which can be easily prepared by hydroboration of alkenes, were reported to form alkoxyamines **XXVIII** by reacting with TEMPO. The Lewis acidic catcholborane forms a complex with TEMPO, which on subsequent fragmentation forms boronate and the transient radical couples with a second equivalent of TEMPO to give the corresponding alkoxyamine **XXVIII** (Scheme 18).[117]

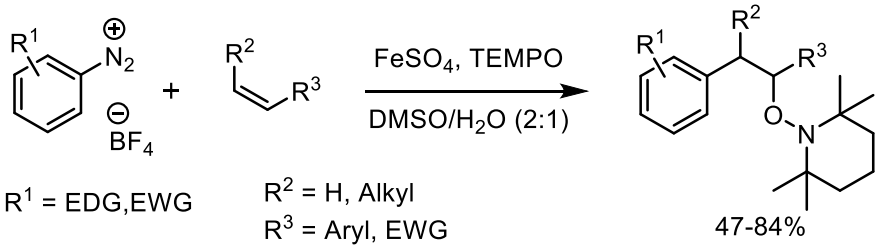


Scheme 18: Formation of alkoxyamine by TEMPO trapping of transient radical

Aryl radical carbon-carbon bond formations with subsequent TEMPO trapping found to be efficient. Boger et al. used the methodology of 5-*exo-trig* aryl radical-alkene cyclization and trapped the C-centered radical with TEMPO to obtain the natural product precursor (Scheme 19).[118-119] Heinrich *et al.* demonstrated intermolecular radical carboaminohydroxylation of olefins with aryl diazonium salts and TEMPO. The formed aryl radical from the diazonium salt couples with olefin to form the C-centered radical which was coupled with TEMPO to obtain the alkoxyamine (Scheme 20).[120]



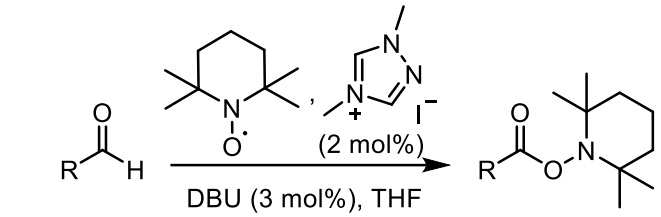
Scheme 19: 5-*exo-trig aryl* radical cyclization.



Scheme 20: Intermolecular radical carboaminohydroxylation of olefins with aryl diazonium salts.

Recent development of novel methodologies utilizing the radical cross coupling with persistent nitroxide radicals allowed easy access to oxygenation reaction of different class of compounds. NHC-catalyzed oxidation of aldehydes **XXIX** with TEMPO as single electron oxidant provides acyl TEMPO derivative **XXX** with can be hydrolyzed to obtain corresponding acid (Scheme 21). The strategy allows oxidation of aldehyde under neutral conditions thus acid-labile

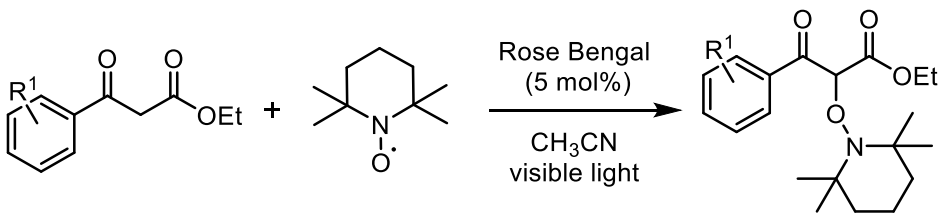
functional groups were tolerated during the reaction conditions.[121] A visible light induced α-oxygenation of 1,3-dicarbonyl compounds **XXXI** using Rose Bengal as the photocatalyst allows a metal free oxidation of electron deficient β-ketoesters (Scheme 22).[122] Mechanistically, photoexcited organic dye, Rose Bengal, acts as a reductant and single electron transfer (SET) occurs to the substrate, β-ketoester **XXXI** and electron rich radical anion is formed. Another SET process recycles Rose Bengal and consecutively forms transient radical of β-ketoester which couples with TEMPO to form the α-oxygenated compound **XXXII**.



XXIX **XXX**

R = aryl, alkenyl 83-98%

Scheme 21: Oxidation of aldehyde catalyzed by NHC.



XXXI **XXXII**

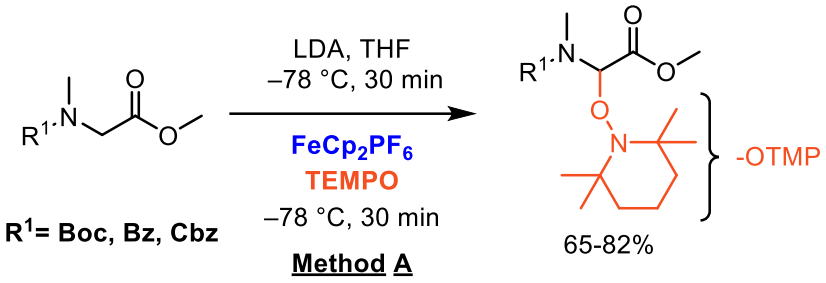
R^1 = H, EDG,EWG 88-95%

Scheme 22: Visible light induced photocatalytic oxidation of 1,3-dicarbonyl compounds.

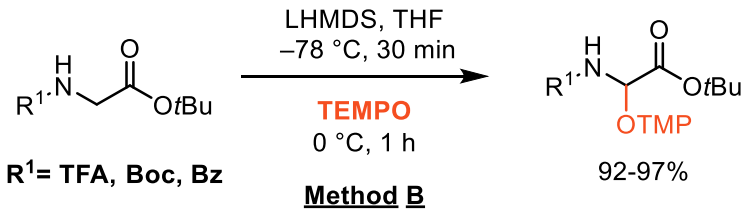
These alkoxyamines formed by coupling the transient α-C centered radical with TEMPO are versatile compounds and further functionalization can be exploited in different applications as radical initiators and precursor for functionalization.[123-124] Given the potential applications of nitroxide radicals in organic synthesis, there exist a substantial opportunity for advancement of nitroxide synthesis to broaden the library of the nitroxide class which will in turn be useful in the development of novel methodology in organic synthesis and their prospective uses.

2. STATE OF THE ART, HYPOTHESIS AND MOTIVATION

The enolate oxidation methodology stands as a potent tool for constructing organic compounds with significant structural intricacy in the realm of synthetic organic chemistry. This methodology has undergone comprehensive investigation and was applied in total synthesis of many natural products as a key step in Jahn's group.[110-116] We hypothesized that this methodology could aid as a tool to modify amino acids (AAs), thereby enabling the synthesis of non-natural amino acids. Interestingly, preliminary experiments conducted by Margaréta Vojtíčková on the sarcosine derivatives utilizing the methodology of enolate oxidation using ferrocenium hexafluorophosphate ($FeCp_2PF_6$) (method A) were successful and the sarcosine alkoxyamines were obtained in good yields.

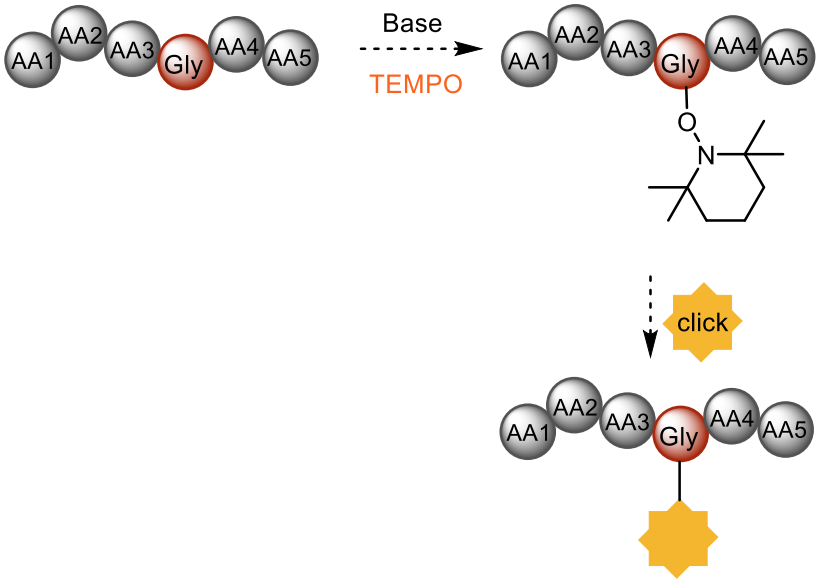


Successful results on oxygenation of sarcosinates raised the question to use glycinates for similar conditions. Johannes Moser studied the applicability of *tert*-butyl glycinates towards the condition of enolate oxidation and found that TEMPO itself can oxidize glycine enolates, making the use an external oxidant such as ferrocenium hexafluorophosphate an unnecessary (scheme 28).[125] This result suggested that the glycine enolates can be oxygenated with 2 equivalents of TEMPO (**2**), without the use of any strong oxidant such as $FeCp_2PF_6$ (method A) to obtain the glycine alkoxyamines.



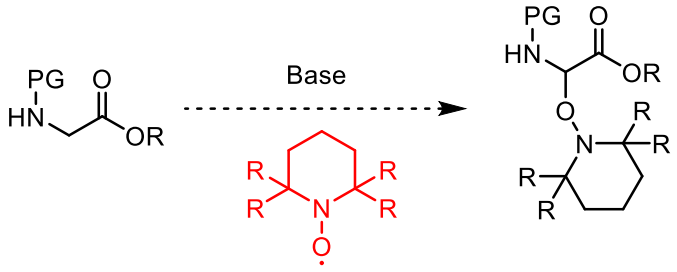
We hypothesized that this methodology holds the potential to be used as a tool to introduce modification on glycine derivatives, thereby enabling the synthesis of non-natural amino acids. Moreover, glycine being distinctive among canonical AAs,[42, 126] the methodology of enolate oxidation holds the potential for inducing a selective reaction at glycine units in the presence of other AA units. This selectivity could be used as a valuable tool for chemical modification in a peptide system and potentially serve as a pivotal step in the modification of a target

peptides in a novel fashion. Alkoxyamines generated by the methodology may serve as versatile intermediates for subsequent functionalization, enabling the synthesis of non-natural peptides and potentially finding applications in biological contexts. These applications include bioconjugation, peptide-protein interactions and bioorthogonal chemistry processes.



Easily accessible nitroxide radicals with different substituents may provide an interesting aspect to the methodology of enolate oxidation of glycine derivatives. A comparison of the stability between different glycine alkoxyamines derived from different nitroxides can be advantageous for utilization of glycine alkoxyamines for peptide modifications.

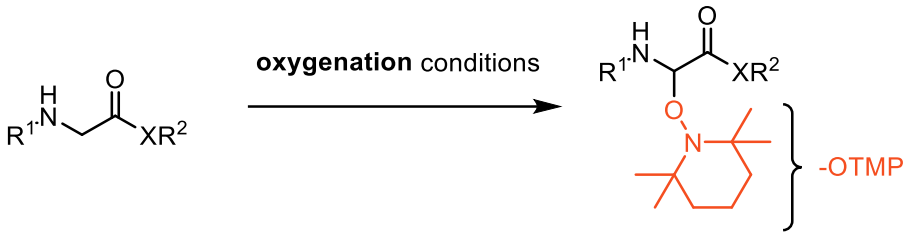
The synthesis of novel nitroxide radicals with bulky substituents at 2- and 6-position can be demanding but serves a dual purpose. Firstly, it expands the spectrum of accessible nitroxide radicals. Secondly, it offers the possibility of imparting a labile C-O bond at the α-position of the resulting alkoxyamines. This, in turn, could influence the selective transformations of these glycine alkoxyamines and expand their potential applications.



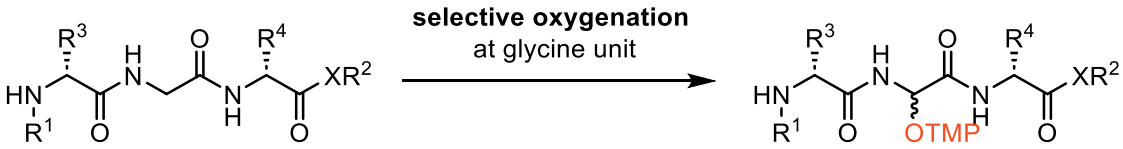
3. AIMS OF THE WORK

The selective and site-specific modification of complex peptide molecules remains a significant challenge in the field of peptide chemistry. Moreover, the viability of a modified peptide as a precursor for multiple functionalization would facilitate an efficient and straightforward approach to chemical modification in peptides. The modification of the glycine unit in a peptide represents a promising strategy for the backbone modification of peptides. This project is aligned with this line of inquiry and aims to achieve the following:

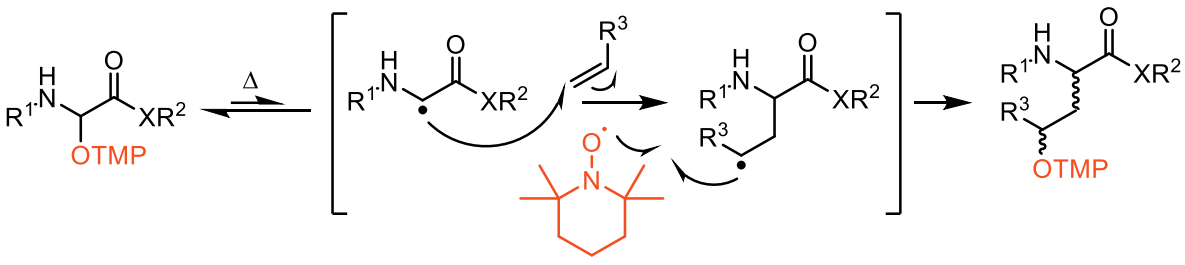
- To study the substrate scope of α-oxygenation methodology in glycine derivatives.



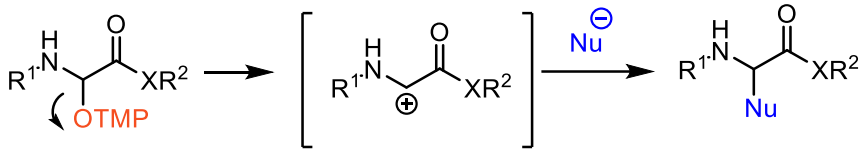
- To extend the methodology in glycine containing short peptides towards the selective α-oxygenation at the glycine unit.



- To study possible transformations of the alkoxyamines generated by the α-oxygenation methodology;
  1) by homolysis of the C-O bond at higher temperature based on Persistent Radical Effect (PRE).



  2) by heterolysis of the C-O bond to make the glycine cationic intermediate and subsequent modification at the α-position of the glycine unit that are unachievable by typical reactivity of glycine enolate. In general, glycine cationic intermediates serve as an umpolung of glycine enolates.



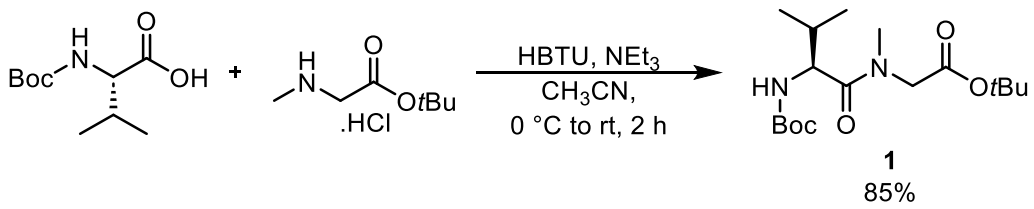
- To provide modification of alkoxyamines at physiological temperature to access non-natural amino acids.
- To demonstrate selective modification of a peptide by the methodology of α-oxygenation and use them for bioconjugation with a protein of interest and establish peptide-protein conjugation.
- To synthesize sterically hindered nitroxide radicals, expand the library of synthetic nitroxides and use them for the methodology of oxygenation.

4. RESULTS AND DISCUSSION

4.1 Oxidation of Sarcosinates

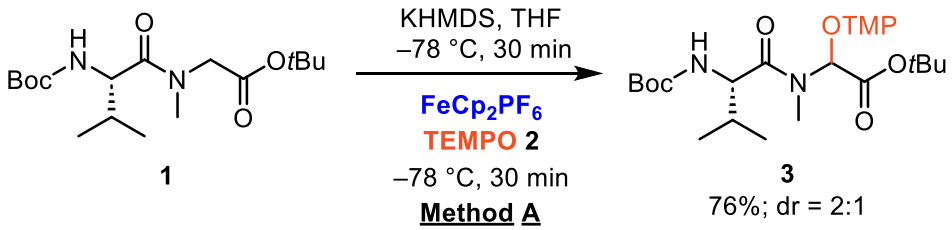
Initial experiments conducted by Vojetickova on the sarcosine derivatives utilizing the methodology of enolate oxidation using ferrocenium hexafluorophosphate ($FeCp_2PF_6$) (method A) were successful and the sarcosine alkoxyamines were obtained in good yields (see state of the art, hypothesis and motivation).

To determine whether selective oxygenation occurs at the sarcosine unit in the presence of other amino acid units, it is important to apply the methodology to peptides. Valine-sarcosine dipeptide, Boc-L-valine-sarcosine-O*t*Bu **1** was obtained by coupling N-Boc-L-valine and sarcosine tert-butyl ester hydrochloride using the standard peptide coupling reaction with hexafluorophosphate-benzotriazoletetramethyluronium (HBTU) in good yield (Scheme 23).



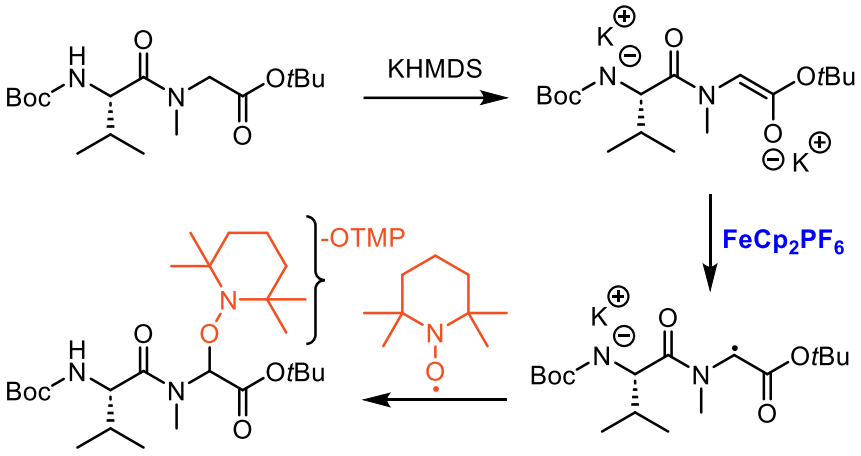
Scheme 23: Synthesis of valine-sarcosine dipeptide **1**.

The methodology of enolate oxidation (method A) was successfully applied to L-valine-sarcosine dipeptide **1** to obtain selective oxidation at the sarcosine unit and the oxygenate dipeptide **3** was isolated in good yield as 2:1 diastereomeric mixture (Scheme 24).



Scheme 24: Oxidation of valine-sarcosine dipeptide **1**.

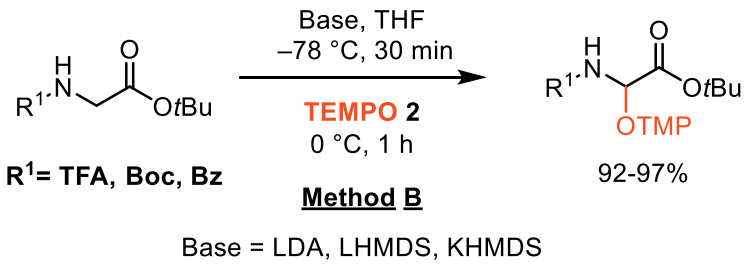
For the deprotonation of the α-proton of the sarcosine moiety in dipeptide **1**, it is important to use two equivalents of base. First, the free NH functionality on the valine moiety, which is unreactive towards oxidation, is deprotonated and then the second equivalent of base generated the enolate, which undergoes oxidation with the oxidant, $FeCp_2PF_6$, and the generated transient α-C-centered radical couples with the persistent radical TEMPO (Scheme 25).



Scheme 25: Mechanism of oxygenation of valine-sarcosine dipeptide

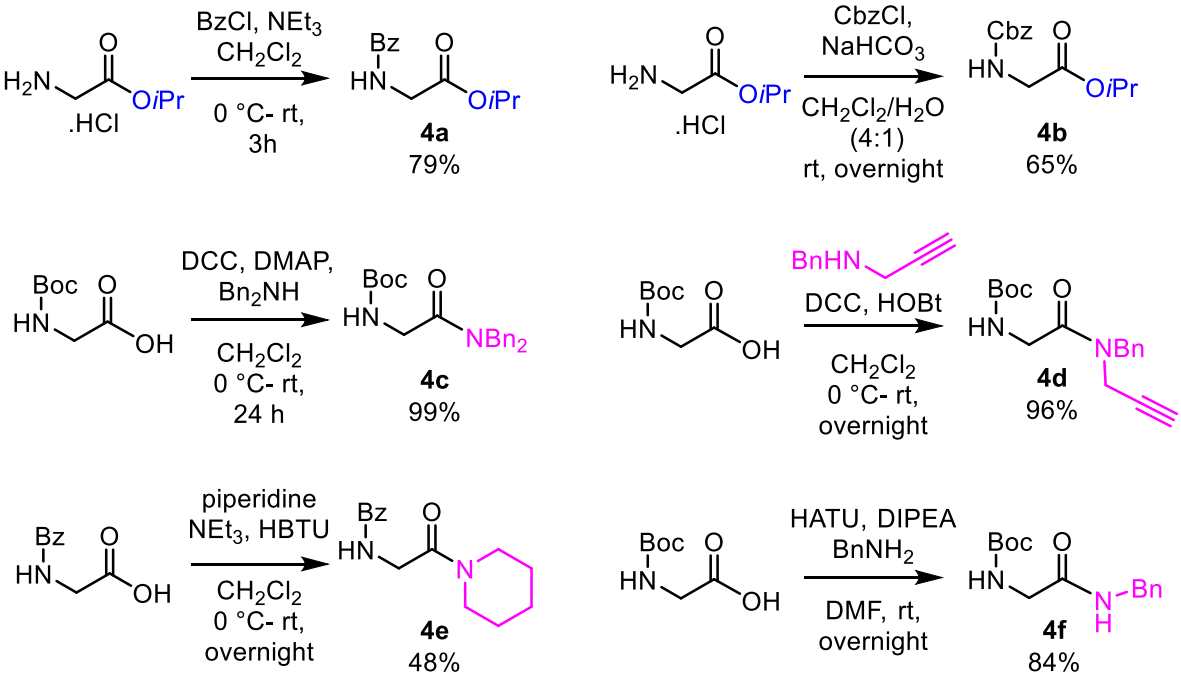
4.2 Oxidation of Glycinates

The results of the study on the oxygenation of glycinates, conducted by Johannes Moser, were intriguing and warrant further exploration (see state of the art, hypothesis and motivation).[125] Protected glycine *tert*-butyl esters were treated with two equivalents of base to generate the enolate and TEMPO **2** itself can oxidize the enolate of *tert*-butyl glycinate and the generated transient radical couples with the second equivalent of TEMPO to obtain the glycine alkoxyamines (Scheme 26). The enolate oxidation of glycine ester is attractive because oxidation does not require an oxidant such as $FeCp_2PF_6$ (method A) as in the case of sarcosine derivatives (*vide supra,* Scheme 24) or any other carboxylate derivatives.[110]



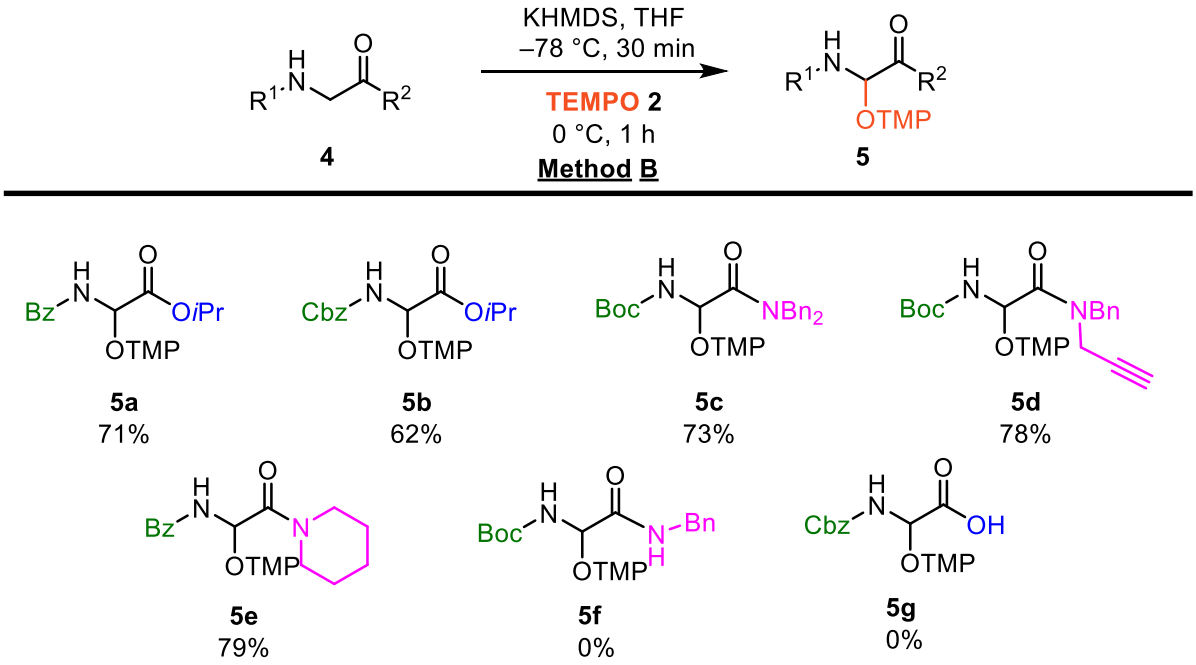
Scheme 26: Oxidation of glycine *tert*-butyl esters.

We further elaborated on this result and explored the substrate scope. Glycine esters and amides **4a-f** were synthesized by standard procedures (Scheme 27). Glycine isopropyl ester was protected with benzoyl and benzyloxycarbonyl to obtain **4a** and **4b** respectively in good yield. The glycinamides **4c-f** were obtained from corresponding protected glycine.



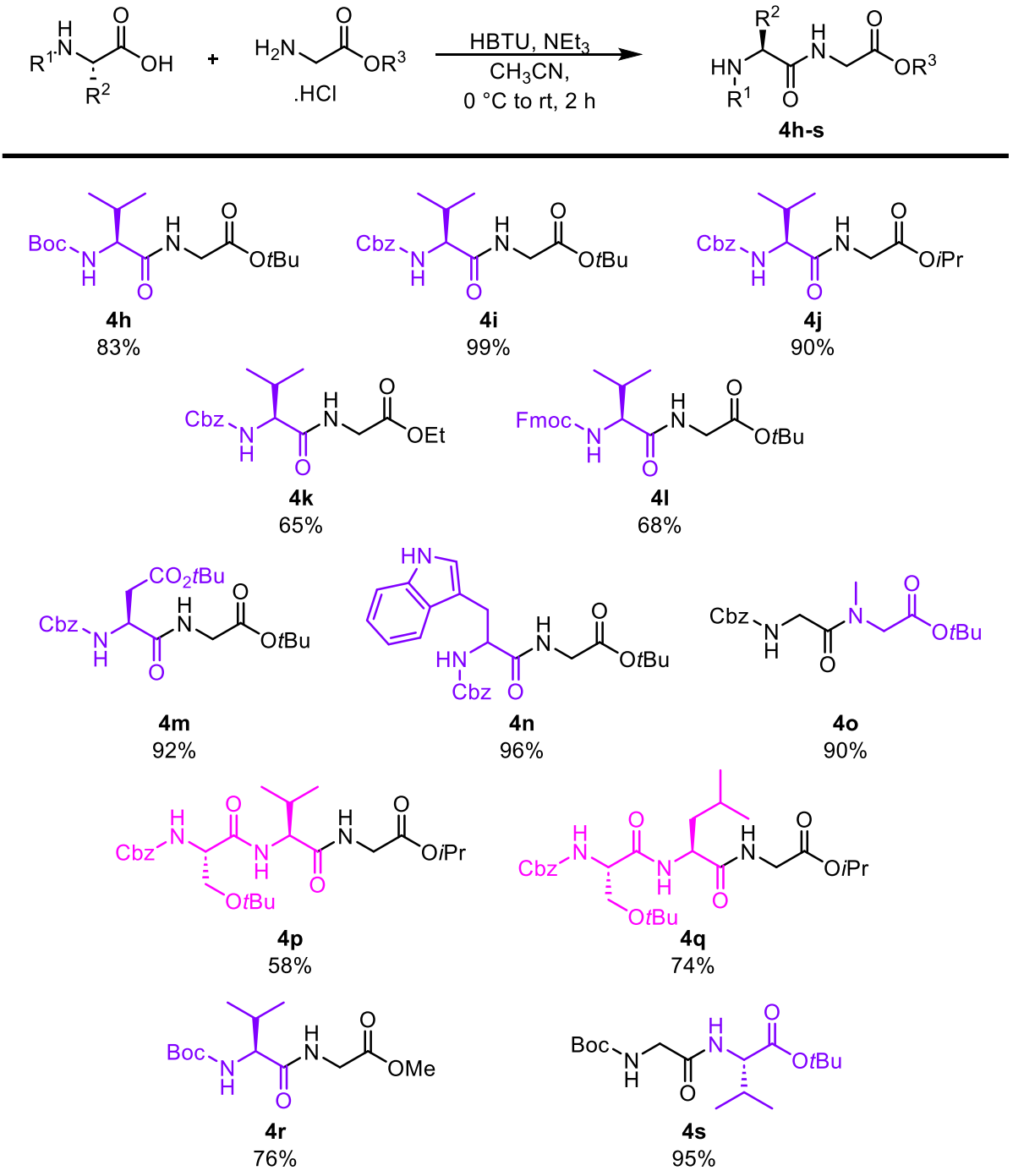
Scheme 27: Synthesis of starting material **4a-f**.

The scope of glycine enolate oxidation by TEMPO (method B) was screened. Glycine isopropyl esters **4a-b** provided oxygenated glycinates **5a-b** in good yields. Glycine amides **4c-e** afforded oxygenated derivatives **5c-e** (Scheme 28), which are suitable starting materials for further functionalization reactions (*vide infra*). However, secondary glycinamide Boc-Gly-NBn **4f** failed to undergo the oxygenation reaction because of unsuccessful enolization of α-proton. Similarly, an attempt to oxygenate Cbz-Gly-OH (**4g**) was unsuccessful. Treatment of Cbz-Gly-OH **4g** with *t*BuLi (3.3 equiv.) provided partial deprotonation of α-proton, however, no oxygenation was observed treating the reaction mixture with TEMPO. Thus, we infer from the result that glycine oxygenation by TEMPO works only for glycine esters or fully substituted glycinamides.

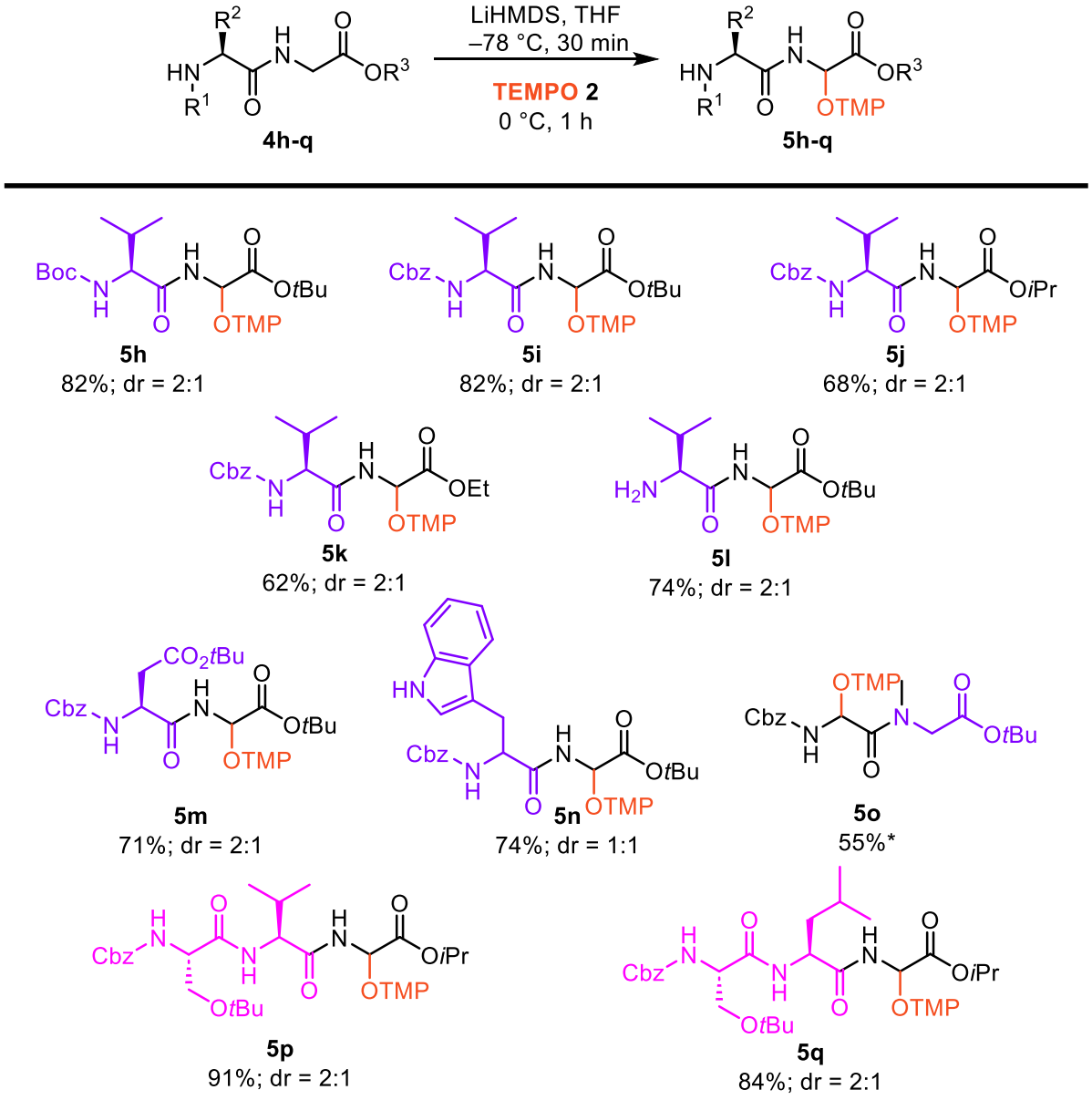


Scheme 28: Substrate scope for glycine enolate oxidation by TEMPO

The methodology of oxygenation of glycine derivatives by TEMPO was extended to glycine containing peptides to test the selectivity of the reaction towards glycine unit. A library of short peptides **4h-s** was synthesized by standard peptide coupling[127] in moderate to excellent yields (Scheme 29). It is important to note that each peptide was obtained as a single diastereomer and no epimerization was observed except in the case of Trp-Gly dipeptide **4n**.[128]



Scheme 29: Synthesis of short peptides **4h-s**.



Scheme 30: Selective oxygenation on short peptides **4h-q**.

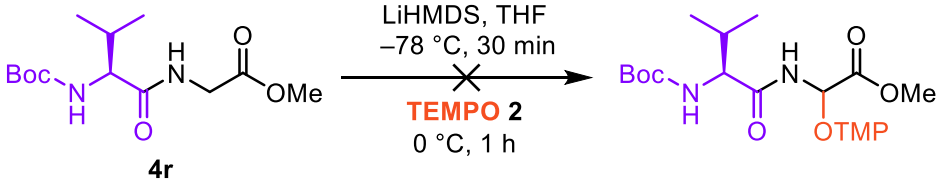
The synthesized peptides (**4h-s**) were subjected to reaction conditions of method B (Scheme 30). The methodology was first attempted on L-Val-Gly dipeptides **4h-l** and required three equivalents of base for deprotonation. The dipeptides **4h-l** contain various N-protecting groups, including Boc, Cbz, and Fmoc, as well as ester groups such as *tert*-butyl ester, isopropyl ester, and ethyl ester groups. Under oxygenation conditions, these dipeptides undergo selective oxygenation of the glycine unit, yielding glycine alkoxyamines **5h-l** in good yields as partially separable 2:1 diastereomeric mixtures. However, it is important to note that the Fmoc-protecting group of the dipeptide **4l** was deprotected under the oxygenation conditions,

resulting in unprotected oxygenated dipeptide **5l**. A detailed study supported the observation that the deprotection of the Fmoc group happened at –78 °C before the oxidation and then on warming to 0 °C the unprotected dipeptide undergoes oxidation by TEMPO. Thus, Fmoc-protected peptides may be used if oxygenated peptides with free N-terminal amino groups are desired for further functionalization.

The reactivity of various dipeptides with functionalized amino acid side chains was further studied. Asp-Gly dipeptide **4m** on oxygenation provided the alkoxyamine **5m** in good yield as 2:1 diastereomeric mixture. Despite the ester group on the aspartate side chain, only glycine enolate undergoes oxidation with TEMPO. A deprotonation study was conducted on dipeptide **4m** by treating it with an additional equivalent of base (4 equiv.). A subsequent deuteration experiment showed no possible enolization at the aspartate side chain. This example demonstrates the specific reactivity for glycine unit under the reaction condition.

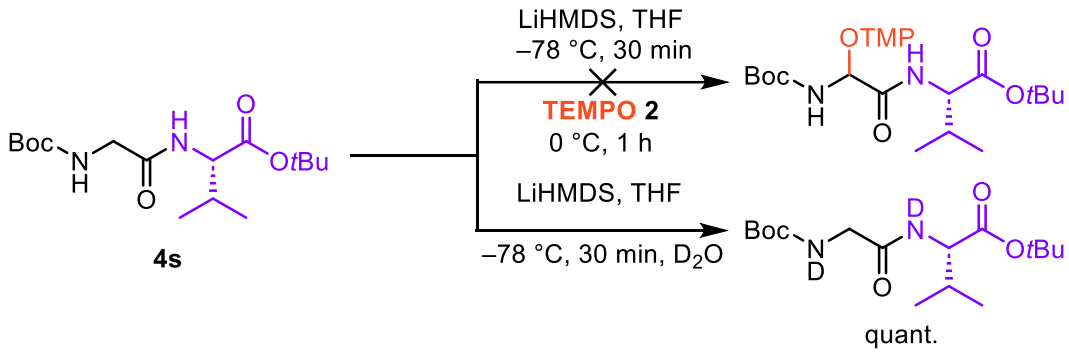
Racemic Trp-Gly dipeptide **4n** with an unprotected indole side chain of the tryptophan undergoes oxygenation selectively at the glycine unit to obtain the alkoxyamine **5n**. Four equivalents of base were utilized for the successful deprotonation of the glycine unit, as the additional equivalent of base is necessary for deprotonation of the indole amine of the dipeptide **4n**. The tryptophan side chain induces no diastereoselectivity at the glycine unit and alkoxyamine was obtained as 1:1 diastereomeric mixtures. To explain the selectivity of the method towards glycine in the presence of a sarcosine unit, we subjected the Gly-Sar dipeptide **4o** to the reaction conditions. This resulted in the selective formation of glycine alkoxyamine **5o**. The methodology also works with tripeptides **4p** and **4q** to obtain the corresponding alkoxyamines **5p** and **5q** respectively. It is important to use LiHMDS as the base for oxygenation of longer peptides as we observed low yields when using KHMDS.

Furthermore, the methyl ester dipeptide **4r** did not undergo oxygenation to provide alkoxyamine and decomposition of the enolate was observed on warming to 0 °C, supported by formation of brown colored reaction mixture. This suggests that methyl esters are not suitable substrates for oxygenation (Scheme 31).



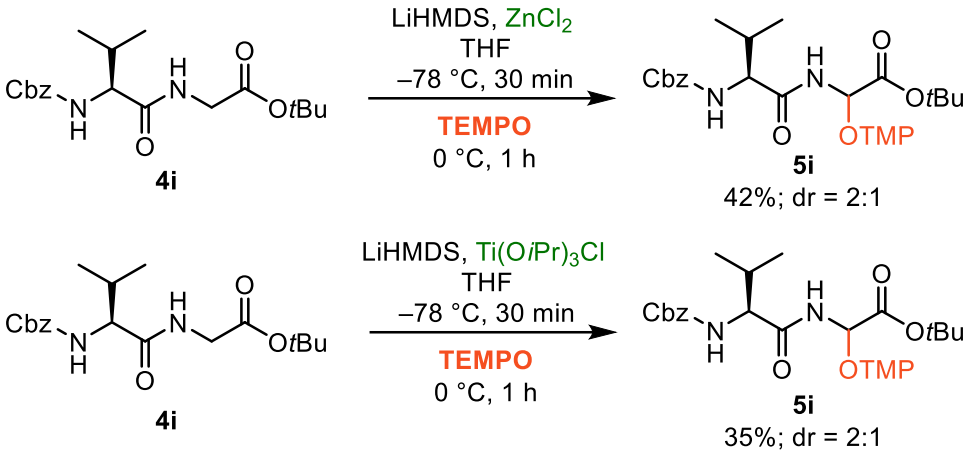
Scheme 31: Unsuccessful oxygenation of the dipeptide Boc-Val- Gly-OMe **4r**.

The dipeptide Boc-Gly-Val-O*t*Bu **4s** failed to undergo the oxygenation reaction because of unsuccessful enolization at the glycine unit. To rationalize the result, a detailed study of deprotonation was performed. The dipeptide was deprotonated with 4 equivalents of LiHMDS, followed by deuteration with D_2O and the crude mixture was analyzed by ^1H NMR spectroscopy. It showed that the amide protons were deuterated but neither the α-protons of the glycine unit and nor the valine unit were deuterated, suggesting that there was no enolization at glycine unit and thus oxygenation was unsuccessful (Scheme 32). This implies that the methodology is limited to the C-terminal glycine peptides so far because it gets difficult to deprotonate the α-position of N-terminal or internal glycine units with the typical bases used for enolization.



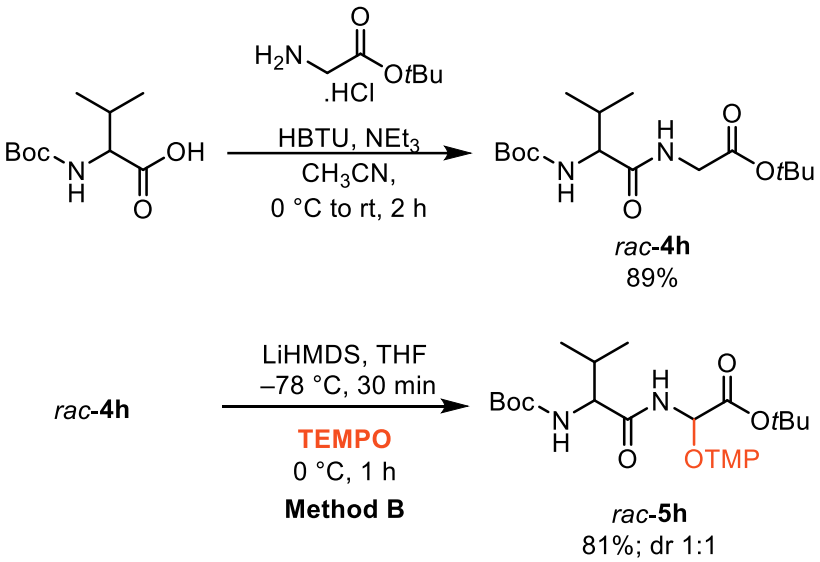
Scheme 32: Unsuccessful oxygenation of the dipeptide Boc-Gly-Val-O*t*Bu **4s**.

α-Oxygenation of the zinc and titanium enolates of Val-Gly dipeptide **4i** was also investigated, as potential chelation of the metal ions may alter the diastereoselectivity. The zinc enolate of dipeptide **4i** reacted sluggishly than its lithium enolate and oxygenated product **5i** was isolated in 42% yield together with 35% recovered starting material. The titanium enolate was oxygenated at an even slower rate accompanied by significant decomposition. The reaction yielded 35% of oxygenated peptide **5i** with 10% starting material recovered (Scheme 33). The 2:1 diastereoselectivity of both reactions was very similar to that of the lithium or potassium enolates (*cf.* Scheme 30).



Scheme 33: Oxygenation of zinc and titanium enolate of glycine dipeptide **4i**.

It is necessary to confirm the non-occurrence of epimerization at the α-position of the other amino acid residues in peptides during the reaction conditions. The dipeptide Val-Gly **4h** was chosen for the illustration. A racemic standard *rac*-**5h** was synthesized by subjecting the racemic valine-glycine peptide *rac*-**4h** to the oxygenation method (method B) (Scheme 34). Investigation of oxygenated dipeptide **5h** by HPLC at a chiral stationary phase with reference to racemic standard *rac*-**5h** confirmed that no epimerization occurred during deprotonation/oxygenation (see experimental section, 6.5 chiral HPLC data).



Scheme 34: Synthesis and oxygenation of racemic dipeptide *rac*-**4h**

The minor diastereomer of **5h** was partially separated by column chromatography, recrystallized and its configuration was unequivocally established by X-ray crystallography. X-ray structure reveals that the minor diastereomer has configuration of [S^{Val},S^{Gly}] (Figure 7) and the Flack parameter was found to be 0.01 (see experimental section, 6.4 X-ray

crystallographic data). This implies that the major diastereomer formed was with configuration [S^{Val},R^{Gly}]. The configurations of **5h-q** as well as that of oxygenated dipeptide **3** (*vide supra,* Scheme 24) were assigned by analogy.

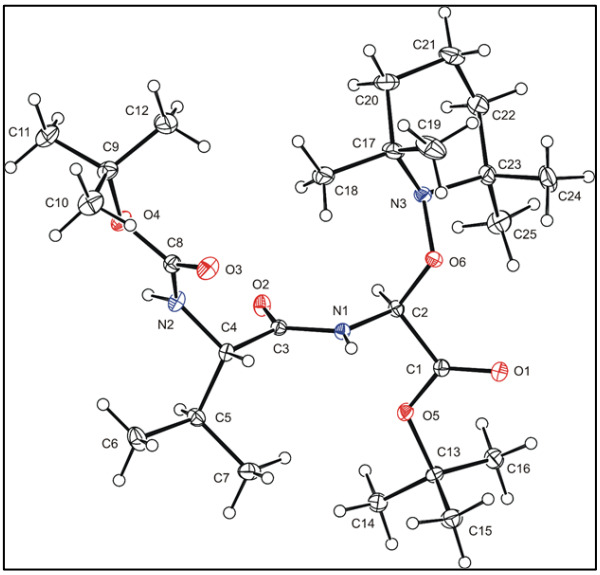
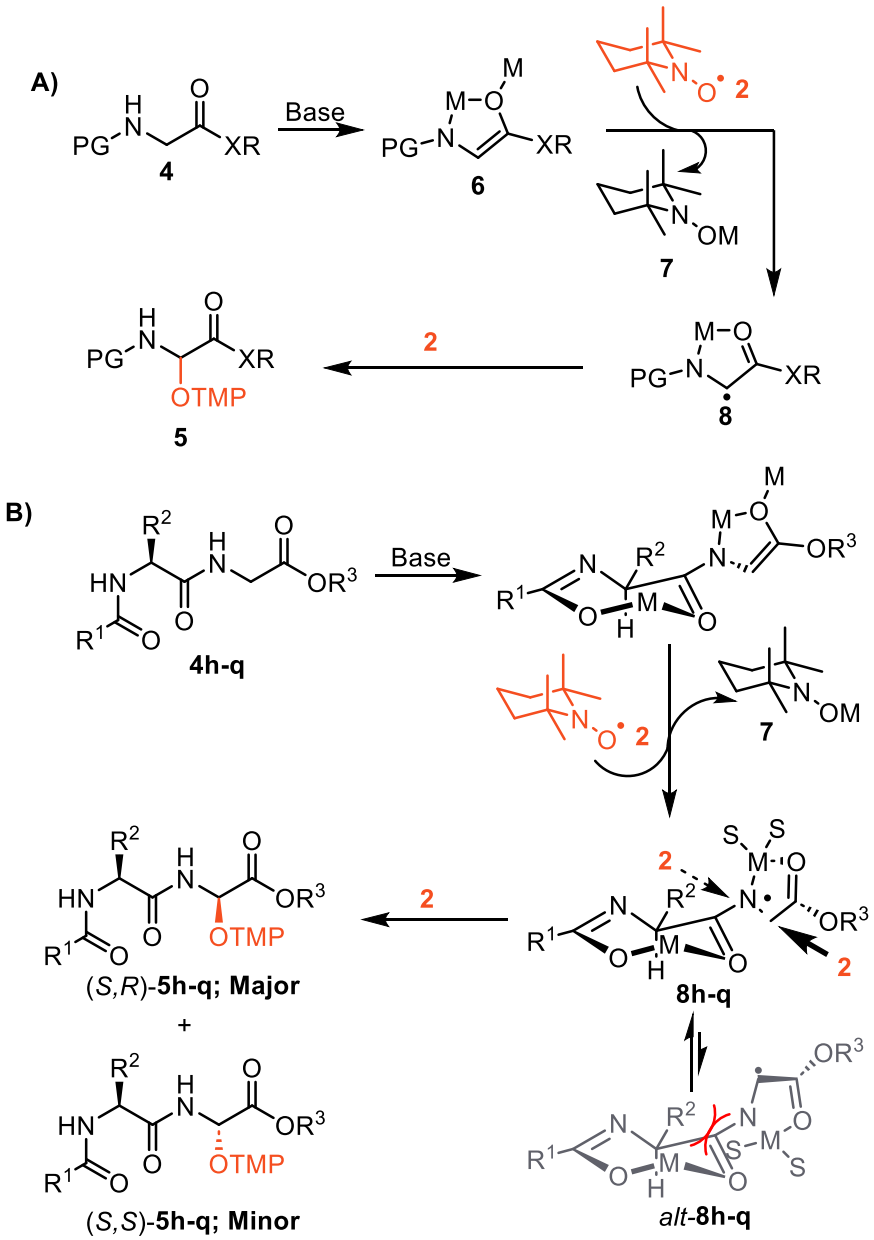


Figure 7: X-ray structure of minor diastereomer **5h** with [S^{Val},S^{Gly}] configuration.

The results for oxygenation of glycine derivatives **4** vs. those of sarcosine **1** differ significantly in that for sarcosine oxygenation an external oxidant, ferrocenium hexafluorophosphate, is mandatory (Scheme 24) as observed before for most major carbonyl classes,[111] whereas for glycinates **4**, TEMPO (**2**) has a dual function as very mild oxidant and oxygenating reagent. The decisive factor for TEMPO serving as oxidant for glycine enolates is the additional negative charge on the nitrogen atom after deprotonation to amide enolate dianions **6**. These chelated extremely electron-rich species have in contrast to other enolates a significantly lower redox potential (+ 0.34 V (irreversible) vs. the saturated calomel electrode)[125] and are thus prone to donate an electron to the weak electron acceptor **2** leading to piperidine N-oxide **7** and chelated radical anions **8**, which smoothly couple with excess **2** providing oxygenated glycinates **5** (Scheme 35A). The mechanism also holds for the peptides **4h-q** (Scheme 35B). The diastereoselectivity of the oxygenated dipeptides and tripeptides can be rationalized by assuming chelated glycinate radical anions **8h-q** that are connected to a chelated seven-membered amide unit at the neighboring amino acid resulting from deprotonation of the N-H function by the third equivalent of base. The chair conformation places the bulky side chain of the neighboring amino acid (R^2 group) in an equatorial orientation thus shielding the α-face of the glycinate radical anion **8h-q**. Therefore, radical coupling with TEMPO (**2**) will proceed predominately from the more accessible β-face providing the major diastereomer (*S, R*)-**5h-q**.

The conformer *alt*-**8h-q** with inverted five-membered chelate is less preferred since it suffers from steric interactions between the solvated metal center and the side chain R^2; moreover, dipole moments are better aligned in intermediates **8h-q** than *alt*-**8h-q**.

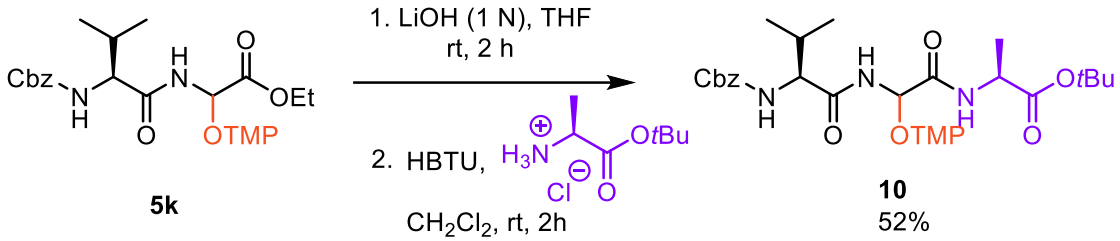


X = O, NH; PG = protecting groups; S= coordinating solvent THF

Scheme 35: A) Mechanism for the formation of alkoxyamine **5**. B) Rationalization of the oxygenation diastereoselectivity in peptides **5h-q**.

The unsuccessful oxygenation reaction of the dipeptide **4s** implies an oxygenation of longer peptides with glycine unit at the internal position is a limitation of our methodology (*cf.* Scheme 32). However, we demonstrate that the oxygenated glycine unit can be coupled with a peptide of interest to access longer peptides containing a glycine alkoxyamine at internal positions. The

oxygenated dipeptide **5k** was easily saponified and hemiaminal at the glycine unit remained unaltered. The residue was directly subjected to the next step, peptide coupling, to obtain the tripeptide containing an internal oxygenated glycine unit **10** (Scheme 36). Therefore, by analogy we propose any longer peptide with the glycine alkoxyamine can be accessible.



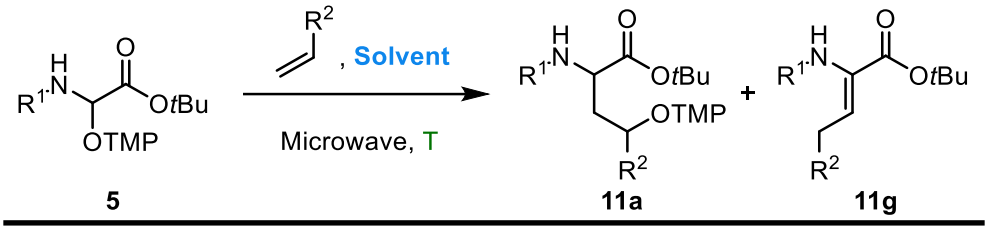
Scheme 36: Constructing linear peptide with glycine alkoxyamine at the internal position.

4.3 Modification of Alkoxyamines by Homolysis

The glycine alkoxyamines are chemically hemiaminals but can be isolated and are stable during purification. We envisage that the α-C-O bond of the glycine alkoxyamine undergoes homolysis at elevated temperatures, similar to other class of alkoxyamines.[87, 129-131] The applicability of homolysis of C-O bond in glycine alkoxyamines **5** and further modifications were explored by exploiting the persistent radical effect.[105]

We performed temperature screening towards homolysis of glycine alkoxyamine **5t** in a microwave reactor**,** starting from 100 °C, and radical addition was observed only at 130 °C. Subsequently, we screened the reactivity of differently protected glycine alkoxyamines **5t-v** [125] with different alkenes at 130 ºC (Table 1). The reaction between benzoyl protected glycine alkoxyamine **5t** with *tert*-butyl acrylate provided the addition product **11a** in good yield (entry 1). We also observed the formation of the β-eliminated product **11g** in small quantity. Boc-protected and TFA-protected glycine alkoxyamines (**5u** and **5v**) were not suitable substrates for the radical addition reaction (entries 2 and 3) as we observed thermal decomposition of Boc and TFA group at the elevated temperature. If the temperature of the radical addition of glycinate **5t** to *tert*-butyl acrylate was raised to 150 °C, dehydroglutamate derivative **11g** was selectively obtained in 53% yield as a single (*Z*)-diastereomer as determined by a ROESY experiment (entry 4). Moreover, radical addition to electron rich alkene, 1-octene, was unsuccessful and the starting material was recovered (entries 5 and 6). The intermolecular radical addition in a non protic solvent, trifluorotoluene was found to be fruitful compared to that in protic solvent *t*BuOH (entry 7).

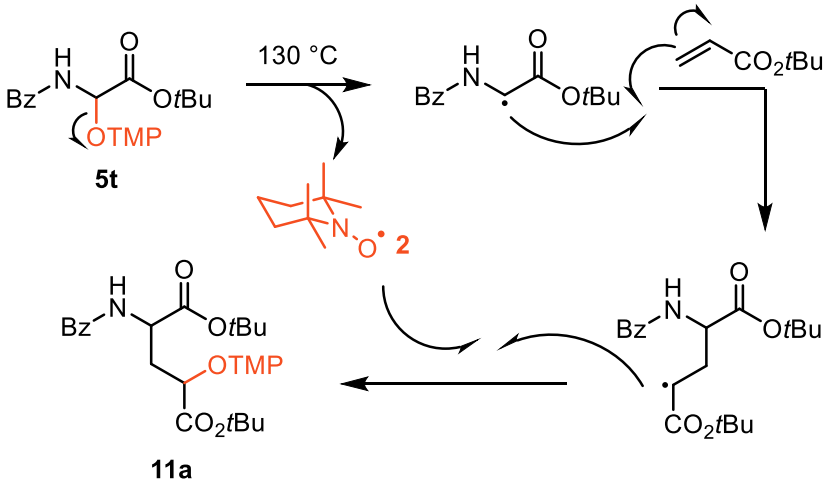
Table 1: Optimization of thermal intermolecular radical addition reaction of glycine alkoxyamines



5 11a 11g

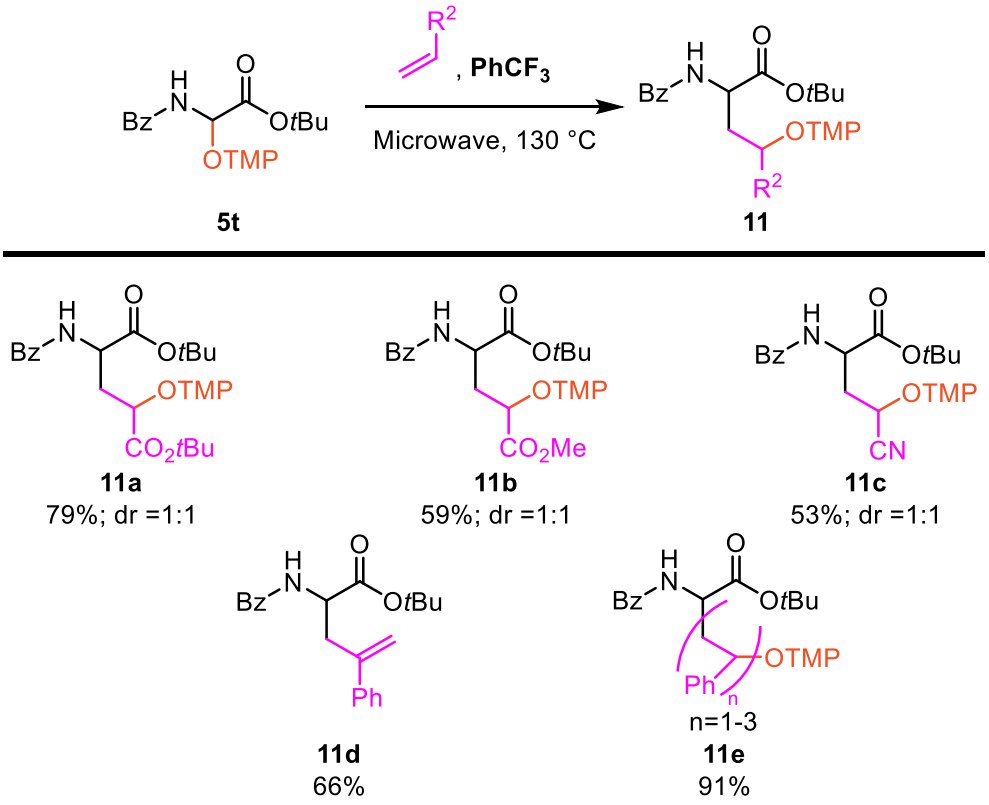
Trial No.	5	R^1	R^2	Solvent	T	11a	11g
1	**5t**	**Bz**	**CO_2tBu**	**$PhCF_3$**	**130 °C**	**79%**	**10%**
2	**5u**	Boc	CO_2tBu	$PhCF_3$	130 °C	-	
3	**5v**	TFA	CO_2tBu	$PhCF_3$	130 °C	-	
4	**5t**	**Bz**	**CO_2tBu**	**$PhCF_3$**	**150 °C**	**-**	**53%**
5	**5t**	Bz	$(CH_2)_5CH_3$	$PhCF_3$	130 °C	-	
6	**5u**	Boc	$(CH_2)_5CH_3$	$PhCF_3$	130 °C	-	
7	**5t**	Bz	CO_2tBu	*t*BuOH	130 °C	13%	

Homolysis of glycine alkoxyamine and further radical addition can be explained by the persistent radical effect (PRE). The optimization study concludes that alkoxyamine **5t** homolyzes at 130 ºC in the microwave generating transient radical and the persistent radical TEMPO. The transient radical undergoes a fast subsequent reaction of radical addition to only electron deficient alkene in trifluorotoluene. The radical addition generates a new transient radical which couples with the persistent radical, TEMPO, present in the system and provides intermolecular addition product **11a** (Scheme 37). A temperature of 130 °C was determined to be a pre-requisite for the reaction, implying that the homolysis of glycine alkoxyamine **5t** does not occur at temperatures below 130 °C.



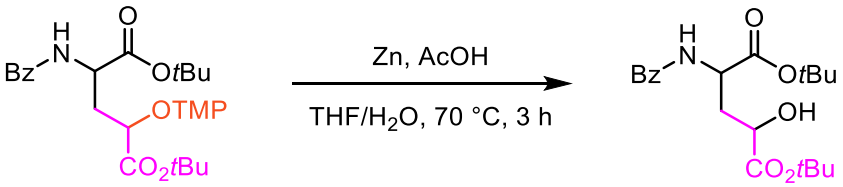
Scheme 37: Mechanism of radical addition of glycine alkoxyamine to olefin.

The further scope of intermolecular radical addition of the glycine alkoxyamine **5t** to alkenes has been explored (Scheme 38). We found that the addition reactions to *tert*-butyl acrylate, methyl acrylate and acrylonitrile proceeded smoothly to obtain the TEMPO adducts **11a**, **11b** and **11c** respectively in good yields as 1:1 diastereomeric mixtures. However, if the resulting alkoxyamine is thermally labile as in the addition to α-methylstyrene, selective elimination took place, furnishing allylglycinate **11d**. Much to our surprise, radical addition of **5t** to styrene resulted in oligomerization providing mixture of mono-, di- and tri-addition products **11e** as 7:10:3 mixture. The oligomerization reaction is characterized by the successive radical addition of the transient radical to excess of styrene molecules by the persistent radical effect (PRE) and polymerization of styrene has been well-studied in literature.[132-135] However, the addition reaction of glycine alkoxyamine **5t** to styrene has not been thoroughly studied to control the addition and obtain a specific product.



Scheme 38: Thermal intermolecular radical addition reactions of glycine alkoxyamine **5t**.

The radical addition products **11a-c** are alkoxyamines and can also be viewed as protected alcohols. The alkoxyamine **11a** was deprotected to liberate the alcohol by reductive cleavage of TEMPO with zinc in acetic acid and 4-hydroxy glutamate **12** was furnished in good yield (Scheme 39).

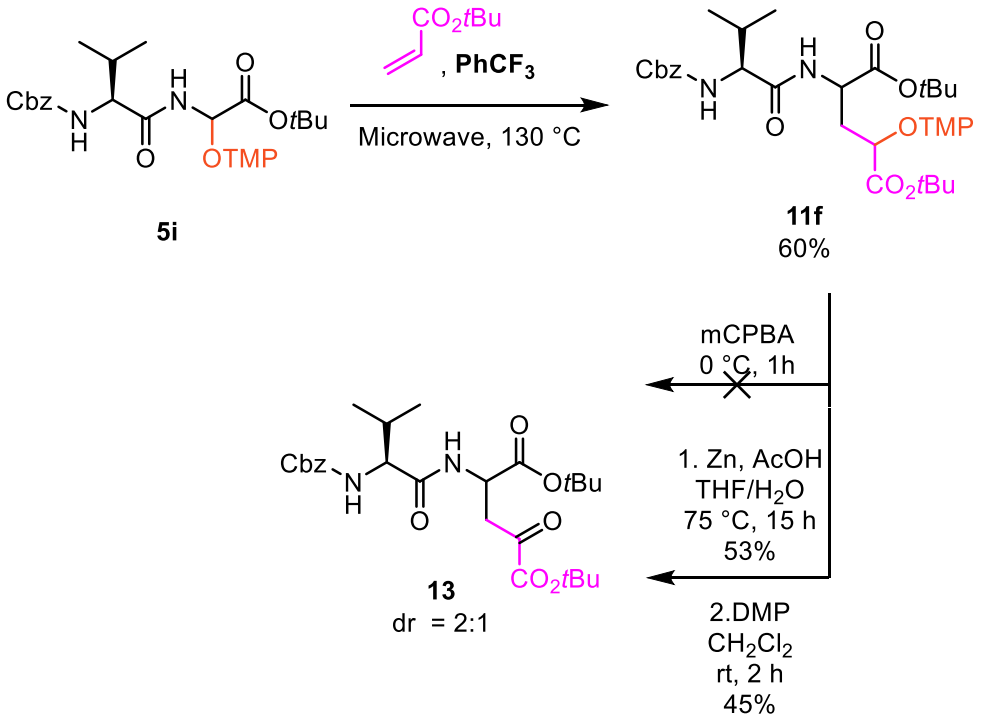


11a
79%; dr =1:1

12
65%; dr =1:1

Scheme 39: Reductive cleavage of alkoxyamine **11a.**

Homolysis is not limited to simple glycine alkoxyamines but can be applied also to dipeptides containing the alkoxyamine unit. Oxygenated dipeptide **5i** undergoes radical addition to *tert*-butyl acrylate and the addition product **11f** has been formed in good yield as an inseparable mixture of four diastereomers approximately of the ratio 2:2:1:1 (Scheme 40). To determine the diastereoselectivity at the α-glycine center towards the radical addition, oxidation of the alkoxyamine **11f** to the corresponding ketone by *m*CPBA [115] was attempted but found to be unsuccessful and the starting material was recovered. A two-step sequence of reduction and subsequent oxidation provided the corresponding ketone **13** in unoptimized 24% yield over two steps and the diastereoselectivity at the glycine α-carbon of **11f** was determined as 2:1 (Scheme 40). This indicates that radical addition takes place with 2:1 diastereoselectivity.



5i

11f
60%

13
dr = 2:1

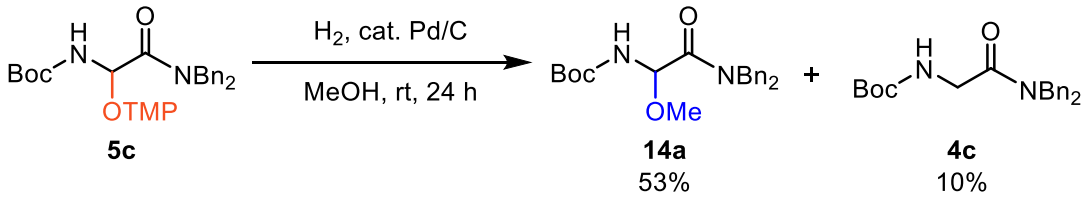
Scheme 40: Thermal intermolecular radical addition reactions of dipeptide alkoxyamine **5i**.

The strategy to modify glycine alkoxyamine **5** by homolysis and subsequent radical addition to olefins was successful to create a library of unnatural amino acids and peptide **11a-f, 12, 13** at a temperature of 130 °C in microwave. However, modification of glycine alkoxyamine units in biologically active peptides and proteins by applying the strategy of thermal homolysis at the elevated temperature would be challenging due to thermal instability of these biomolecules.

4.4 Modification of Glycine Alkoxyamines by Heterolysis

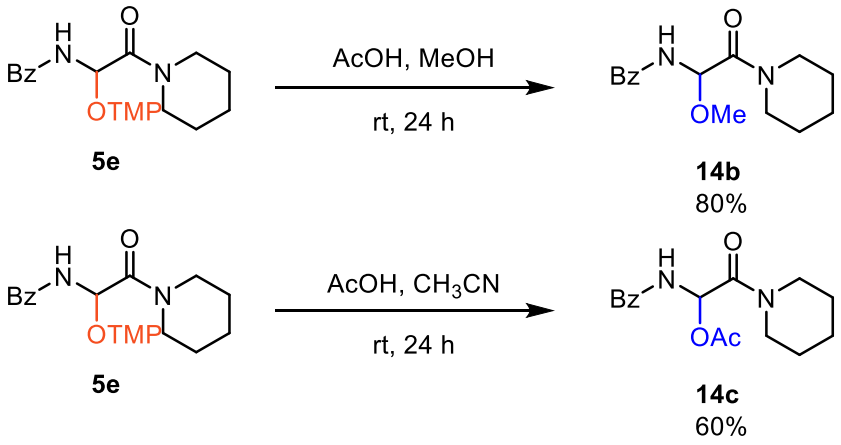
4.4.1 Alkoxide Exchange

While attempting hydrogenolysis to deprotect benzyl groups of the alkoxyamine **5c**, we serendipitously observed an alkoxide exchange to **14a** in moderate yield. The unintentional exchange of the tetramethylpiperidinyloxy group for a methoxy group was observed along with 10-20% of N,N-dibenzylglycine amide **4c**. This suggests that hemiaminal reduction competes to some extent with alkoxide exchange, but both processes are faster than intended benzyl group hydrogenolysis (Scheme 41). The alkoxide exchange reaction works similarly if the reaction was performed under a nitrogen atmosphere in the absence of dihydrogen. The exchange was found to be promoted by acid and the nucleophilic substitution takes place to modify the alkoxyamine. This result adds a new method to modify the alkoxyamines under mild conditions. Thus, we started to explore more of these attractive polar coupling reactions by C−C, C−O, C−N and C−S bond formation reactions.



Scheme 41: Unintended alkoxide exchange during hydrogenolysis

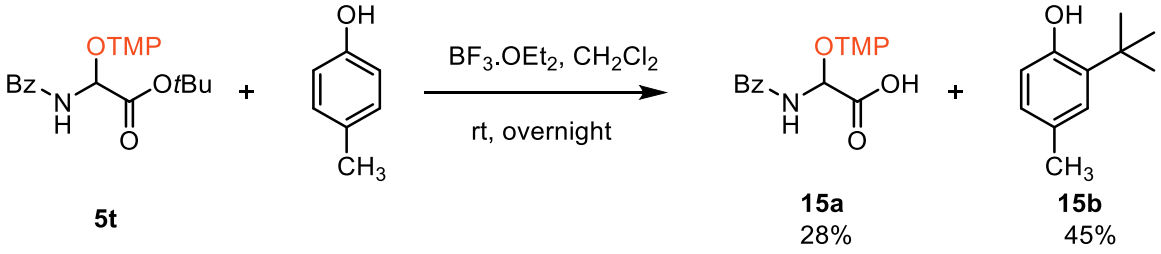
We explored the C-O exchange reaction of glycine alkoxyamines. Glycinamide alkoxyamine **5e** in methanol with stochiometric acetic acid provided α-methoxyglycinamide **14b** in good yield (Scheme 42). Interestingly, the reaction in non-nucleophilic solvent, acetonitrile with excess of acetic acid provided α-acetoxyglycinamide **14c** in moderate yield (Scheme 42).



Scheme 42: Alkoxide exchange of glycine alkoxyamine **5e**

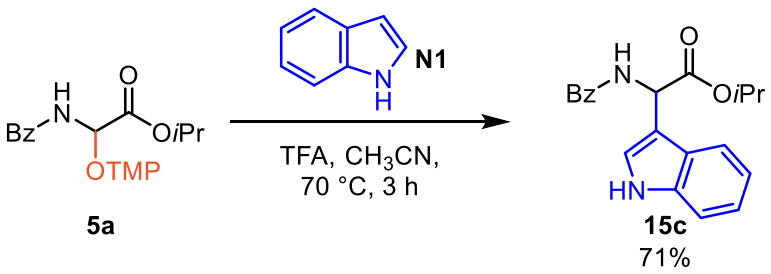
4.4.2. C-C Bond Formation

A Friedel-Crafts-type reaction of *tert*-butyl *N*-benzoyl-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)glycinate (**5t**) with *p*-cresol in the presence of $BF_3{\cdot}OEt_2$ was attempted. Instead, *tert*-butyl ester cleavage and Friedel-Crafts alkylation reaction of *tert*-butyl cation to *p*-cresol was observed (Scheme 43). Thus, *tert*-butyl glycinates are not appropriate for acid/Lewis acid mediated exchange of the tetramethylpiperidinyloxy group.



Scheme 43: Attempted Friedel-Craft type reaction

The alkoxyamine **5a** was reacted with indole (**N1**), mediated by TFA at 70 °C and the nortryptophan derivative **15c** was obtained in good yield (Scheme 44). The reaction at 40 °C was found to be slower and 40% conversion was observed in 12 h.



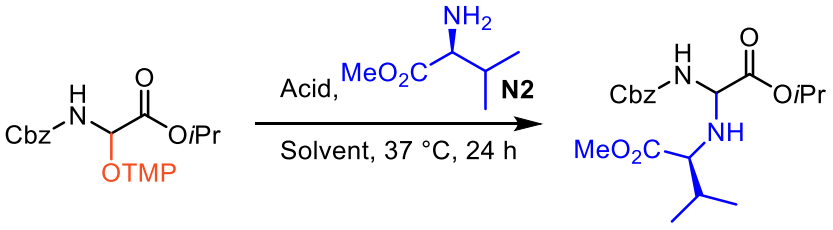
Scheme 44: Nucleophilic substitution of alkoxyamine by indole.

4.4.3. Cross-Conjugation of Amino Acids and Peptides

The nucleophilic addition to α-imonoesters is reported in the literature to access non-natural amino acids.[136-139] However, the use of amino acids as nucleophiles remains less explored.[61, 63] With our strategy of nucleophilic substitution to glycine alkoxyamine at lower temperature, we attempted the substitution reactions with amino acids as nucleophiles, to access the class of α-aminal compounds derived from amino acids.

Glycine alkoxyamine **5b** was used as the substrate for the optimization of the substitution reaction with L-valine methyl ester (**N2**) as the nucleophile at physiological temperature. Table 2 summarizes the results of optimization with different acids in different solvents and all entries provided the aminal derivative **16a** as 1:1 diastereomeric mixture. The reaction in CH_3CN provided aminal **16a** in high yield (entry 1). It is noteworthy that the reaction can also be catalytic in acid to obtain the substituted product in high yield (entry 2). Interestingly, the substitution reaction also takes place in methanol (entry 3), under aqueous conditions (entry 4) as well as in slightly acidic buffer solution (entry 5). However, under these conditions the aminal derivative **16a** was obtained in lower yields, because of the possible side reactions such as hydrolysis. Nonetheless, the reaction in aqueous media or buffer solutions might be beneficial while using the strategy in longer peptides or proteins because of their insolubility in organic solvents.

Table 2: Optimization of substitution reaction with L-valine methyl ester

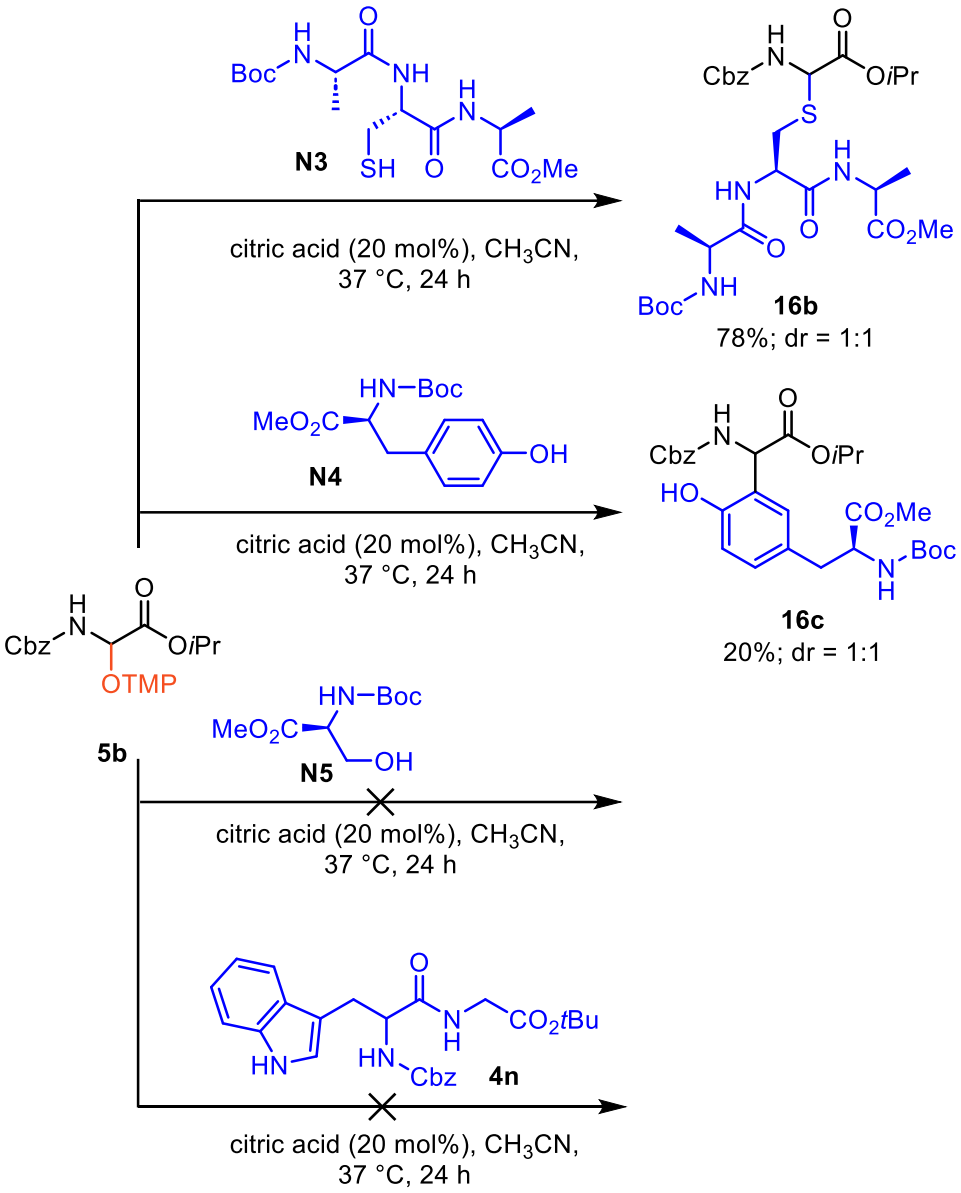


5b **16a**

Trial No.	Acid	Solvent	Yield **16a**
1	Citric acid	CH_3CN	75% (dr = 1:1)
2	Citric acid (cat.)	CH_3CN	87% (dr = 1:1)
3	AcOH	MeOH	52% (dr = 1:1)
4	AcOH	$MeOH/H_2O$	55% (dr = 1:1)
5	-	phosphate buffer pH-6.2	52% (dr = 1:1)

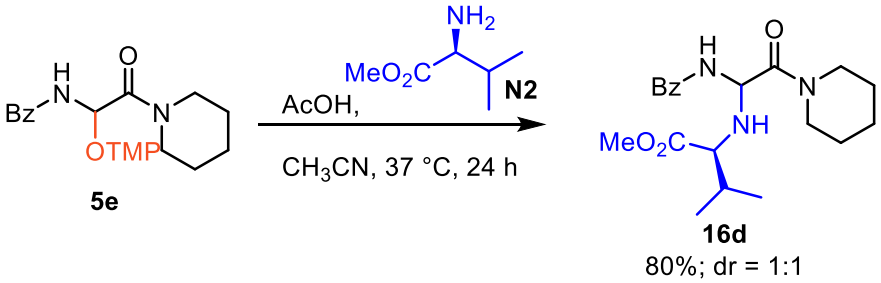
Conjugation of L-valine methyl ester at the glycine α-position reveals that a free N-terminal of an amino acid or a peptide acts as a potential nucleophile for the substitution reaction. However, within the set of natural amino acids, there are also other nucleophilic positions in amino acid side chains which may act as potential nucleophiles for substitution reactions. The reactivity of various amino acids bearing nucleophilic sidechains was investigated to study the substitution of the glycine alkoxyamine **5b** at optimized reaction conditions (Scheme 45). The tripeptide Boc-Ala-Cys-Ala-OMe (**N3**), which contains a thiol group in the cysteine side chain, acts as a nucleophile in the substitution reaction to the alkoxyamine **5b**. The reaction provided thioheminaminal derivative **16b** in good yield as 1:1 diastereomeric mixture. Tyrosine is an amino acid bearing phenol as a side chain, and the potential substitution reaction of the tyrosine side chain was tested using Boc-Tyr-OMe (**N4**) as the nucleophile under similar conditions. Interestingly, a Friedel-Crafts-type reaction was observed to form the C-C bond and the product **16c** was obtained in 20% yield as 1:1 diastereomeric mixture. However, the reaction is slow and low yielding and unreacted starting material was recovered. Serine is another amino acid bearing hydroxy functional group on the side chains. Boc-Ser-OMe (**N5**) was subjected to the nucleophilic substitution reaction with the glycine alkoxyamine **5b**, but no reaction was observed, and the starting materials were recovered. The amino acid tryptophan contains an indole moiety as a side chain, which may be another potential nucleophile for the reaction. The substitution reaction of glycine alkoxyamine **5b** was attempted with Try-Gly dipeptide (**4n**) as nucleophile under similar reaction conditions and found to be unsuccessful and the starting materials were recovered.

The experiments, aimed to test the potential conjugation site in a peptide side chain, indicated that thiol and amino groups are good nucleophiles for substitution reactions at the α-position of the glycine alkoxyamines **5**. Furthermore, it has been observed that tyrosine sidechain may interfere to a smaller extent with peptide conjugation reaction of glycine alkoxyamine. Nevertheless, these experiments have shown that hydroxy and indole groups on amino acid side chains are unreactive towards substitution reactions at physiological temperature.



Scheme 45: Substitution reactions of alkoxyamine **5b** with various amino acids/peptides.

The glycinamide alkoxyamine **5e** also acts as a suitable substrate for the substitution and the reaction with L-valine methyl ester **N2** provided the aminal compound **16d** in 80% yield as 1:1 diastereomeric mixture (Scheme 46).



Scheme 46: Substitution of glycinamide alkoxyamine with L-valine methyl ester

In order to understand the reaction kinetics of the glycine alkoxyamine substitution, we studied the reaction of alkoxyamine **5b** with two nucleophiles, L-valine methyl ester (**N2**) and Boc-Ala-Cys-Ala-OMe (**N3**), mediated by acetic acid-D_4 (stochiometric) at 37 °C in CD_3CN by ^1H NMR spectroscopy. The reaction progress was continuously monitored over an extended period, and nearly complete conversion was observed.

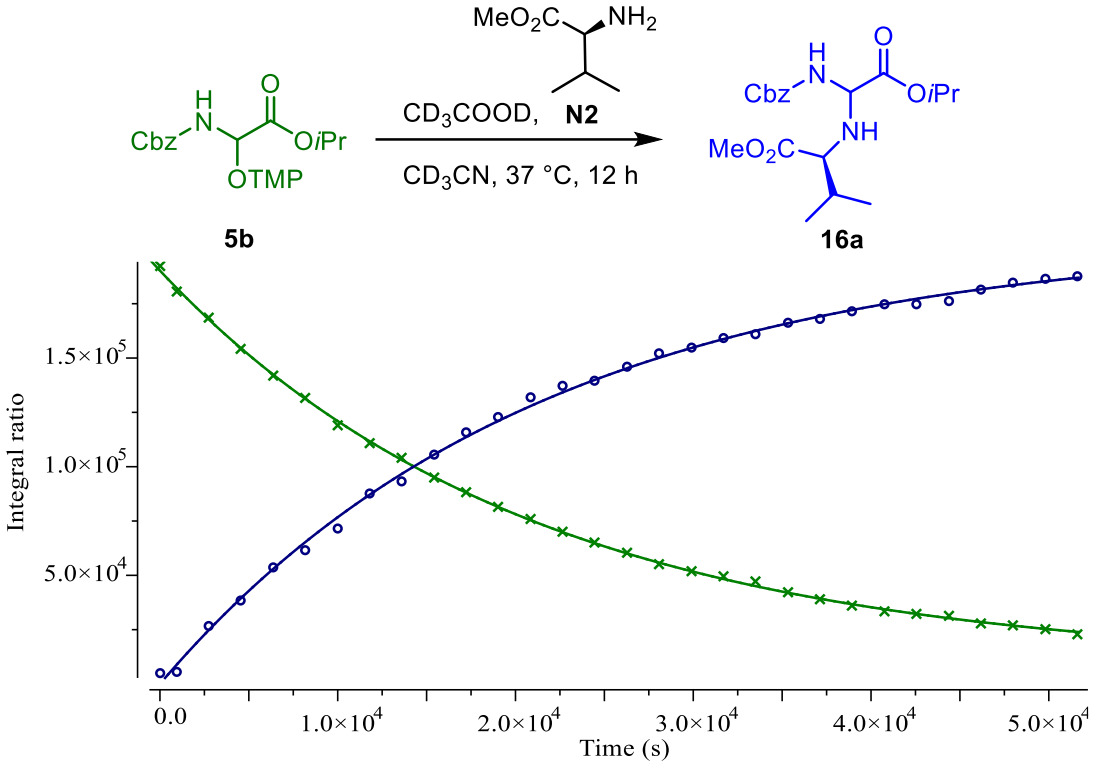


Figure 8: Reaction kinetics of aminal formation from the glycine alkoxyamine of concentration 0.05 M.

In the case of alkoxyamine **5b** with L-valine methyl ester (**N2**) as the nucleophile, the concentration of the starting material reached a minimum, while that of the product attained a maximum within the 11-hour timeframe. Two distinct initial concentrations of the alkoxyamine were investigated, namely 0.05 M and 0.005 M. The kinetic analysis of the rate plot for each concentration yielded a rate constant of $5x10^{-5}$ s^{-1} (Figure 8). The substitution reaction of glycine alkoxyamine **5b** with Boc-Ala-Cys-Ala-OMe (**N3**) as the nucleophile under same reaction conditions provided a similar reaction plot and a rate constant of $6x10^{-5}$ s^{-1} (Figure 9). The reactions with different nucleophiles provided similar rate constants and thus imply that the nucleophiles, amine or thiol, do not influence the reaction rate differently (for reaction procedures, see experimental section, 6.6 Reaction Kinetics by ^1H NMR Spectroscopy).

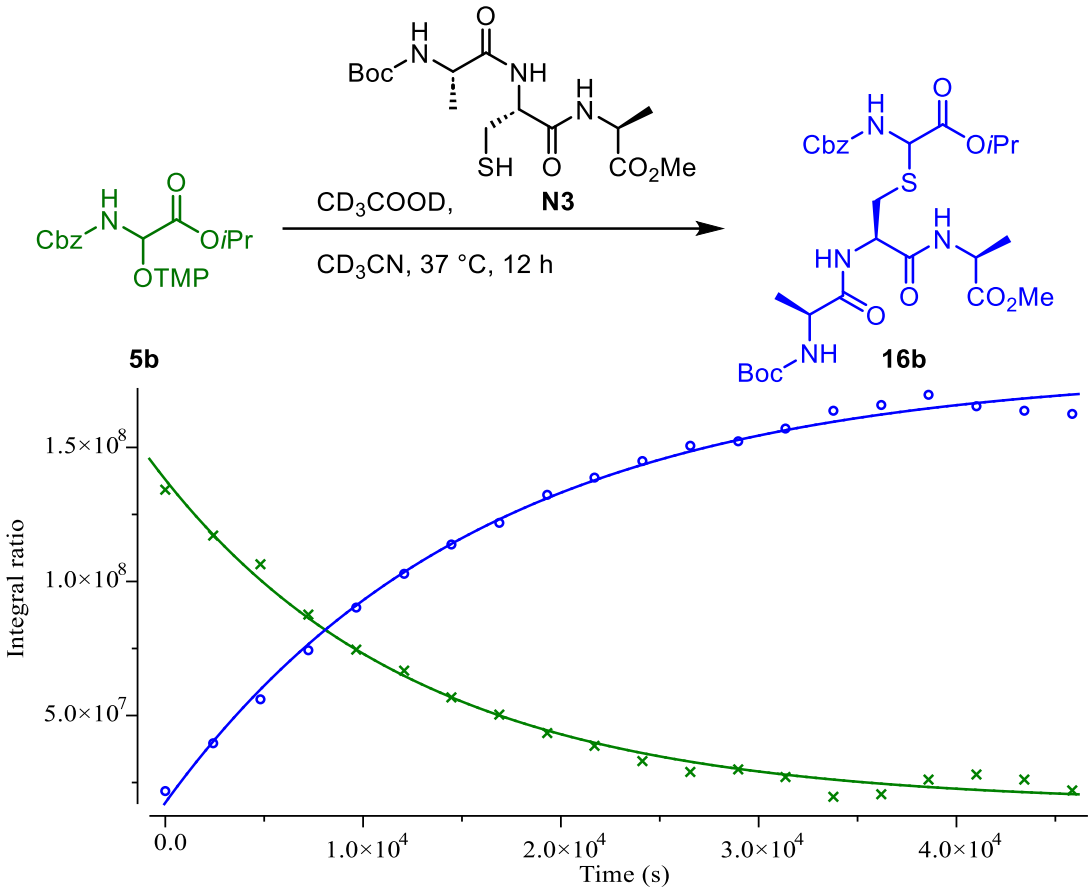


Figure 9: Reaction Kinetics of thiohemiaminal formation from the glycine alkoxyamine of concentration 0.05 M.

To compare the reaction kinetics of the elimination reaction with that of the substitution reaction, we conducted another kinetic experiment under identical reaction conditions by omitting the use of any nucleophile. The objective was to determine the rate of formation of the iminium intermediate and to compare it to that of substitution. However, no iminium or imine intermediate was detected by NMR spectroscopy. Instead, α-hydroxy glycinate was formed with a rate constant of $2x10^{-4}$ s^{-1}, and equilibrium was attained between the starting material and the α-hydroxy glycinate over time (Figure 10). It is suspected that the water content in the deuterated acetonitrile was reactive enough to form the observed product. The kinetic studies (Figure 8, 9, 10) do not confirm unambiguously that the reaction is unimolecular and follows the S_N1 type reaction, as we were unable to detect the iminium intermediate by ^1H NMR spectroscopy. However, we hypothesize that the intermediate was highly reactive and subsequent substitution was fast enough, even with water, to form the substituted product in equilibrium with the starting material.

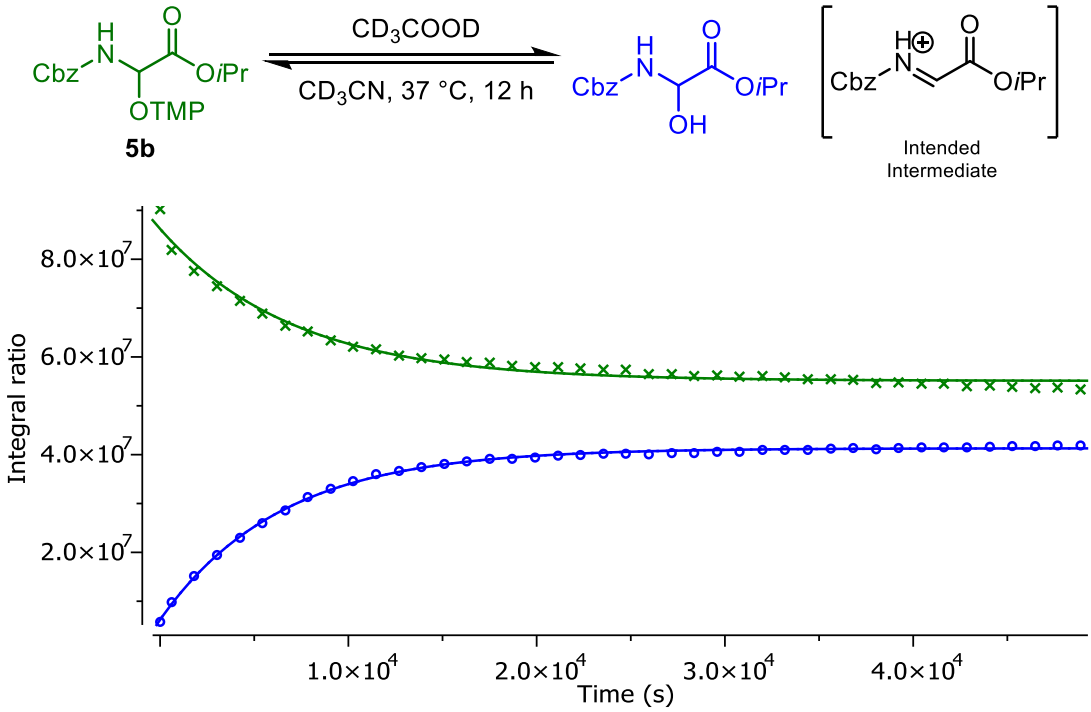
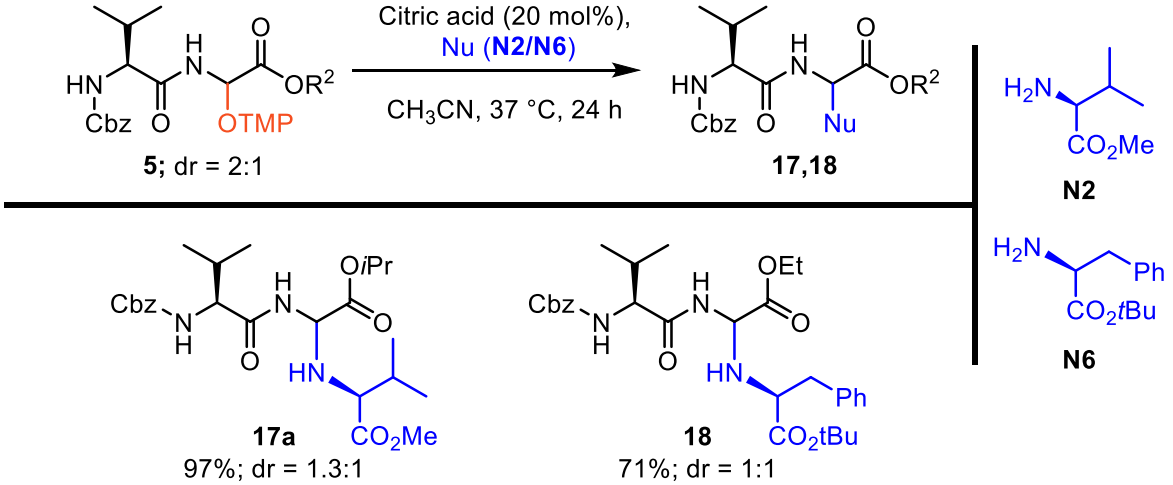


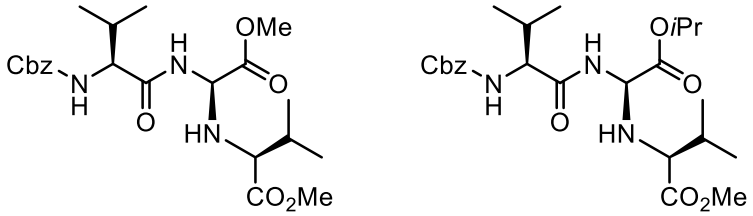
Figure 10: Reaction kinetics of the glycine alkoxyamine without the addition of nucleophile.

The substitution reaction was also tested in glycine alkoxyamine dipeptides (Scheme 47). The substitution reaction of Val-Gly dipeptide **5j** (dr = 2:1) with L-valine methyl ester (**N2)** catalyzed by citric acid (20 mol%) gave the aminal product **17a** in 97% yield as 1.3:1 diastereomeric mixture. The configuration of the major diastereomer of the aminal compound **17aD1** was found to be [S^{Val}, S^{Aminal}, S^{Val}] by comparing the ^{1}H NMR spectrum to that of previously reported aminal product **XV**[61] (Figure 11) (*cf.* Scheme 9). Similarly, the reaction of Val-Gly dipeptide **5k** (dr = 2:1) with L-phenyl alanine *tert*-butyl ester (**N6)** gave the aminal product **18** in 71% yield with 1:1 diastereomeric ratio.



Scheme 47: Substitution reaction with dipeptides containing glycine alkoxyamine

Our strategy of nucleophilic addition to glycine alkoxyamine allowed us to obtain the aminal compound **17a** in just two steps from the dipeptide Cbz-Val-Gly-O*i*Pr (**4j**). In contrast, the previously reported strategy required a longer synthetic route to synthesize **XV**, starting from Cbz-Val-Ser-OMe (*vide supra*, Scheme 9).



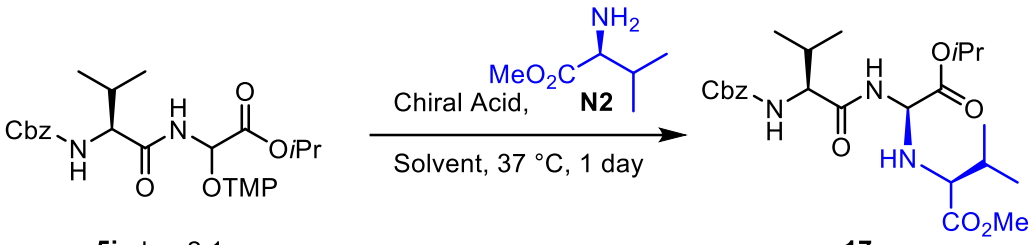
XV
dr = 5:1

17aD1
[S^{Val}, S^{Aminal}, S^{Val}]

Figure 11: Comparison between already reported aminal compound **XV** and **17a**.

We attempted to induce diastereoselectivity in the aminal products by using chiral acids. The glycine alkoxyamine dipeptide **5j** (dr = 2:1) was reacted with L-valine methyl ester (**N2**) at 37 ºC for 24 h catalyzed by different chiral acids such as (*R*)-TRIP, (*S*)-TRIP, L-tartaric acid, and D-tartaric acid (Table 3). The reactions catalyzed by (*R*)-TRIP as well as (*S*)-TRIP in CH_3CN resulted in the formation of aminal compound **17a** as 1.7:1 diastereomeric mixture (entry 1 and 2). Reactions catalyzed by L-tartaric acid, and D-tartaric acid in CH_2Cl_2 also provided **17a** in 1.7:1 diastereomeric ratio (entry 3 and 4). The reaction in toluene with (*S*)-TRIP, gave only 15% conversion to the product (by ^{1}H NMR spectrum) but 5:1 diastereoselectivity was observed (entry 5). However, changing the solvent to CH_3CN/toluene (1:1) resulted 54% conversion but the diastereoselectivity remained 1.6:1. Surprisingly, all the entries provided same stereoselectivity, preferring the configuration [S^{Val}, S^{Aminal}, S^{Val}] as the major diastereomer regardless of the enantiomer of the chiral acid used. The aminal derivative **17a** was partially separable by column chromatography and the major diastereomers **17aD1** was isolated.

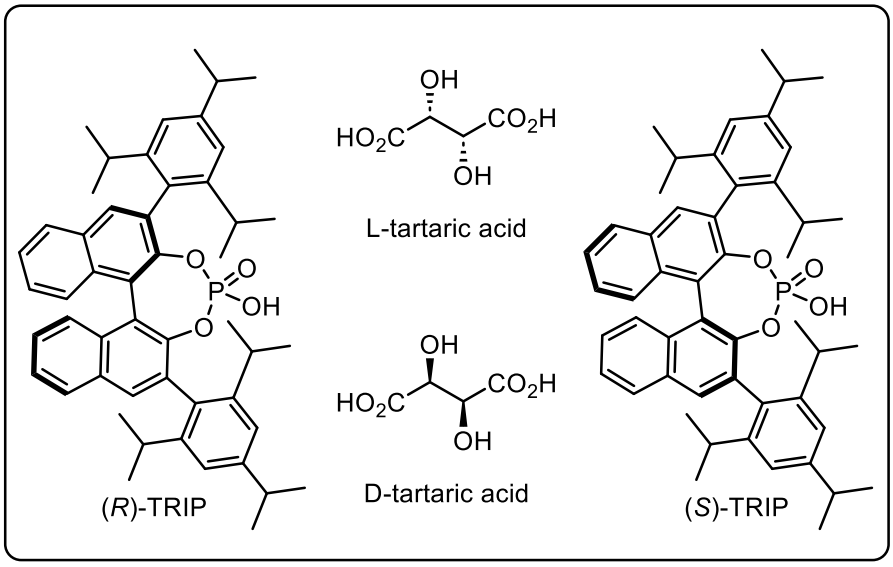
Table 3: Screening of stereoselectivity in substitution of alkoxyamines 5j with L-valine methyl ester



5j; dr = 2:1 **17a**

Trial No.	Acid (20 mol%)	Solvent	Yield	dr
1	(*R*)-TRIP	CH_3CN	85%	1.7:1
2	(*S*)-TRIP	CH_3CN	85%	1.8:1
3	L-tartaric acid	CH_2Cl_2	64%	1.7:1
4	D-tartaric acid	CH_2Cl_2	74%	1.7:1
5	(*S*)-TRIP	toluene	15%*	5:1
6	(*S*)-TRIP	CH_3CN/toluene (1:1)	54%*	1.6:1

* Reaction conversion analyzed by ^{1}H NMR spectroscopy



We also performed electronic circular dichroism (ECD) spectroscopy for cross-conjugated peptide **17a** and was compared with that of the alkoxyamine derivative **5j** (Figure 12). ECD spectra of the 2:1 diastereomeric mixture of the cross-conjugated peptide **17a** (Figure 12A, red) revealed positive Cotton effects at ~ 190 nm ($\Delta\varepsilon$ = +6.8 $Lmol^{-1}cm^{-1}$) and at ~ 214 nm ($\Delta\varepsilon$ = +2.9 $Lmol^{-1}cm^{-1}$). ECD spectrum of the major diastereomer **17aD1** (Figure 12A, black) was characterized by positive Cotton effects at ~ 192 nm ($\Delta\varepsilon$ = +1.5 $Lmol^{-1}cm^{-1}$) and ~ 210 nm ($\Delta\varepsilon$ = +8.6 $Lmol^{-1}cm^{-1}$) accompanied by negative Cotton effect at ~ 240 nm with low intensity ($\Delta\varepsilon$ = −0.6 $Lmol^{-1}cm^{-1}$). ECD spectrum of the 2:1 diastereomeric mixture of the alkoxyamine **5j**

(Figure 12A, green) showed positive Cotton effects at ~ 192 nm ($\Delta\varepsilon$ = +2.2 $Lmol^{-1}cm^{-1}$) and ~ 215 nm ($\Delta\varepsilon$ = +6.3 $Lmol^{-1}cm^{-1}$) accompanied by negative Cotton effect at ~ 236 nm with low intensity ($\Delta\varepsilon$ = −3.4 $Lmol^{-1}cm^{-1}$). To add, as expected, all three compounds had rather similar UV-vis spectra as well (Figure 12B).

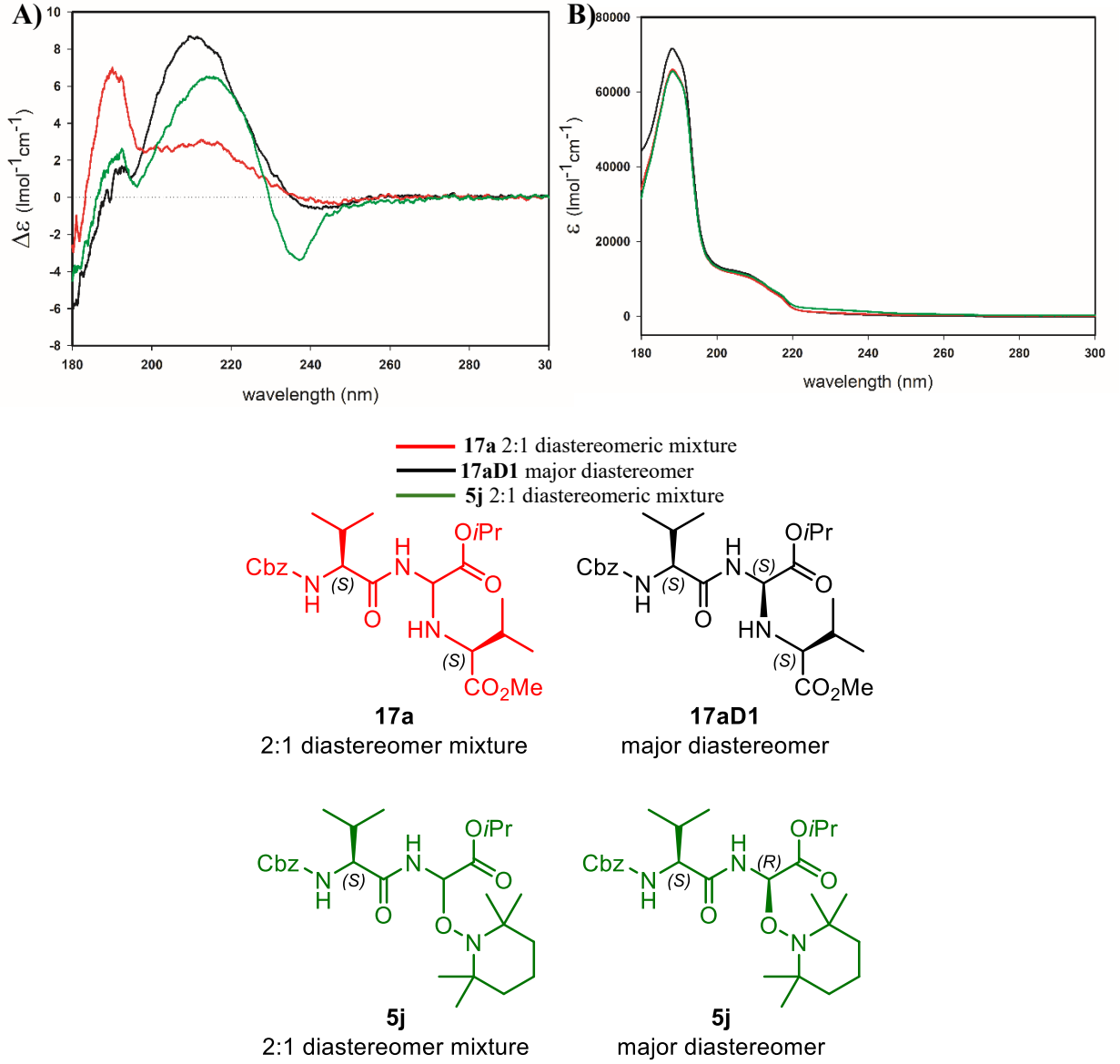
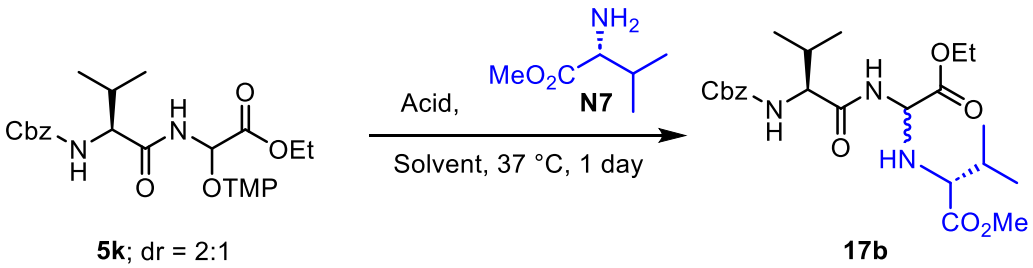


Figure 12: A) ECD spectra of **17a, 17aD1** and **5j**; B) UV-vis spectra of **17a, 17aD1** and **5j**

We suspect that the reaction of glycine alkoxyamine **5j** with L-valine methyl ester (**N2**) catalyzed by chiral acids exhibits a match-mismatch situation, given that both substrates possess fixed stereocenters. Thus, we also explored the selectivity of the reaction with D-valine methyl ester (**N7**) as the nucleophile (Table 4). The substitution reaction of glycine alkoxyamine **5k** catalyzed by (*R*)-TRIP in CH_3CN/toluene (1:1) resulted 53% conversion to aminal **17b** as 5:1 diastereomeric mixture (entry 1). Surprisingly, the reaction, catalyzed by (*S*)-TRIP also provided **17b** (dr = 5:1), favoring the same diastereomer (entry 2). The result

implied that the stereochemical outcome was not determined by the chiral acid. To prove it, we performed the reaction catalyzed by the achiral acid citric acid and it resulted the formation of **17b** with the same diastereomeric outcome and better conversion to the product (entry 3). The stereoselectivity of the reaction is thus substrate-controlled and depends on the incoming nucleophile. The aminal derivative **17b** was also partially separable by column chromatography and the major diastereomer **17bD1** was isolated, but recrystallization attempts were unsuccessful.

Table 4: Screening of the stereoselectivity in the substitution of alkoxyamine 5k by D-valine methyl ester



5k; dr = 2:1 **17b**

Trial No.	Acid (20 mol%)	Solvent	Conversion*	dr
1	(*R*)-TRIP	CH_3CN/toluene (1:1)	53%	5:1
2	(*S*)-TRIP	CH_3CN/toluene (1:1)	62%	5:1
3	Citric acid	CH_3CN/toluene (1:1)	77%	5:1

*Conversion analyzed by ^{1}H NMR spectroscopy.

To propose a transition state and rationalize the stereochemical outcome, it is necessary to understand the preferred confirmation of the glycine alkoxyamine **5** in the solution. To gain insight into the occurrence of intramolecular hydrogen bonding in the glycine alkoxyamine dipeptides, a titration experiment was conducted for the $CDCl_3$ solution of the 2:1 diastereomeric mixture of Val-Gly dipeptide glycine alkoxyamine **5k** (10 mM $CDCl_3$) with the hydrogen bonding-interrupting co-solvent DMSO-d6 (5-50 µL) and ^{1}H NMR spectra were measured (Figure 13).[140] Upon the addition of DMSO the amide proton NH-2 (green) of the major diastereomer of the glycine alkoxyamine **5k** exhibited a prominent downfield chemical shift, while the amide proton NH-1 (red) exhibited a small downfield chemical shift. The addition of 5 µL of DMSO-d6 (12 equiv.) resulted a downfield chemical shift of 0.3 ppm for the amide protons NH-2 (green) and with increasing volume of DMSO, a gradual change in the chemical shift was observed (see experimental section, 6.7 DMSO titration experiment by 1H NMR Spectroscopy). The amide protons NH-1 (orange) and NH-2 (blue) of the minor diastereomer **5k** also follow similar trend with the addition of DMSO. This indicates the

occurrence of intramolecular hydrogen bonding networks for the amide proton NH-2 in the solution of glycine alkoxyamine dipeptide **5k**.

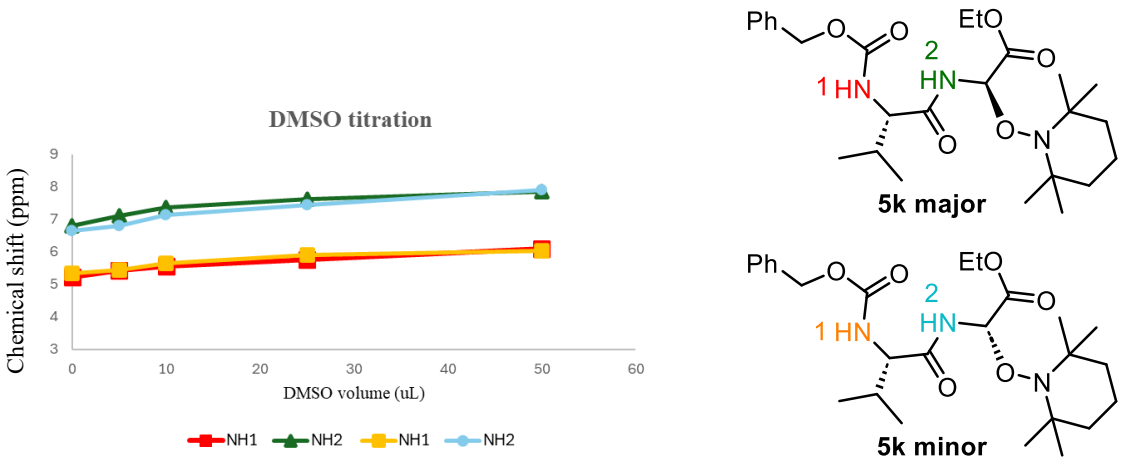


Figure 13: Addition of DMSO-d6 to 10 mM $CDCl_3$ solution of the 2:1 diastereomeric mixture of glycine alkoxyamine **5k**.

A similar study was also conducted in the aminal compound **17b** with the hydrogen bonding-interrupting co-solvent DMSO. A $CDCl_3$ solution of the major diastereomer of the aminal compound **17bD1** (10 mM) was treated with DMSO (5-50 µL) and ^{1}H NMR spectra were measured (Figure 14). The amide proton NH-2 (green) exhibits a prominent downfield chemical shift with the addition of DMSO (5 µL, 10 µL and 25 µL and 50 µL) up to 1.2 ppm. This indicates the interruption of hydrogen bonding for the amide proton NH-2 in the presence of DMSO. In contrast, a downfield chemical shift of only 0.5 ppm was observed for the amide proton NH-1 (red) upon the addition of 50 µL of DMSO, while the amide proton NH-3 (blue) exhibited almost no change. These observations suggest minimal interactions between DMSO and the amide protons NH-1 and NH-3. A comparable outcome was also observed in the titration experiment with the diastereomeric mixture of **17b**. This suggests that intramolecular hydrogen bonding networks are preferred in solution for the amide proton NH-2 of the aminal compound **17b**, similar to that in glycine alkoxyamine dipeptide **5k**. We also measured a correlation spectrum to observe any predominant structural confirmation for the compound **17b**, but no correlation was observed except NH-1 with benzyl protons of Cbz group.

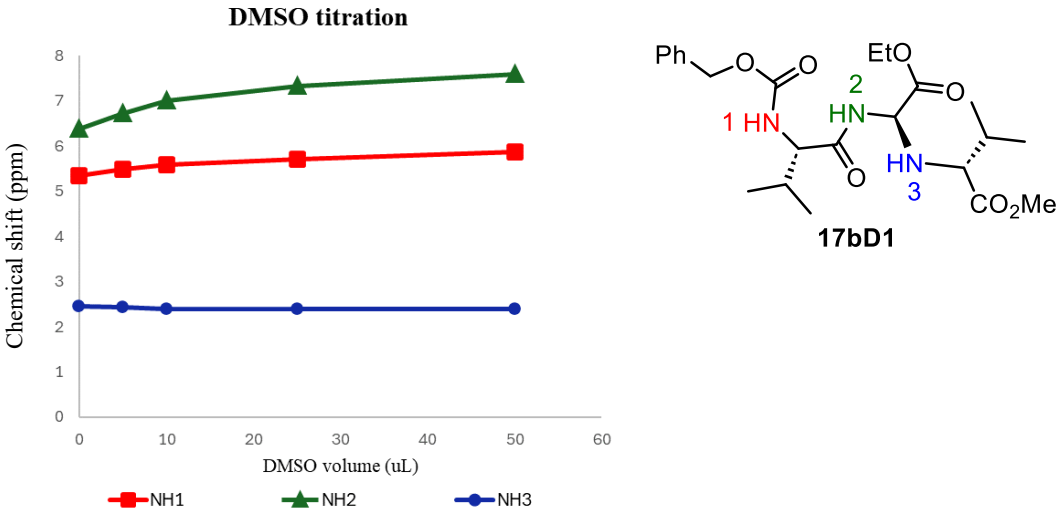
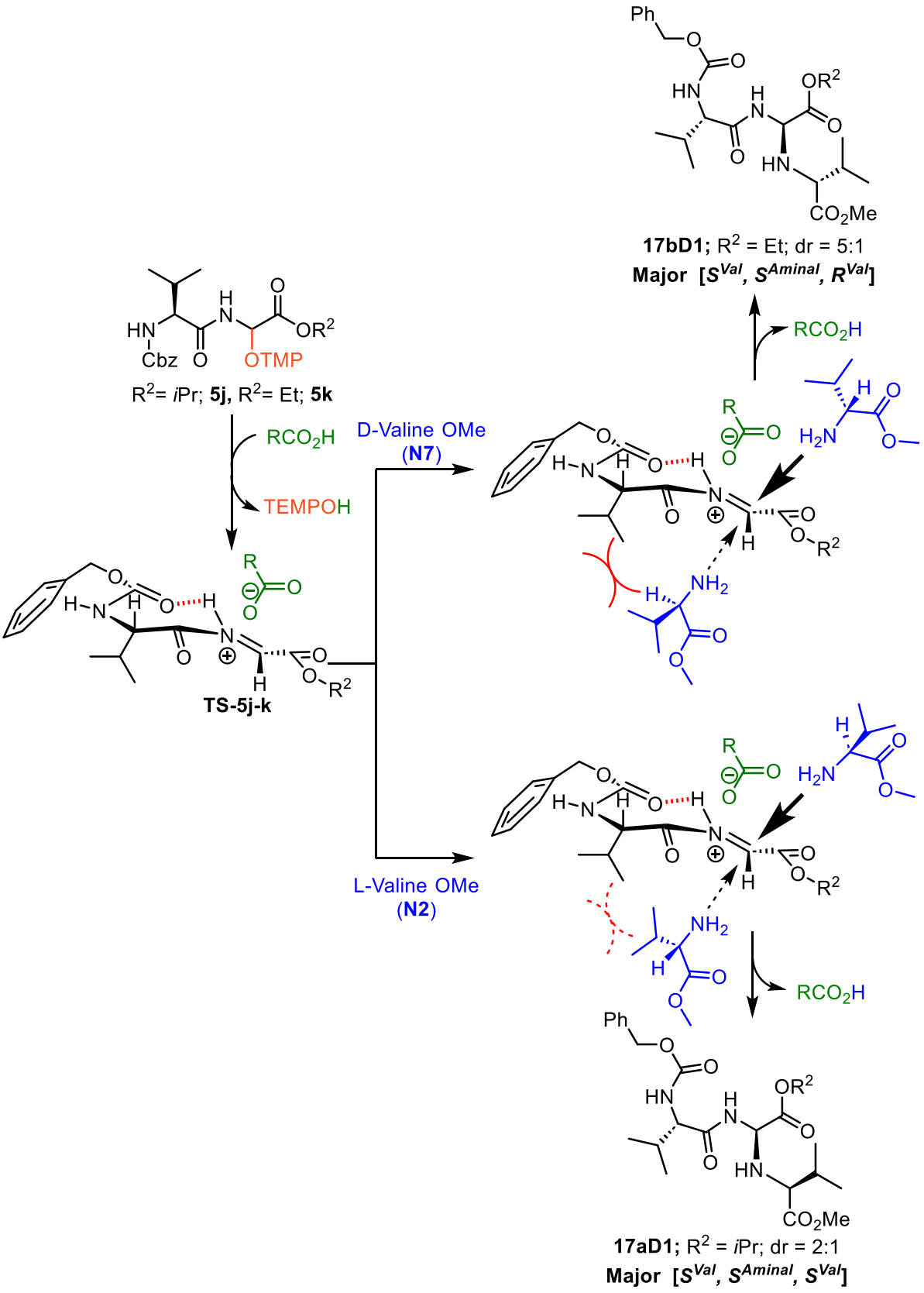


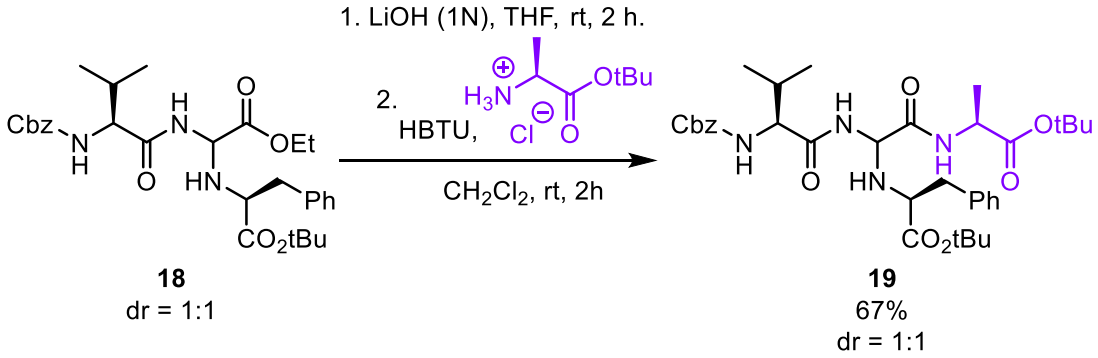
Figure 14: Addition of DMSO-d6 to 10 mM $CDCl_3$ solution of **17bD1**

The following mechanism and preferred transition state are proposed to rationalize the stereochemical outcome of the acid-catalyzed substitution reaction of the dipeptide glycine alkoxyamines **5** (Scheme 48). The experimental results demonstrate that the stereochemical outcome of the reaction depends on the substrate as well as the incoming nucleophile. Under acidic conditions, the dipeptide glycine alkoxyamines (**5j-k**) undergo the elimination of the N-hydroxy-2,2,6,6-tetramethyl piperidine (TEMPOH) to form the corresponding iminium intermediate **TS-5j-k**. It is likely that **TS-5j-k** will have an intramolecular hydrogen-bonded seven-membered ring, which confers structural rigidity and the valine side chain, isopropyl, is situated in the preferred equatorial position. For the incoming nucleophiles, the β-face of the iminium intermediate **TS-5j-k** is more accessible than the α-face due to the steric hinderence by the isopropyl group in the equatorial position of the seven-membered ring. The attack of the D-valine methyl ester (**N7**) on the iminium **TS-5j-k** occurs selectively from the more accessible β-face. The addition from the α-face is not preferred due to the strong repulsion between the isopropyl groups on the equatorial position of the seven-membered ring and the side chain of valine methyl ester. This preferential attack from the β-face results in a diastereoselectivity of 5:1 and the major diastereomer, **17bD1**, is proposed to possess a [S^{Val}, S^{Aminal}, R^{Val}] configuration. In the case of L-valine methyl ester (**N2**) as the nucleophile, it attacks the iminium **TS-5j-k** from the more accessible β-face, but the α-face is not completely restricted, as there is not much steric repulsion between the isopropyl group on the equatorial position of the seven-membered ring and the side chain of L-valine methyl ester. This results in the formation the aminal compound **17a** with a slight preference for the diastereomer **17aD1** with a configuration [S^{Val}, S^{Aminal}, S^{Val}].



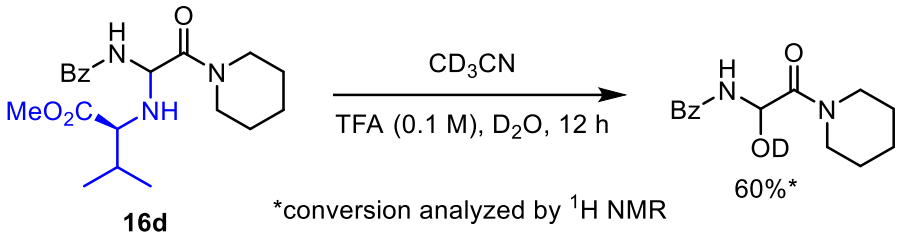
Scheme 48: Proposed mechanism and stereoselective rationalization for the substitution reaction of alkoxyamine **5j-k** with D- valine methyl ester and L-valine methyl ester.

Unmasking the C-terminal carboxylic acid of the aminal derivatives **16-18** would be interesting for subsequent peptide chain elongation to feature a new class of peptide derivatives. We attempted saponification of the aminal derivative **18** under basic condition. The ethyl ester was selectively saponified and no base hydrolysis at the aminal moiety was observed in 2h (Scheme 49). Subsequent peptide coupling of the carboxylic acid with alanine *tert*-butyl ester gives the long peptide chain **19** in good yield. Similarly, these aminal derivatives can be coupled with different peptides of interest. The peptide **19** also showcases its structural complexity as a branched peptide, which are a new class of peptide family. To add, an attempt to saponify α-aminal derivative with isopropyl ester **17** resulted in the formation of mixture of base hydrolysed products and carboxylic acid derivative was not obtained. Thus, the aminal derivatives with isopropyl esters are not suitable for saponification and further fuctionalization.



Scheme 49: Peptide coupling of aminal ester to construct branched peptide.

Acid hydrolysis can be a fatal pathway for these aminal derivatives **16**, which might limit their use for extended biological applications. To test the stability, the aminal derivative **16d** was treated with TFA/D_2O for 12 h and the mixture was analyzed by ^{1}H NMR spectroscopy. The aminal found to be hydrolyzed partially over the period (Scheme 50) and concludes that aminal products are less stable under acidic conditions.



Scheme 50: Stability of aminal towards acid hydrolysis

Effectively, the modification of glycine alkoxyamines under acidic conditions by heterolysis of α-C-O bond allows easy access to construct a library of non-natural amino acids. It also

allows cross conjugation between amino acids or peptides by a simple substitution reaction under physiological conditions. Here, the strategy only requires two steps to access the conjugation product from the corresponding peptide derivatives. With the results from preliminary studies comparing the reactivity of a few amino acids and their side chain on the substitution reaction, we can put forward that amines and thiols act as effective linking sites for the coupling with glycine alkoxyamines **5** to give the cross-conjugated peptide products in good yields whereas weaker nucleophiles such as alcohols give no reaction. Thus, a peptide containing thiol or amino group on side chains or the free amino group at the N-terminal acts as suitable cross-linking candidates to glycine alkoxyamines under physiological conditions. The method also enables construction of the new compound classes such as aminal, hemiaminal as well as thiohemiaminal amino acid derivatives and access to a new class of branched peptides.

4.5 Synthesis of hindered nitroxides and their application in glycine oxygenation

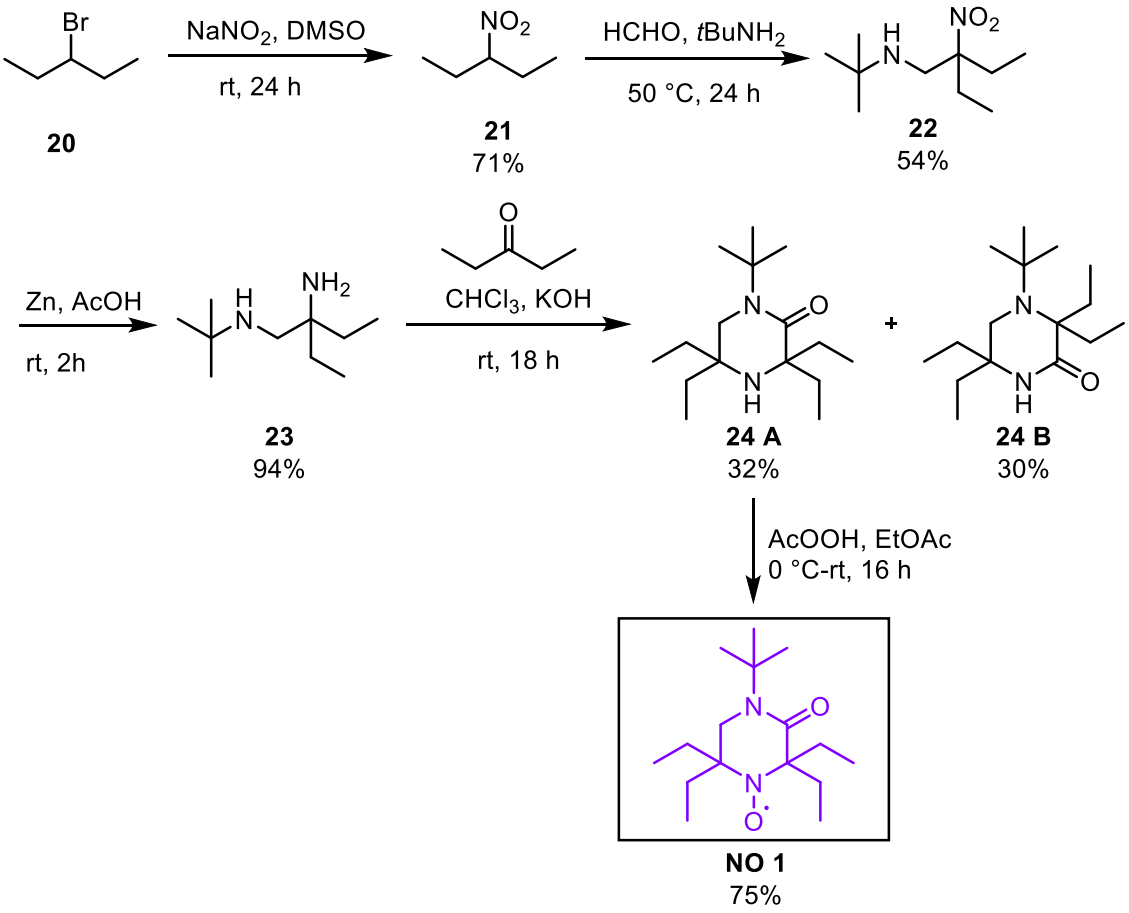
Glycine alkoxyamines with weaker C-O bond would act as ideal candidates to apply the strategy of alkoxyamine modification by homolysis at lower temperature compared to glycine alkoxyamines derived from TEMPO. We hypothesis that the strategy can be then extended to modify glycine alkoxyamines in high-temperature sensitive biologically active peptides. We envisioned that piperidine nitroxides with sterically hindered substituents at the 2,6-positions might be a promising compound class to replace TEMPO in the methodology of α-oxygenation of glycine derivatives. The resulting alkoxyamines may present an opportunity for modification of glycine derivatives by homolysis at lower temperatures.

4.5.1 1-*tert*-Butyl-3,3,5,5-tetraethyl-2-piperazinon-4-oxyl (**NO1**)

A. Studer reported a nitroxide 1-*tert*-butyl-3,3,5,5-tetraalkyl-2-piperazinon-4-oxyl **NO1** being highly efficient for controlled radical polymerization and the alkoxyamine derived from the nitroxide found to have homolysis at 105 ºC for styrene polymerization.[141] These 6-membered nitroxides are having bulky substituents in α-position to the N atom and we chose to explore our methodology with this nitroxide as oxygenating reagent instead of TEMPO. The reported procedures were followed for the synthesis of the target nitroxide.

We started the synthesis with the commercially available 3-bromopentane **20** and transformed it to corresponding nitro compound **21**. Nitro-Mannich reaction of 3-nitropentane **21** with *tert*-

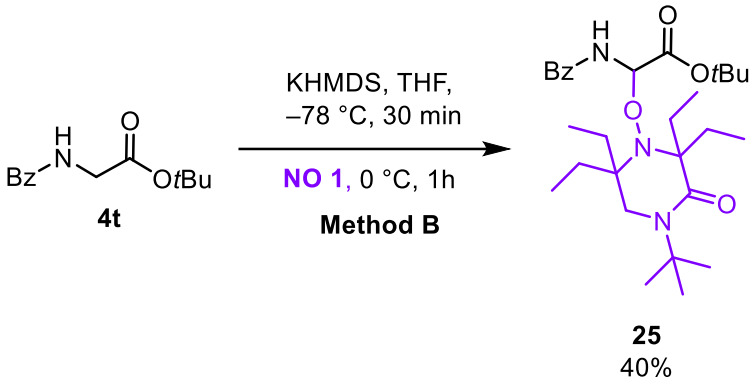
butyl amine and formaldehyde afforded β-nitroamine **22** in moderate yield. Moreover, compounds **21** and **22** possessed challenges in handling due to their volatile characteristics, resulting in diminished yields. However, pursuing the reported synthesis, subsequent reduction of the nitro group with zinc in acetic acid provided diamine **23** in excellent yield. The cyclization of diamine with KOH and $CHCl_3$ in 3-pentanone to give the piperazinone, found to be the key step in the synthesis. The regioselectivity of the addition reaction to 3-pentanone becomes crucial and the poor selectivity resulted in a low yielding cyclized products **24A** and **24B**. The targeted piperazinone **24A** was subjected to oxidation with AcOOH and the nitroxide **NO1** was obtained as red liquid (Scheme 51).



Scheme 51: Synthesis of hindered nitroxide 1-*tert*-butyl-3,3,5,5-tetraethyl-2-piperazinon-4-oxyl **NO1**

The synthesis sequence has its limitation at the cyclization step where the preferred piperazinone was obtained in a very low yield and the resulting target nitroxide **NO1** was obtained in overall yield of 5%. With nitroxide **NO1** in hand, the methodology of glycine enolate oxidation was explored (Scheme 52). The enolate of glycinate **4t** was generated with KHMDS, which reacted with two equivalents of the nitroxide **NO1** to provide alkoxyamine **25**

in moderate yield. The oxygenation reaction with **NO1** compared to TEMPO is low yielding and unreacted starting material was recovered. However, the result shows the possibility to use bulky nitroxides as oxidant for the methodology of glycine oxygenation. Nevertheless, synthetically demanding nitroxide **NO1** does not appear to be a feasible reagent for the methodology because of its challenging and low yielding synthesis. Thus, the substrate was not explored for further studies.



Scheme 52: Oxidation of glycine enolate with **NO1**

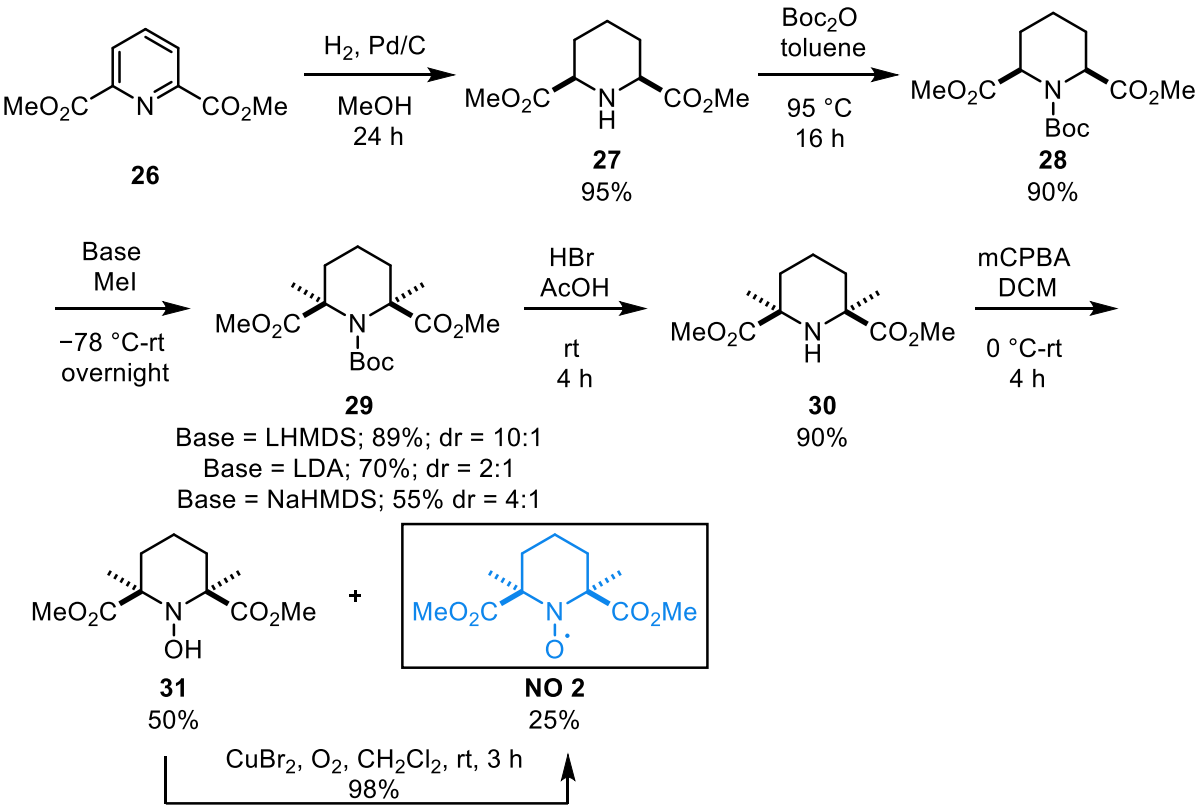
A different nitroxide compound, bearing bulky substituents, must be designed with the aim of evaluating their suitability for glycine enolate oxygenation and further modifications. In addition, the nitroxide must be readily synthesizable and accessible for the methodology.

4.5.2 *cis*-2,6-Bis(methoxycarbonyl)-2,6-dimethylpiperidine-1-oxyl **(NO2)**

Synthesis of another piperidine nitroxide *cis*-2,6-dimethylpiperidine-2,6-dicarboxylic acid dimethyl ester-1-oxyl radical[142] (**NO2**) was reported and an alkoxyamine derived from this nitroxide was utilized as radical initiators for the nitroxide-mediated polymerization (NMP).[143] The nitroxide is structurally similar to TEMPO but bearing methyl ester substituents at the 2- and 6- position instead of two methyl units. We hypothesized that the sterically hindered and the electron-deficient substituents at the 2- and 6- positions of the nitroxide may result in a weaker α-C-O bond on the projected glycine alkoxyamine, which would lead to a lower homolytic temperature compared to TEMPO-derived glycine alkoxyamine **5**.

Exchange student Gael Le Berre worked on the synthesis of nitroxide **NO2** (Scheme 53). The synthesis commenced with commercially available pyridine-2,6-dimethyl ester **26**. The pyridine was subjected to hydrogenation conditions yielding piperidine **27**. Subsequent Boc protection afforded N-Boc piperidine-2,6-dimethylester **28** in excellent yield. The reported

strategy[142] of enolization with LDA and addition of MeI afforded the piperidine **29** in 70% yield (dr = 2:1; *cis*/*trans*) and a mixture of unreacted starting material and monomethylated product. Double methylation step was attempted with different bases for better results. Enolization with NaHMDS provided the piperidine **29** in 55% yield as 4:1 diastereomeric mixture favoring the *cis* diastereomer, whereas LHMDS gave **29** in 89% yield as 10:1 diastereomeric mixture favoring the *cis* diastereomer. The major *cis* diastereomer was then subjected to the subsequent stage of the process. Deprotection of Boc under acidic condition afforded piperidine **30** which was subjected to oxidation by *m*CPBA to obtain the nitroxide **NO2** in 25% yield. Interestingly, we observed the formation of N-hydroxylamine **31** in 50% yield as the major product of the oxidation reaction, but the N-hydroxylamine **31** can be converted to the nitroxide **NO2** under oxygen atmosphere with catalytic amount of $CuBr_2$.



Scheme 53: Synthesis the nitroxide **NO2**

Nitroxide **NO2** is a meso compound having two stereocenters at 2- and 6- positions with a plane of symmetry. A freshly prepared solution of **NO2** in toluene (c = 4 mM) shows a typical triplet signal with a g factor of 2.0059 (Figure 15).

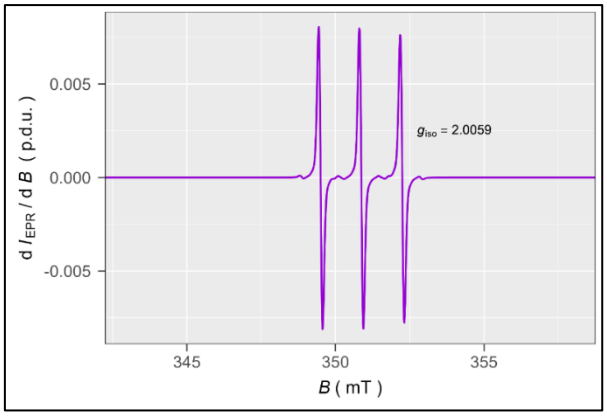
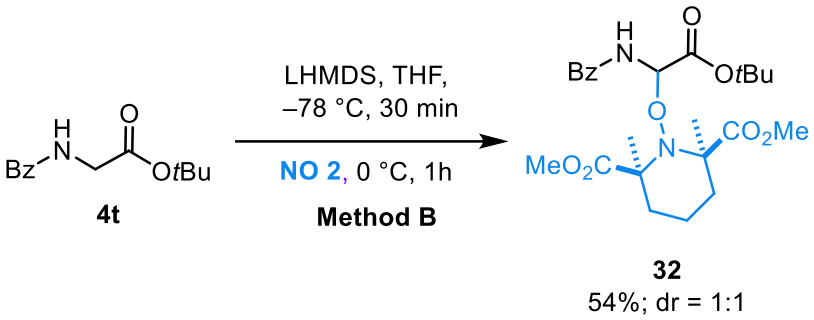


Figure 15: EPR spectra of **NO2**

With nitroxide **NO2** in hand we exploited our methodology of enolate oxidation (method B) in the glycinate **4t** to obtain the glycine alkoxyamine **32** in moderate yield as 1:1 diastereomeric mixture (Scheme 54). The diastereomeric mixture of the alkoxyamine **32** was recrystallized from 5% EtOAc/pentane and X-ray structure was obtained (Figure 16). The alkoxyamine **32** is racemic and the X-ray structure does not provide the absolute configuration.



Scheme 54: Oxygenation of glycinate by nitroxide **NO2**

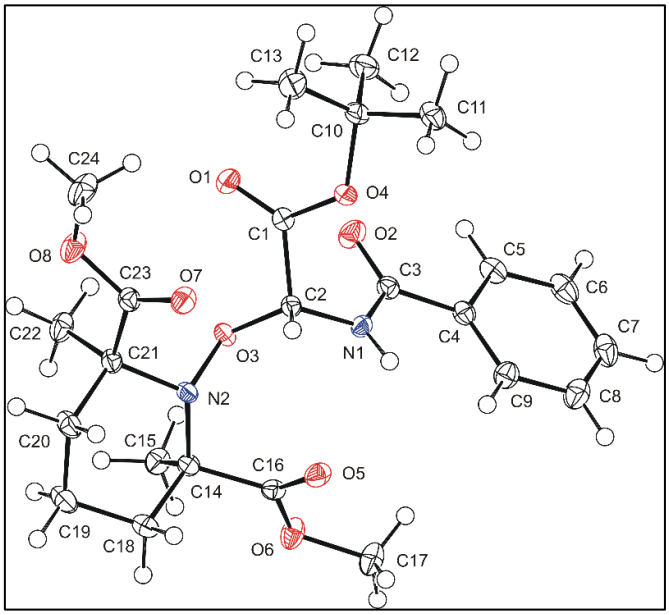
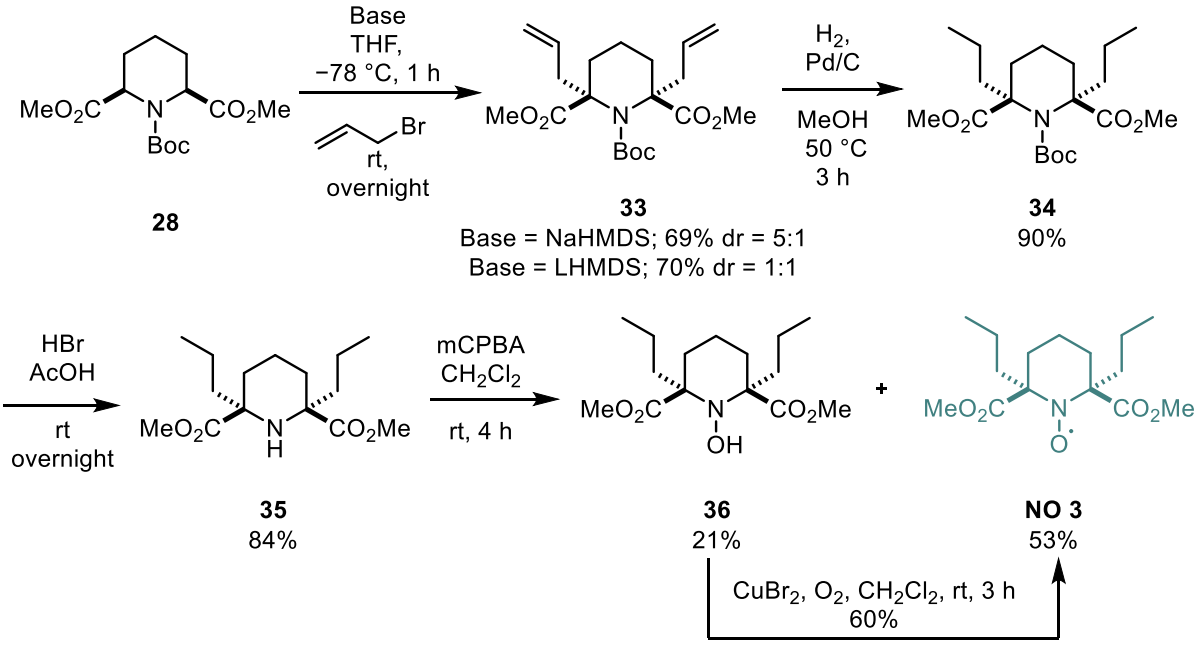


Figure 16: X-ray structure of glycine alkoxyamine **32**

4.5.3 *cis*-2,6-Bis(methoxycarbonyl)-2,6-dipropylpiperidine-1-oxyl (**NO3**)

We postulate that the substitution of methyl groups at 2- and 6- position in **NO2** with larger substituents would be intriguing, leading to the synthesis of a novel piperidine nitroxides.

The alkylation of **28** with various alkyl halides was attempted in order to access differently substituted piperidine esters. Ethyl iodide and isopropyl iodide were found to be low-yielding when used as alkylating reagents. It can be postulated that the 2- and 6- positions of the piperidine are hindered, resulting in lower reactivity to bulkier alkyl halides. On the other hand, allylation with allyl bromide was successful to obtain the allylated piperidine **33** as an inseparable 5:1 diastereomeric mixture, with a clear preference for the *cis* diastereomer when NaHMDS was used as the base (Scheme 55). The allylation reaction with LHMDS as the base resulted in the formation of the piperidine **33** as an inseparable 1:1 diastereomeric mixture. The diastereoselective reaction with NaHMDS was utilized and the 5:1 mixture of diastereomers was carried forward in the synthesis. Reduction of the alkene **33** was catalyzed by Pd/C under a hydrogen atmosphere (15 bar) and propyl derivative **34** was obtained in 90% yield. Boc deprotection of **34** with HBr/AcOH afforded the amine **35**. Oxidation with *m*CPBA gave the corresponding nitroxide **NO3** as well as N-hydroxylamine **36** in 53% and 21% yields, respectively. The N-hydroxylamine **36** was converted to nitroxide **NO3** under oxygen atmosphere catalyzed by $CuBr_2$. The nitroxide was recrystallized from pentane to obtain *cis* diastereomer.



Scheme 55: Synthesis of nitroxide **NO3**

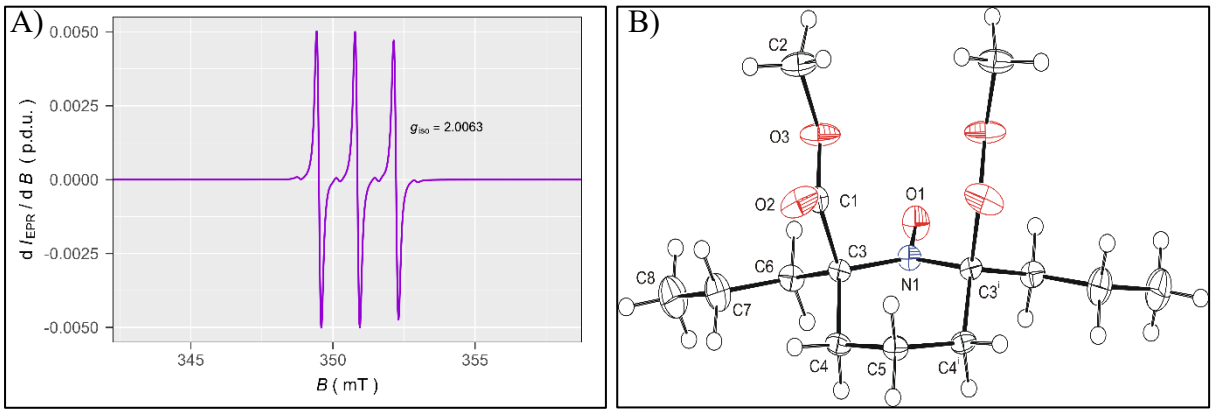
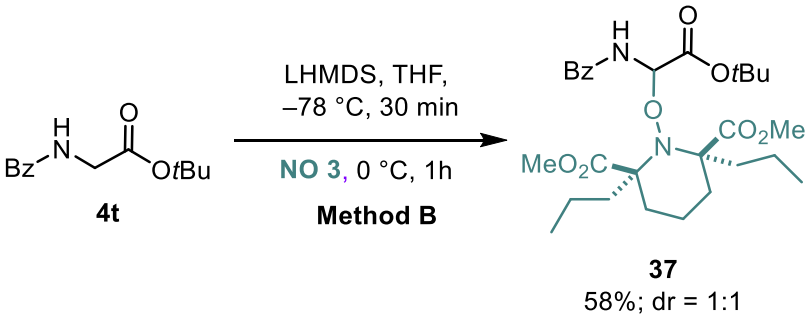


Figure 17: A) EPR spectrum of **NO3**, B) XRD structure of the nitroxide **NO3**

Nitroxide **NO3** is also a meso compound having two stereocenters at 2- and 6- position with a plane of symmetry. It differs only by the propyl substituents at 2- and 6- position compared to the methyl substituents in **NO2** (*cf.* Scheme 53). A freshly prepared solution of **NO3** in toluene (c = 3.3 mM) shows a typical triplet signal with a g factor of 2.0063 (Figure 17A). The recrystallized nitroxide **NO3** in pentane was subjected to X-ray crystallography and the X-ray structure was obtained (Figure 17B).

With the nitroxide **NO3** in hand we exploited our methodology of enolate oxidation (method B) in the glycinate **4t** to obtain the glycine alkoxyamine **37** in moderate yield as 1:1 diastereomeric mixture (Scheme 56). The glycine alkoxyamine was recrystallized from a 5% EtOAc/pentane and the structure was determined by X-ray crystallography (Figure 18). The alkoxyamine **37** is racemic and the X-ray structure does not provide the absolute configuration.



Scheme 56: Oxygenation of glycinate by the nitroxide **NO3**

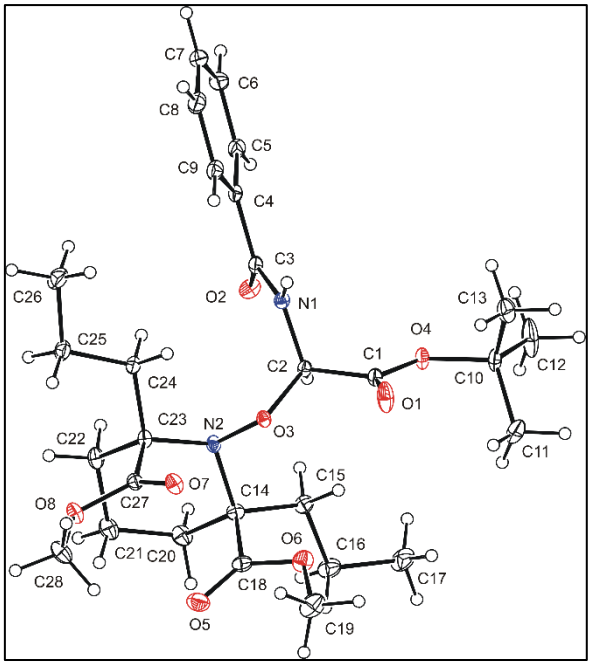


Figure 18: X-ray structure of glycine alkoxyamine **37**

4.5.4 Homolysing temperature Screening of Glycine Alkoxyamines

Glycine alkoxyamine **5t** derived from TEMPO found to undergo homolysis at 130 °C (*cf.* Table 1) and transient glycyl α-radical were reacted to olefins to obtain modified glycine derivatives (*vide supra* Scheme 38). The alkoxyamines **32** and **37** derived from the hindered nitroxides **NO2** and **NO3** might be potential candidates for the modification by homolysis at lower temperature compared to the alkoxyamine **5t**.[143] The stability of these alkoxyamines **32** and **37** were studied at a range of temperature and the homolysing temperature was determined by EPR spectroscopy.

The alkoxyamine **32** in toluene (c = 2 mM) was screened at temperatures in a range from 305 K to 370 K and the EPR spectra were analyzed. Homolysis started to appear from 325 K and a gradual increase of the concentration of the nitroxide was found up to 370 K (Figure 19). The alkoxyamine **37** in toluene (c = 2 mM) was also screened in the temperature range towards homolysis and EPR spectra were analyzed at temperatures in a range from 305 K to 370. The manifestation of nitroxide signals commenced at 325 K and a concentration vs temperaure plot distinctly indicates the initiation of homolysis at 325 K, with a gradual rise in nitroxide concentration observed until reaching 370 K (Figure 20) (see experimental section, 6.8 Homolysing Temperature Screening of The Glycine Alkoxyamines)

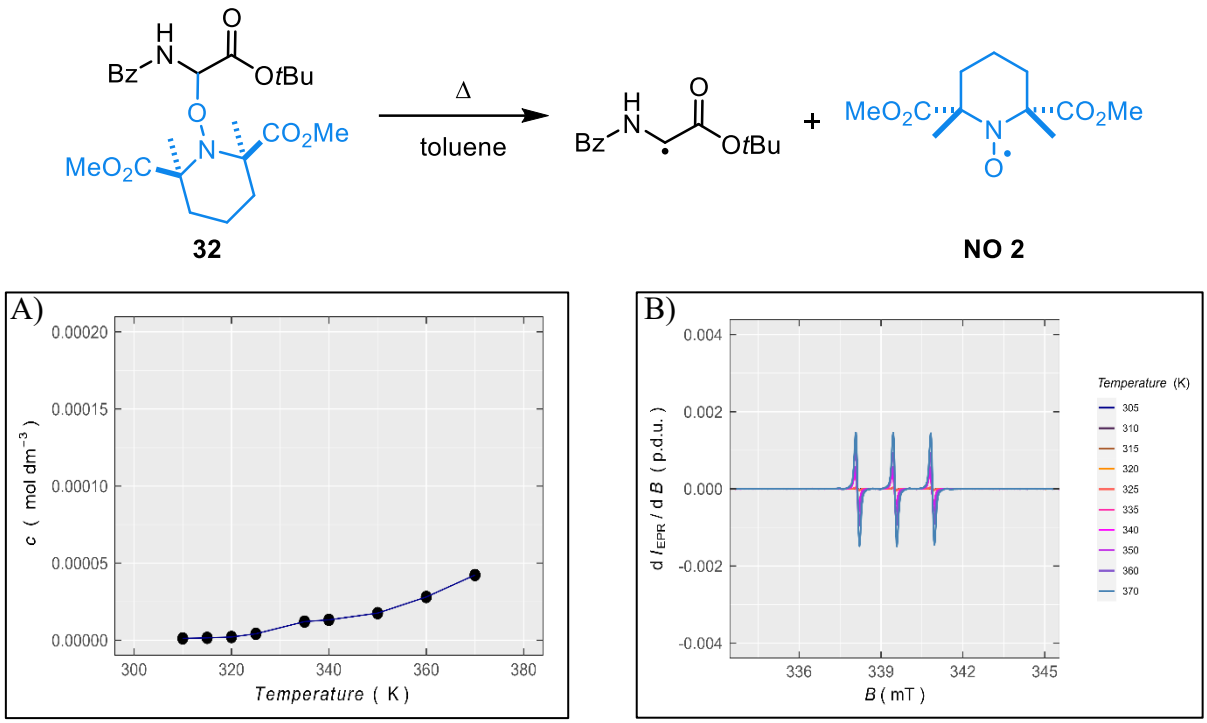


Figure 19: A) Homolyzing temperature screening of the glycine alkoxyamine **32** by EPR signal of **NO2**, B) Temperature overlay of EPR signals of the solution of the glycine alkoxyamine **32**.

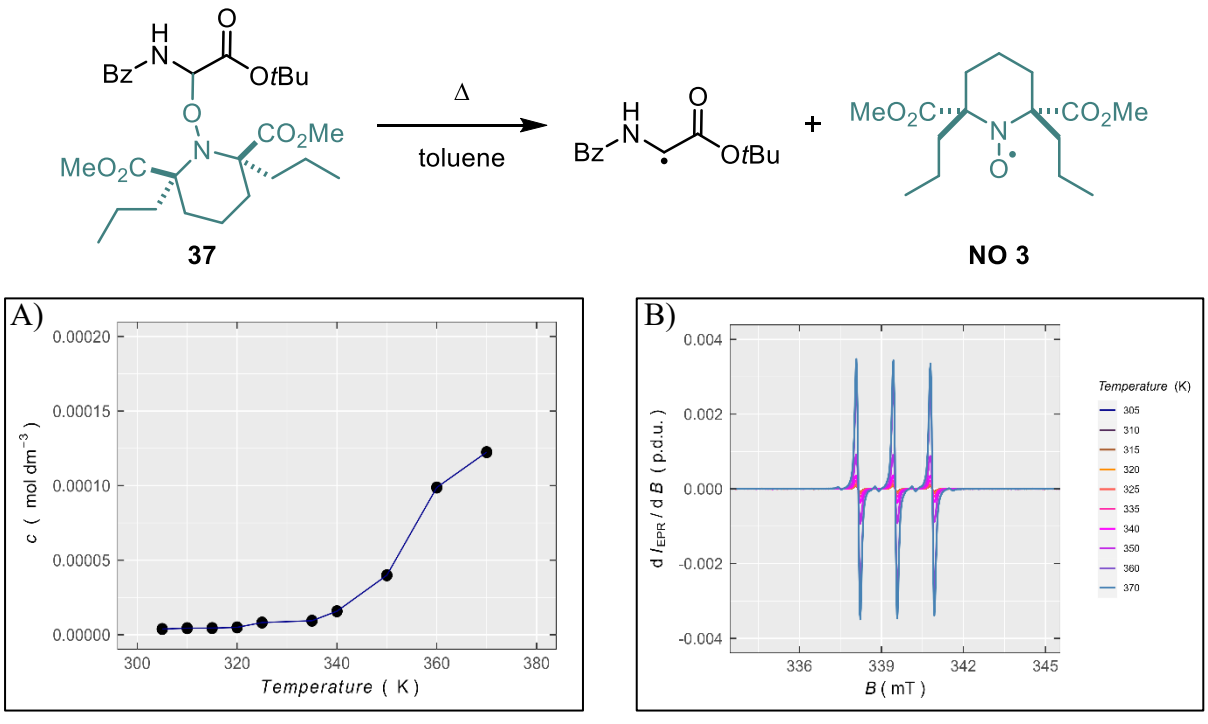


Figure 20: A) Homolyzing temperature screening of the glycine alkoxyamine **37** by EPR signal of **NO3**, B) Temperature overlay of EPR signals of the solution of the glycine alkoxyamine **37**.

The EPR spectroscopy experiments conclude that the glycine alkoxyamines **32** and **37** are not stable above the temperature 325 K (52 °C) and start homolyzing to the transient α-glycine

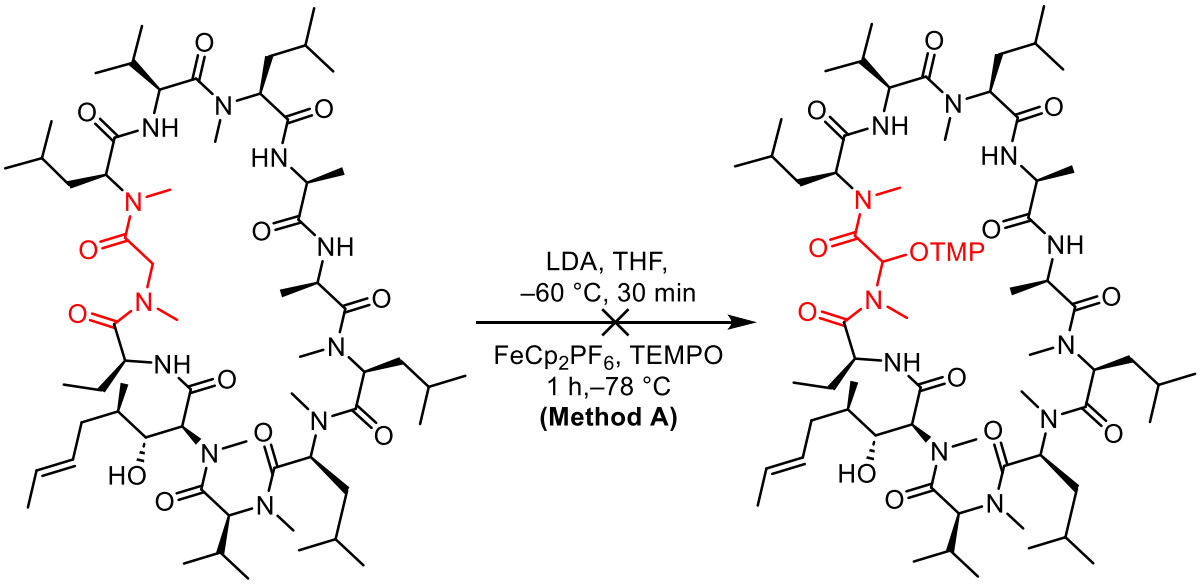
radical and corresponding nitroxide **NO2** and **NO3** respectively. Even though, both alkoxyamines **32** and **37** start dissociating to the corresponding nitroxides at 325 K, the concentration of the nitroxide formed at 370 K is higher for the alkoxyamine **37**. The data indicates that, in comparison to the glycine alkoxyamine generated from TEMPO (**5t**) (*vide supra*, Table 1), glycine alkoxyamines **32** and **37**, derived from hindered nitroxide radicals, may be susceptible to modification by homolysis at lower temperatures. Additionally, **37** was identified as a promising candidate for modification at 370 K between the alkoxyamines **32** and **37**. Future investigations will focus on exploring modifications and functionalization of these hindered glycine alkoxyamines by homolysis.

4.6. Applications

The methodology of selective modification of glycine derivatives by enolate oxidation, using nitroxide radicals, generates alkoxyamines and their further modifications at the glycine α-position enable to construct a library of non-natural amino acids and peptides. Moreover, the methodology demonstrates potential applications in peptide chemistry.

4.6.1 Attempted Modification of Cyclosporin A

Cyclosporin A is a cyclic undecapeptide and a potent immunosuppressive agent. We hypothesized that the polypeptide serves as a candidate to demonstrate the methodology of enolate oxidation to oxygenate the sarcosine unit. The peptide was treated with base, as precedented for alkylation[31] and the enolate was treated with ferrocenium hexafluorophosphate and TEMPO (method A). However, we were unsuccessful in obtaining the oxygenated peptide (Scheme 57). To confirm the formation of enolate, we added D_2O to the base treated cyclosporin A. The residue was analyzed by ^1H NMR spectroscopy and deuterated sarcosine unit was observed, implying successful enolization. It is presumed that the enolate oxidation becomes difficult or if it occurs, the oxidized α-radical exchanges H atom from a position in close proximity. Thus, further investigations were not conducted.

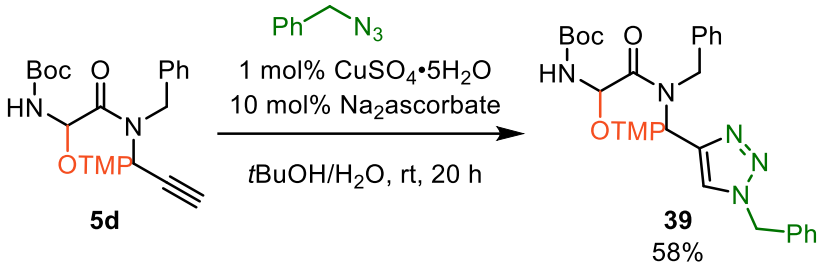


III 38

Scheme 57: Attempt to oxygenate the sarcosine unit in cyclosporin A.

4.6.2 Employing Glycine Alkoxyamines for Click Reactions

We demonstrate that the glycine alkoxyamine **5d**, possessing an alkyne group, can undergo copper catalyzed click reaction. Typical reaction conditions were used with benzyl azide as click partner and triazole **39** was formed in moderate yield (Scheme 58). The hemiaminal was found to be stable under the reaction conditions and was preserved. In future, the applicability of this reaction can be exploited with biological substrates for conjugation to glycine alkoxyamines and employ the new amino acid-derived triazole compounds for modification, exploiting the reactivity of α-C-O bond of glycine alkoxyamines.

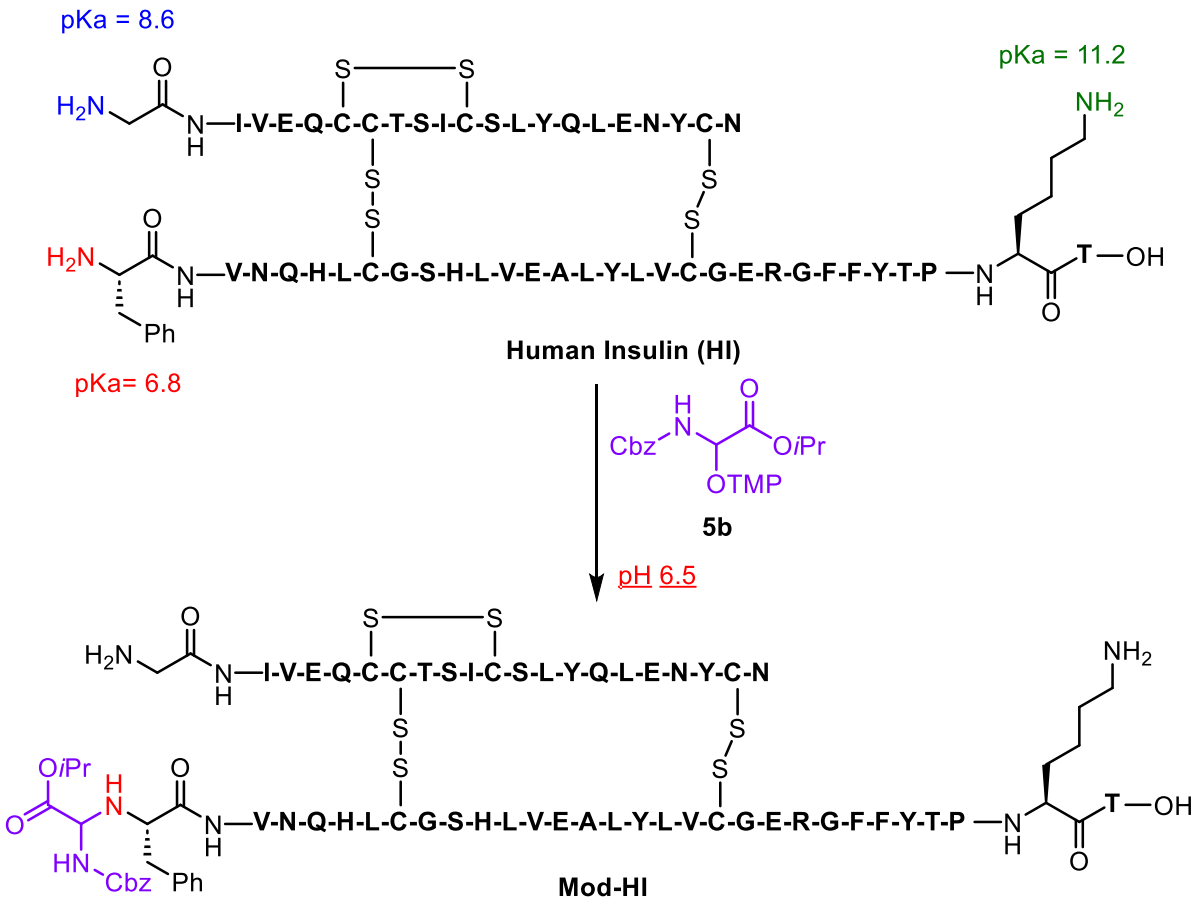


Scheme 58: Modification of glycine alkoxyamines by click reaction.

4.6.3 Protein Conjugation

Insulin is a small protein containing two peptide chains A and B, linked by disulfide bridges. The A chain consists of 21 AAs and the B chain consists of 30 AAs. The main modification sites for the protein are the two N-terminal positions and the lysine sidechain. Insulin presents as a potential candidate for protein conjugation to glycine unit using our strategy of glycine alkoxyamine modification. A collaboration with the group of Dr. Jiri Jiracek helped us to study the *in vitro* protein conjugation to glycine alkoxyamines, mediated by acid.

The glycine alkoxyamine **5a** was chosen as an initial substrate and the conjugation was studied at different conditions. The substrate was found to react with insulin at room temperature in 3 h in a phosphate buffer solution of pH 6.5 obtaining the conjugated product (Scheme 59). The analysis of the reaction was conducted by liquid chromatography-mass spectrometry (LCMS) and showed the conjugated product as the major compound (Figure 21). Peak one corresponds to unreacted insulin and peak 2 and peak 3 corresponds to diastereomers of the conjugated product **Mod-HI**. However, isolation of the product was unsuccessful, and decomposition of the conjugated product was observed with standard HPLC isolation conditions (80% CH_3CN/H_2O with 0.1 M TFA).



Scheme 59: Insulin conjugation to glycine alkoxyamine.

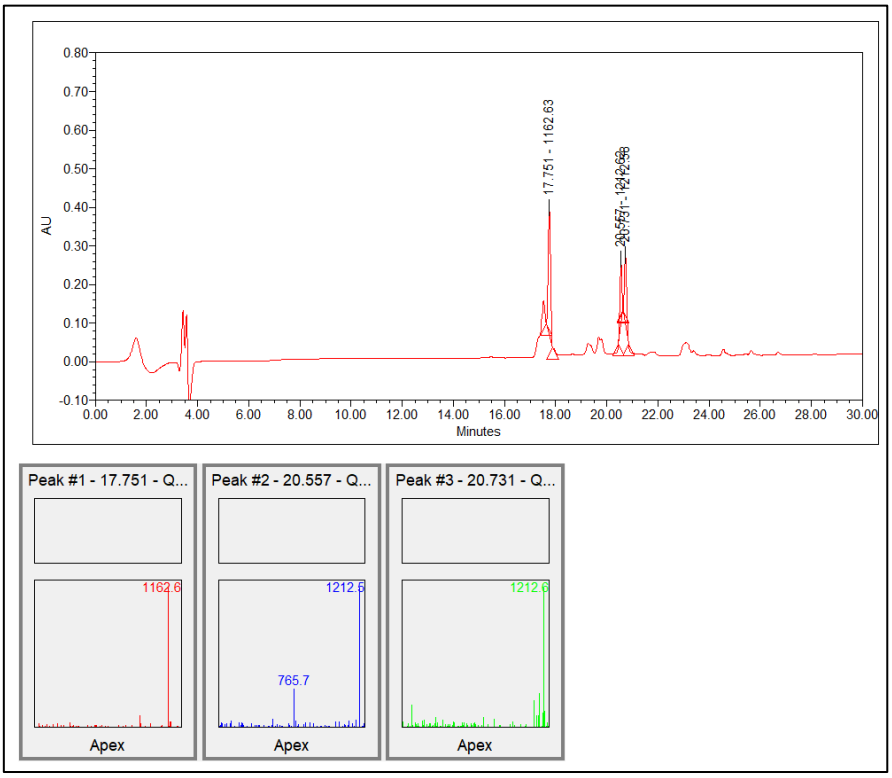


Figure 21: Analysis of conjugated molecule by LCMS

Further stability testing on different solvent systems revealed that the conjugated product **Mod-HI** are unstable under acidic conditions and relatively stable under neutral and basic conditions (Figure 22). Further analysis and attempts to isolate the conjugated product are underway in the Jiracek laboratory.

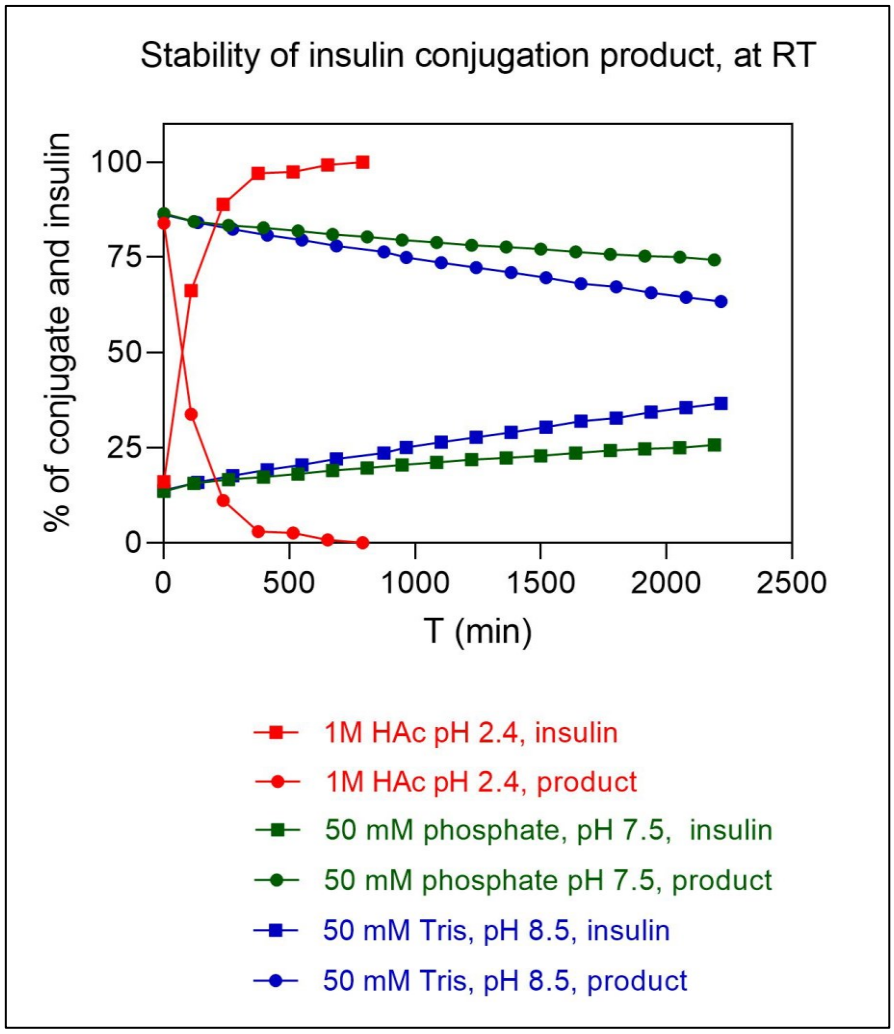
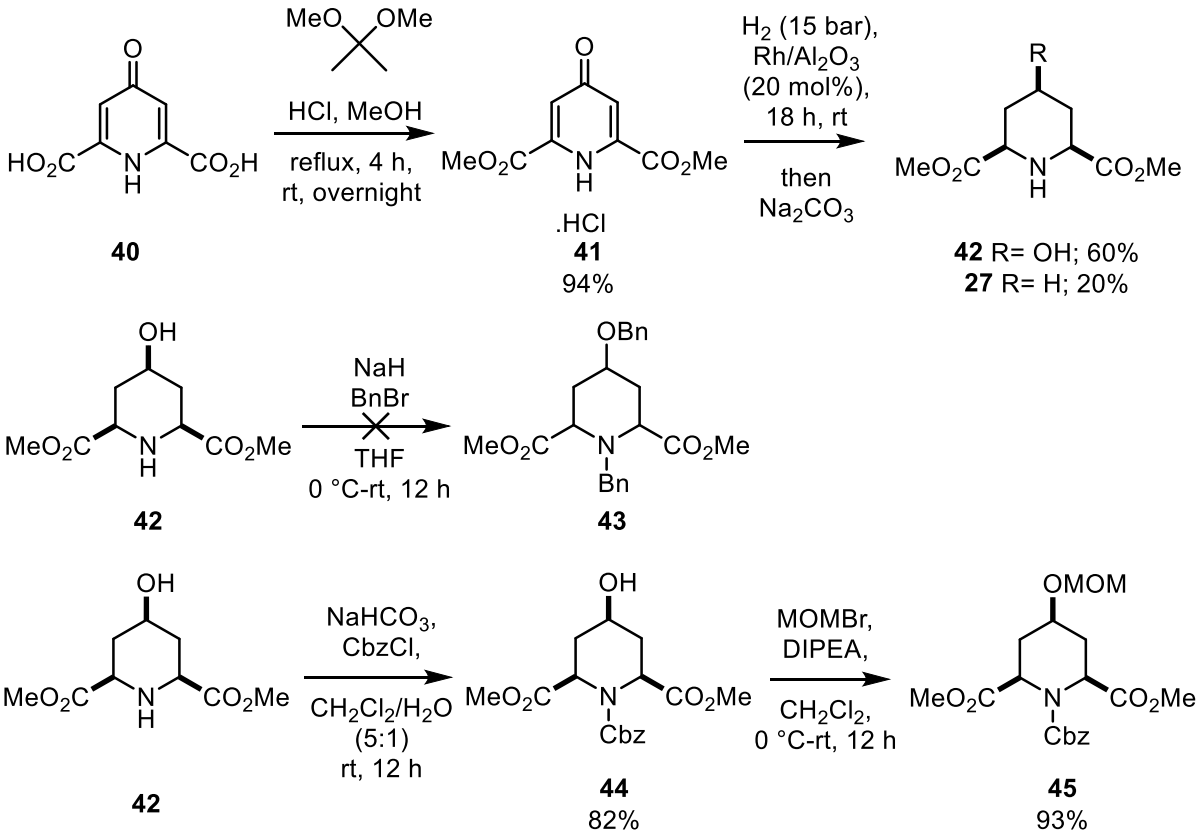


Figure 22: Stability of insulin conjugation product **Mod-HI**

4.6.4 Functionalized Nitroxide for The Application of Conjugation

The nitroxide **NO2** with methyl ester group at 2- and 6- position as an analogue of TEMPO was found to be interesting and a coupling group at 4th position would enable the use of these nitroxides for the conjugation to biomolecules or macromolecules of interest and an introduction of an azide group at 4-position to the nitroxide would allow conjugation by click reactions. In collaboration with Dr. Petr Cigler at IOCB, we designed the synthesis of a piperidine based nitroxide, featuring azide group at 4-position and envisioned that the nitroxide-conjugated nanomaterials will be used for its application such as spin labels in material chemistry.

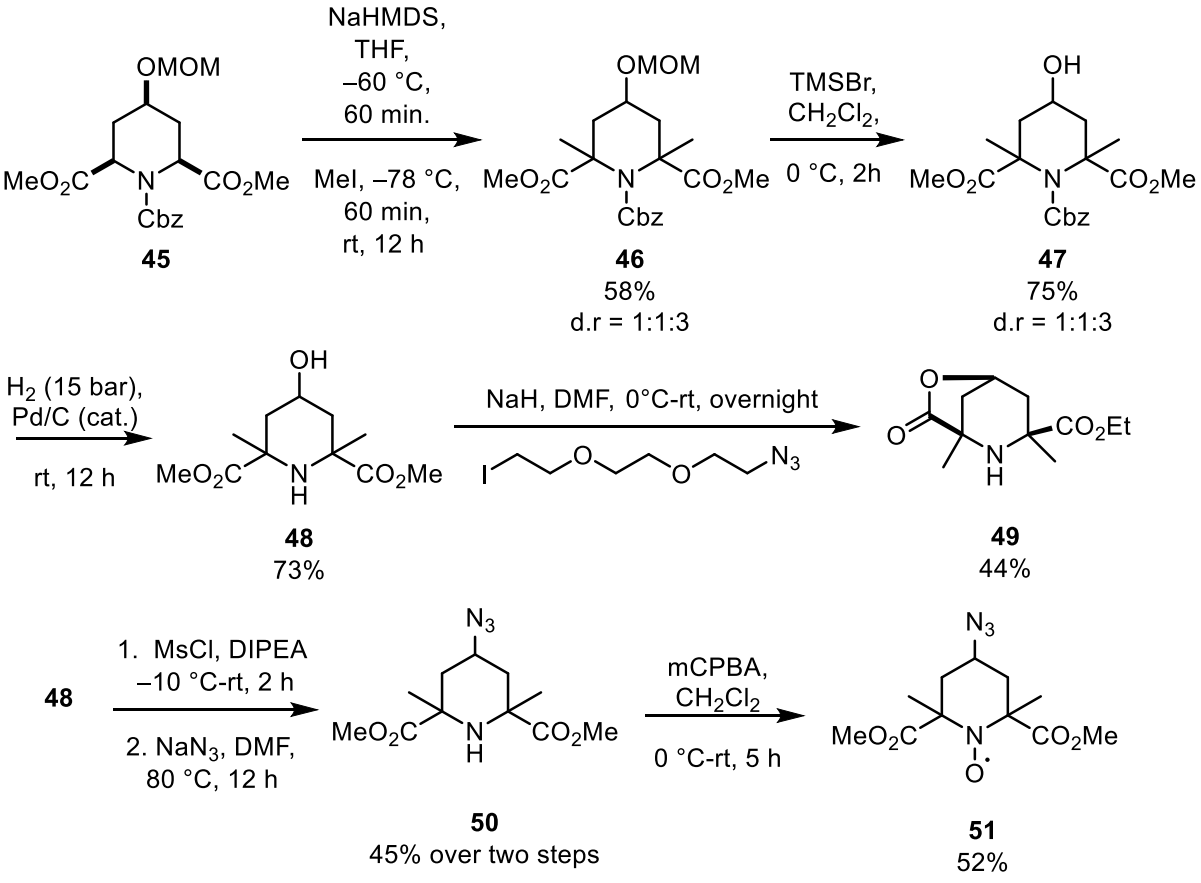


Scheme 60: Synthesis of protected 4-hydroxy piperidine **45**.

The synthesis started with commercially available chelidamic acid **40** (Scheme 60). Esterification provided methyl ester salt **41** and the salt was used for the next step without purification. Reduction of **41** catalyzed by rhodium on alumina resulted in the formation of two compounds. Alcohol **42** was obtained as a major product but a complete reduction, including the hydroxy group provided saturated piperidine **27** as a minor product. The major and minor products were separated by column chromatography and selectively obtained as *cis*

compounds.[144] The piperidine **27** was employed in the synthesis of **NO2** and **NO3** (*cf.* Scheme 53, 55).

The synthesis was carried forward with the major product 4-hydroxy piperidine **42**. Before the alkylation at the 2- and 6- position of the piperidine we envisage protecting both amine and hydroxy groups with benzyl protection group (Bn) to obtain protected piperidine **43**. The piperidine **42** was treated with NaH followed by BnBr, however protection of amino group and hydroxy groups with Bn was found to be unsuccessful and the starting material was recovered. Thus, we followed separate protections of amine and alcohol with different protecting groups. A common amine protecting group Cbz was chosen and the reaction of **42** with CbzCl provided protected 4-hydroxy piperidine **44**. A subsequent MOM protection of the alcohol **44** with MOMBr as well as MOMCl were attempted. Protection with MOMBr provided better results and the protected piperidine **45** was obtained in 70% yield.



Scheme 61: Synthesis of functionalized nitroxide

Double methylation of compound **45** at 2- and 6- position was performed using 2.5 equivalents of NaHMDS as a base for deprotonation, followed by alkylation with methyl iodide (Scheme 61). The double methylated piperidine **46** was obtained in good yield as inseparable mixture of

diastereomers in 1:1:3 diastereomeric ratio. The diastereomeric mixture was carried forward and MOM deprotection using TMSBr in CH_2Cl_2 provided the alcohol **47** in 2 h and subsequent Cbz deprotection under hydrogenolytic conditions resulted in the formation of dimethyl-2,6 dimethyl-4-hydroxy-piperidine-2,6-dicarboxylate **48**. An attempted deprotonation of the alcohol with NaH towards the etherification with PEG azide resulted in the formation of the lactone **49.** The observation can be explained mechanistically as intramolecular lactonization of the deprotonated alcohol with one of the ester groups. The lactone **49** was recrystallized from 10% EtOAc/pentane and the structure was determined by X-ray crystallography (Figure 23). The X-Ray structure with absolute structure parameter 0.2 implies that the lactone has *cis* configuration of the substituents. Moreover, a transesterification of the remaining methyl ester took place to provide the corresponding ethyl ester. The deprotonation of the free alcohol became fatal here and no etherification was possible. Thus, we decided to eliminate the linker group and the 4th position of the nitroxide was functionalized with azide group in two steps. Mesylating the alcohol **48** with MsCl followed by substitution with sodium azide at 80 ºC provided the azide functionalized piperidine **50** in two steps. The oxidation with *m*CPBA resulted in the formation of nitroxide **51** in good yield (Scheme 61). The piperidine nitroxide **51** consists of ester functionality at positions 2- and 6- and the azide group at the 4-position, that can act as a handle for useful conjugation. Furthermore, saponification of this nitroxide may result in a water-soluble compound functionalized with carboxylic acid groups, which could lead to new applications for the compound. The synthesized nitroxide was handed over to Dr. Petr Cigler for the further exploration of its applications.

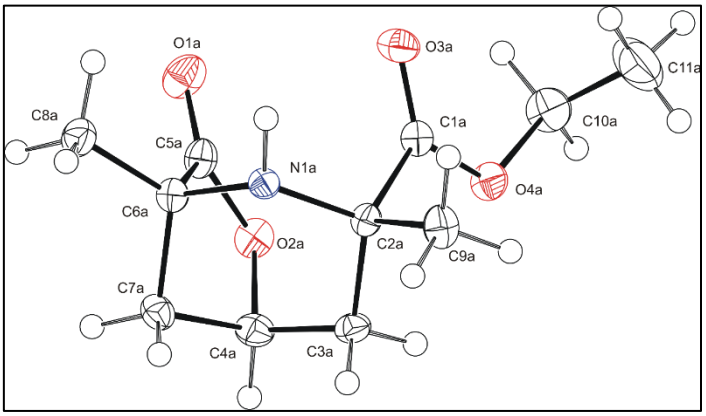


Figure 23: X-Ray structure of lactone **49**

5. CONCLUSION AND OUTLOOK

We have developed a novel methodology for enolate oxidation of glycine derivatives, using a mild oxidant, a nitroxide radical, to provide α-oxygenated glycinates. We differentiated between the reaction conditions for both the sarcosine derivatives and glycine derivatives and found that an external oxidant is necessary to oxidize the sarcosine enolate, as with any other class of carbonyl compounds. Interestingly, the glycine enolates are oxidized directly by the mild oxidant TEMPO, and coupling of the α-glycine radicals with a second equivalent of TEMPO provides the glycine alkoxyamines. The substrate scope of the methodology was studied to synthesize differently protected glycine alkoxyamines. The methodology was then extended to glycine containing peptides, and the selectivity of the reaction to different amino acids was studied. The methodology was found to be selective for glycine units, providing the alkoxyamines and no other amino acid oxidation was observed during the investigation. However, only C-terminal glycine peptides act as suitable substrates for the methodology.

The glycine alkoxyamines, derived from TEMPO, are chemically under the class of hemiaminals and act as versatile compounds for further modifications at the glycine α-position. The labile C-O bond undergoes homolysis at a temperature of 130 °C and adds to electron-deficient alkenes to obtain non-natural amino acids and peptides. The C-O bond of the hemiaminal can also undergo heterolysis mediated by acid. The strategy allows constructing C-C, C-O, C-N and C-S bonds at the α-position to provide glycine modifications. The conjugation of amino acids to glycine alkoxyamines was also demonstrated to construct a class of amino acid-derived aminal compounds.

Furthermore, we synthesized two already reported piperidine nitroxide compounds and a novel nitroxide, functionalized with esters and propyl substituents at the 2- and 6- positions. These three nitroxides were used as the oxidant for the developed methodology of glycine enolate oxidation, instead of using TEMPO, and new derivatives of glycine alkoxyamines were obtained. Studies were conducted using EPR spectroscopy to investigate the temperature dependent homolysis of novel glycine alkoxyamines. Further studies and applications of these promising alkoxyamines can be explored in the future.

The synthesized glycine alkoxyamines were used as a key to establish amino acid-protein conjugation. Insulin was modified by coupling it with glycine alkoxyamine, resulting in the addition of the glycinate unit to the β chain and producing glycine-conjugated human insulin protein. However, the modified insulin was found to be unstable for purification under acidic conditions.

Inspired by the nitroxides we synthesized for glycine oxygenation, which have ester groups at the 2- and 6- positions of the piperidine ring, we developed a new piperidine nitroxide. This nitroxide has a functional group at the 4th position that can conjugate with molecules of interest. Compared to previously reported nitroxides, the new structural features of this nitroxide potentially allow it to be used as a water-soluble spin label in hydrophilic conditions. The newly synthesized nitroxide will be used and applied in the laboratory of Dr. Petr Cigler for the studies of nanodiamonds.

In future, the group will explore the peptide conjugation strategy involving glycine alkoxyamines for the purpose of achieving previously unattainable conjugations with complex biomolecules. Additionally, sterically hindered nitroxides and their alkoxyamine derivatives will be employed to elucidate modifications through homolysis at temperatures conducive to biological processes.

6. EXPERIMENTAL SECTIONS

6.1 General Experimental Information

Reactions not involving aqueous conditions were performed in flame-dried glassware under an argon atmosphere. Microwave-assisted reactions were performed using a CEM Discover® SP instrument. Solvents and additives were dried prior to use according to standard procedures. TLC analyses were performed on POLYGRAM SIL G/UV254 plates and visualization was performed either by UV light (254 or 366 nm) or chemical staining with potassium permanganate, vanillin or ninhydrin. Chromatographic separations were carried out either manually on silica gel 60 (Fluka, 230-400 mesh) or on a CombiFlash® NextGen 300+ instrument using prefilled columns RediSep Gold®. IR spectra were measured on a Bruker ALPHA-FT-IR spectrometer as neat samples using an ATR device.

NMR spectra were recorded on Bruker Advance 400 spectrometers at working frequencies of 400 MHz for ^{1}H NMR spectra and 100.1 MHz for ^{13}C NMR spectra. The signals of inseparable mixtures of diastereoisomers are assigned in a single data set and those of the minor diastereoisomers are indicated by an * and **. In rotameric mixtures, signals of the major rotamer are assigned by 'A' and those of the minor by 'B'.

EPR spectra were acquired on Bruker EMX^{plus} 10/12 CW (continuous wave) spectrometer equipped with a Premium-X-band microwave bridge. The g_{iso} value of radicals was determined using a built-in spectrometer frequency counter and the ER-036TM NMR-Teslameter (Bruker). All *g*-values were determined with the precision of ± 0.0002. VT (variable temperature) experiments were controlled by the liquid/gas nitrogen unit ER 4141VT-U (Bruker). Once the desired temperature was set, equilibration time of usually ≈ 1 min was used prior to recording of an EPR spectrum at that temperature. Samples (corresponding toluene solutions of the investigated compounds) were measured in common EPR quartz tubes (with an i.d. = 3 mm and a length of 250 mm). Typical EPR spectra were recorded using the following instrumental parameters: microwave frequency ≈ 9.53 GHz, modulation amplitude = 0.05 mT, modulation frequency = 100 kHz, microwave power = 2.5 mW, resolution (number of points) = 2222, conversion time = 8.96 ms. Usually, each spectrum was recorded as an accumulation of several sweeps ≈ 10. The sensitivity *Q*-factor was ≥ 3000 for all experiments.

The electronic circular dichroism (ECD) and absorption spectra were measured on a Jasco-1500 instrument (Tokyo, Japan). The spectra were measured over a spectral range of 180 nm to 300 nm in acetonitrile of a concentration $1x10^{-3}$ molL^{-1}. Measurements were performed in a cylindrical quartz cell with a 0.2 cm path length using a scanning speed of 10 nm/min, a

response time of 8 seconds, standard instrument sensitivity and with 0.3 accumulations. After a baseline correction, spectra were expressed in terms of differential molar extinction ($\Delta\varepsilon$) and molar extinction (ε), respectively.

Mass spectra were measured on a Q-TOF micro mass spectrometer (Waters, source temperature 120 °C, desolvation temperature 200 °C, sample cone voltage 20 V) at the mass spectrometry facility of IOCB CAS. Optical rotations were performed on AUTOPOL VI (*Rudolph Research Analytical*, USA) at the analytical laboratory of IOCB CAS.

6.2 General Procedures for Modification of Glycine Derivatives

General procedure A: Peptide coupling[145]

Amino acid-1 (5.0 mmol) and amino acid-2 (5.0 mmol) were dissolved in CH_3CN (30 mL) and cooled to 0 °C. HBTU (1.97 g, 5.2 mmol) and triethylamine (1.5 mL, 10.8 mmol) were added, the mixture was warmed to rt and stirred for 2 h. Brine (20 mL) was added and the reaction mixture was extracted with EtOAc (3x40 mL). The combined organic extracts were washed with HCl solution (1 N, 5 mL), followed by sat. $NaHCO_3$ solution (10 mL), the organic layer was dried over $MgSO_4$, the solvent was removed at reduced pressure and the residue was purified by flash column chromatography (20% EtOAc/cyclohexane) to give the coupling products (**1, 4h-s, 10, 19**).

General procedure B: α-Oxygenation of sarcosine derivatives (Method A)

KHMDS solution (1.4 mL, 1.35 mmol, 1M in THF) was added dropwise to a solution of the sarcosine derivative **1** (1.23 mmol) in THF (3 mL) at −78 °C and the mixture was stirred for 30 min. TEMPO (173 mg, 1.11 mmol) was added followed by portion-wise addition of $Cp_2Fe^+PF_6^-$ until a blue color persisted (ca 340 mg, 1.05 mmol). After 30 min, the reaction mixture was quenched by a few drops of sat. NH_4Cl solution, filtered through a short pad of Celite®, which was washed with EtOAc. The solvents were removed at reduced pressure and the crude product was purified by column chromatography (10% EtOAc/hexane) to give oxygenated products (**3**).

General procedure C: α-Oxygenation of glycine derivatives (Method B)

A freshly prepared LiHMDS solution (HMDS (0.23 mL, 1.1 mmol) and *n*-BuLi (0.69 mL, 1.1 mmol, 1.6 M in hexane) in THF (1 mL) at −78 °C) was added dropwise to a solution of glycine derivative **4** (0.44 mmol) in THF (3 mL) at −78 °C and the mixture was stirred for 30 min. TEMPO (151 mg, 0.98 mmol) was added, the solution was warmed to 0 °C and stirred for 1 h. The reaction was quenched by the addition of a few drops of sat. NH_4Cl solution and the mixture was filtered through a short pad of Celite®, which was washed with EtOAc. The solvent

was removed at reduced pressure and the crude product was purified by column chromatography (10% EtOAc/cyclohexane) to obtain the α-oxygenated glycinates (**5**).

General procedure D: Microwave-assisted intermolecular radical addition of alkoxyamines

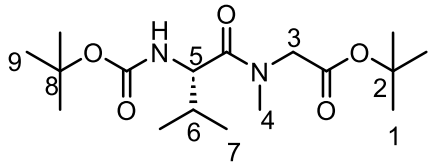
Acrylate derivatives or α-methyl styrene (2.56 mmol) were added to a solution of alkoxyamine **5q** or **5f** (0.26 mmol) in degassed α,α,α-trifluorotoluene (2 mL) and the mixture was heated in a microwave reactor at 130 °C for 1 h. After completion, the solvent was removed in vacuum and the residue was purified by column chromatography (5% EtOAc/cyclohexane gradient to 10% EtOAc/cyclohexane) to obtain the addition products (**11a-f**).

General procedure E: Nucleophilic substitution at the α-oxygenated glycine derivative

Nucleophile (**N1**-**N6**) (0.60 mmol) was added to a solution of glycine alkoxyamine **5** (0.50 mmol) and acid (0.50 mmol or 0.10 mmol for catalysis) in preferred solvent and was stirred at 37 °C until the reaction completes. After completion, the solvent was removed in vacuum and the product was purified by flash column chromatography to give the α-substitution glycinates (**15c**, **16a-d, 17a-b, 18**).

6.3 Synthetic Procedures and Spectroscopic Characterization

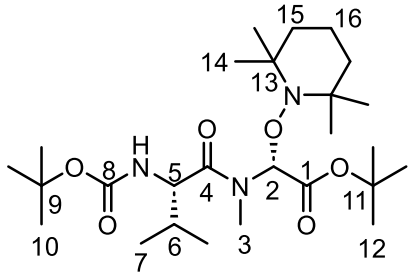
tert-Butyl *N*-(*N*-(*tert*-butyloxycarbonyl)-L-valyl)sarcosinate (1)



Prepared according to general procedure **A** by coupling *N*-(*tert*-butyloxycarbonyl)-L-valine and *tert*-butyl sarcosinate•HCl, providing **1** (633 mg, 85%) as 4.5:1 rotameric mixture as colorless oil.

R_f = 0.6 (50% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 5.28 (d, *J* = 9.0 Hz, 2H, NH, A+B), 4.52 (dd, *J* = 9.2, 5.6 Hz, 1H, CH-5, A), 4.39 (d, *J* = 17.0 Hz, 1H, CH_2-3, A), 4.30 (dd, *J* = 9.2, 6.1 Hz, 1H, CH-5, B), 4.22 (d, *J* = 18.2 Hz, 1H, CH_2-3, B), 3.97 (d, *J* = 18.1 Hz, 1H, CH_2-3, B), 3.65 (d, *J* = 17.1 Hz, 1H, CH_2-3, A), 3.13 (s, 3H, CH_3-4, A), 2.97 (s, 3H, CH_3-4, B), 2.05-1.97 (m, 1H, CH-6, A), 1.96-1.88 (m, 1H, CH-6, B), 1.47 (s, 9H, CH_3-1, B), 1.45 (s, 9H, CH_3-1, A), 1.43 (s, 9H, CH_3-9, A), 1.42 (s, 9H, CH_3-9, B), 1.01 (d, *J* = 6.8 Hz, 3H, CH_3-7, A), 0.94 (d, *J* = 6.9 Hz, 3H, CH_3-7, B), 0.91 (d, *J* = 6.8 Hz, 3H, CH_3-7, A), 0.88 (d, *J* = 6.5 Hz, 3H, CH_3-7, B). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 172.7 (C=O, B), 172.6 (C=O, A), 168.0 (C=O, A), 167.7 (C=O, B), 155.8 (C=O, A), 155.7 (C=O, B), 82.7 (C2, B), 81.9 (C2, A), 80.1 (C8, B), 79.4 (C8, A), 54.9 (C5, B), 54.8 (C5, A), 52.3 (C3, B), 50.4 (C3, A), 36.5 (C4, A), 34.9 (C4, B), 31.5 (C6, B), 31.3 (C6, A), 28.3 (C1, A+B), 28.1 (C9, A), 28.0 (C9, B), 19.60 (C7, B), 19.56 (C7, A), 17.3 (C7, B), 17.1 (C7, A). **IR**: v [cm^{-1}] 3320, 2976, 2934, 1740, 1709, 1646, 1487, 1366, 1228, 1154, 1118, 1042, 1016, 946, 886, 841, 749. **LRMS** (ESI+) *m/z*, (%): 345 (5, $[M+H]^+$), 367 (100, $[M+Na]^+$), 711 (10, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{17}H_{32}N_2O_5Na$: 367.2203, found: 367.2203.

***tert*-Butyl *N*-(*S*)-(*N*-(*tert*-butyloxycarbonyl)valyl)-(2*R* and 2*S*)-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)sarcosinate (3)**



Prepared according to general procedure **B**, starting from *tert*-butyl *N*-(*N*-(*tert*-butyloxycarbonyl)-L-valyl)sarcosinate (**1**) (100 mg, 0.29 mmol) and KHMDS (0.7 mL, 0.65 mmol, 1 M in THF) as base. The crude product was purified by column chromatography (10% EtOAc/hexane) to give **3** (110 mg, 76%) as partially separable 2:1 diastereomeric mixture as pale yellow oil.

Major diastereomer (S^{Val},R^{Gly}) as 4.3:1 rotameric mixture

R$_f$ = 0.7 (20% EtOAc/PE). $[\alpha]^{20}_{589}$ = −71.1 (c 0.35, $CHCl_3$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.49 (s, 1H, CH-2, A), 5.72 (s, 1H, CH-2, B), 5.42 (d, *J* = 9.0 Hz, 1H, NH, B), 5.20 (d, *J* = 9.6 Hz, 1H, NH, A), 4.65 (dd, *J* = 9.1, 4.1 Hz, 1H, CH-5, B), 4.43 (dd, *J* = 9.7, 6.9 Hz, 1H, CH-5, A), 3.05 (s, 3H, CH_3-3, A), 2.81 (s, 3H, CH_3-3, B), 2.66-2.54 (m, 1H, CH-6, B), 2.01-1.93 (m, 1H, CH-6, A), 1.60-1.31 (m, 12H, CH_2-15, CH_2-16, A+B), 1.48 (s, 18H, CH_3-12, A+B), 1.44 (s, 18H, CH_3-10, A+B), 1.28 (s, 3H, CH_3-14, A), 1.23 (s, 3H, CH_3-14, A), 1.19 (s, 3H, CH_3-14, B), 1.18 (s, 3H, CH_3-14, A), 1.16 (s, 3H, CH_3-14, B), 1.10 (s, 3H, CH_3-14, A), 1.04 (d, *J* = 6.8 Hz, 3H, CH_3-7, B), 1.00 (d, *J* = 6.6 Hz, 3H, CH_3-7, A), 0.96 (d, *J* = 6.8 Hz, 3H, CH_3-7, A), 0.94 (s, 6H, CH_3-14, B), 0.87 (d, *J* = 6.8 Hz, 3H, CH_3-7, B). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 172.6 (C=O, A+B), 167.0 (C=O, A+B), 155.7 (C=O, A+B), 89.1 (C2, A+B), 82.3 (C11, A+B), 79.4 (C9, A+B), 60.9 (C13, A+B), 59.2 (C13, A+B), 55.7 (C5, B), 55.0 (C5, A), 40.5 (C15, B), 40.0 (C15, A), 32.8 (C14, A+B), 32.7 (C14, A+B), 32.4 (C6, A+B), 31.4 (C3, A), 30.7 (C3, B), 28.3 (C12, A+B), 28.0 (C10, A), 27.8 (C10, B), 20.4 (C14, A+B), 20.0 (C14, A+B), 19.3 (C7, A+B), 17.7 (C7, A+B), 17.1 (C16, A), 17.0 (C16, B).

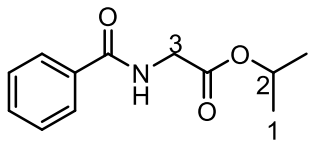
Minor diastereomer (S^{Val}, S^{Gly}) as 3.3:1 rotameric mixture

R$_f$ = 0.75 (20% EtOAc/PE). $[\alpha]^{20}_{589}$ = +82.7 (c 0.35, $CHCl_3$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 5.77 (s, 1H, CH-2, A), 5.65 (s, 1H, CH-2, B), 5.50 (d, *J* = 8.6 Hz, 1H, NH, A), 5.20 (d, *J* = 9.6 Hz, 1H, NH, B), 5.06 (dd, *J* = 8.7, 3.5 Hz, 1H, CH-5, A), 4.42 (dd, *J* = 9.6, 5.0 Hz, 1H, CH-5, B), 3.03 (s, 3H, CH_3-3, B), 2.86 (s, 3H, CH_3-3, A), 2.20-2.12 (m, 1H, CH-6, A), 2.04-1.99 (m, 1H, CH-6, B), 1.68-1.27 (m, 12H, CH_2-15, CH_2-16, A+B), 1.51 (s, 9H, CH_3-12, A),

1.47 (s, 9H, CH_3-12, B), 1.46 (s, 9H, CH_3-10, A), 1.44 (s, 9H, CH_3-10, B), 1.25 (s, 3H, CH_3-14, A), 1.22 (s, 6H, CH_3-14, A+B), 1.18 (s, 3H, CH_3-14, B), 1.12 (s, 3H, CH_3-14, B), 1.10 (s, 3H, CH_3-14, A), 1.04-1.00 (m, 9H, CH_3-7, A+B, CH_3-14, B), 0.89 (d, J = 6.7 Hz, 3H, CH_3-7, B), 0.86-0.82 (m, 6H, CH_3-7, CH_3-14, A). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 173.0 (C=O, A), 172.1 (C=O, B), 165.8 (C=O, A+B), 155.6 (C=O, A+B), 89.3 (C2, A+B), 83.0 (C11, A+B), 79.4 (C9, B), 79.0 (C9, A), 61.5 (C13, A+B), 59.5 (C13, A+B), 55.4 (C5, A+B), 39.7 (C15, A+B), 39.6 (C15, A+B), 32.7 (C6, A+B), 32.6 (C14, A+B), 31.5 (C3, A+B), 28.5 (C12, B), 28.4 (C12, A), 28.3 (C10, B), 27.9 (C10, A), 20.4 (C14, A+B), 20.04 (C7, A+B), 20.01 (C14, A+B), 17.0 (C16, B), 16.9 (C16, A), 16.2 (C7, A+B).

IR: v [cm^{-1}] 2974, 2932, 1751, 1718, 1658, 1495, 1366, 1155, 1133, 1082, 1056, 1044, 1017, 992, 958, 842, 760, 707. **LRMS** (ESI+) *m/z*, (%): 500 (100, $[M+H]^+$), 522 (95, $[M+Na]^+$), 1021 (5, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{26}H_{49}N_3O_6Na$: 522.3514, found: 522.3512.

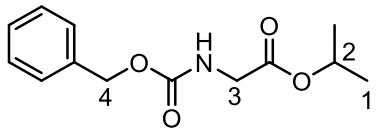
Isopropyl *N*-benzoylglycinate (4a)



Benzoyl chloride (0.6 mL, 4.88 mmol) was added dropwise to a solution of glycine isopropyl ester hydrochloride (500 mg, 3.25 mmol) and triethylamine (1 mL, 7.16 mmol) in CH_2Cl_2 (15 mL) at 0 °C. The resulting mixture was warmed to rt and stirred for 3 h. After completion, brine (10 mL) was added to the reaction mixture, the layers were separated and the aqueous was extracted with DCM (2x10 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated at reduced pressure. The resulting residue was purified by column chromatography (20% EtOAc/cyclohexane) to obtain **4a** (570 mg, 79%) as amorphous white solid.

R_f = 0.6 (50% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.89-7.78 (m, 2H, CH_{Ar}), 7.56-7.49 (m, 1H, CH_{Ar}), 7.47-7.41 (m, 2H, CH_{Ar}), 6.80 (br s, 1H, NH), 5.12 (sept, J = 6.3 Hz, 1H, CH-2), 4.21 (d, J = 5.0 Hz, 2H, CH_2-3), 1.30 (d, J = 6.3 Hz, 3H, CH_3-1), 1.29 (d, J = 6.3 Hz, 3H, CH_3-1). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 169.6 (C=O), 167.4 (C=O), 133.8 (C_{Ar}), 131.7 (CH_{Ar}), 128.6 (CH_{Ar}), 127.1 (CH_{Ar}), 69.5 (C2), 42.1 (C3), 21.8 (C1). **IR**: v [cm^{-1}] 3338, 3063, 2981, 2936, 1737, 1647, 1535, 1489, 1375, 1205, 1107. **LRMS** (ESI+) *m/z*, (%): 180 (50, $[M+H–propene]^+$), 222 (50, $[M+H]^+$), 244 (100, $[M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{12}H_{15}NO_3Na$: 244.0944, found: 244.0945.

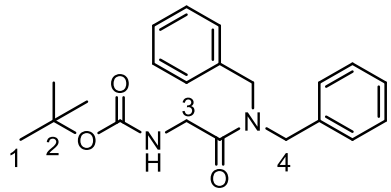
Isopropyl *N*-benzyloxycarbonylglycinate (4b)



Benzyl chloroformate (1.4 mL, 9.77 mmol) was added to a mixture of glycine isopropyl ester hydrochloride (1 g, 6.51 mmol) and $NaHCO_3$ (0.82 g, 9.77 mmol) in CH_2Cl_2 (20 mL) and H_2O (5 mL). The reaction mixture was stirred overnight at rt. Brine (20 mL) was added and the mixture was extracted with CH_2Cl_2 (3x20 mL), the combined organic layers were dried over $MgSO_4$, the solvent was removed at reduced pressure and the residue was purified by flash column chromatography (20% EtOAc/cyclohexane) to give the product as colorless solid (1.06 g, 65%).

R_f = 0.6 (40% EtOAc/PE). **m.p.** 58-60 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.43-7.30 (m, 5H, CH_{Ar}), 5.28 (br s, 1H, NH), 5.16 (s, 2H, CH_2-4), 5.09 (sept, *J* = 6.2 Hz, 1H, CH-2), 3.97 (d, *J* = 5.4 Hz, 2H, CH_2-3), 1.28 (d, *J* = 6.2 Hz, 6H, CH_3-1). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 169.5 (C=O), 156.2 (C=O), 136.3 (C_{Ar}), 128.5 (CH_{Ar}), 128.2 (CH_{Ar}), 128.1 (CH_{Ar}), 69.3 (C2), 67.1 (C4), 43.0 (C3), 21.8 (C1). **IR**: ν [cm^{-1}] 3353, 2981, 1705, 1519, 1374, 1273, 1200, 1147, 1104, 1051, 991, 736, 697. **LRMS** (ESI+) *m/z*, (%): 252 (10, $[M+H]^+$), 274 (100, $[M+Na]^+$), 525 (5, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{13}H_{17}NO_4Na$: 274.1050, found: 274.1051.

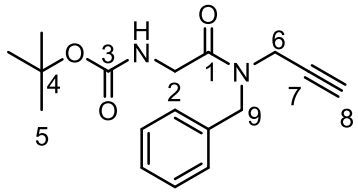
***N*'-(*tert*-Butyloxycarbonyl)-*N*,*N*-dibenzylglycinamide (4c)**



N-Boc-glycine (577 mg, 3.29 mmol), DCC (679 mg, 3.29 mmol) and DMAP (40.3 mg, 0.33 mmol) were added to a stirred solution of dibenzylamine (0.5 mL, 2.53 mmol) in CH_2Cl_2 (13 mL) at 0 °C. The reaction mixture was slowly warmed to rt and stirred for 24 h. After completion, the precipitate was filtered off, washed with CH_2Cl_2 (25 mL) and the filtrate was treated with sat. $NaHCO_3$ solution (10 mL). The layers were separated and the aqueous was extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated at reduced pressure. The pale yellow residue was purified by column chromatography (20% EtOAc/PE) giving **4c** (900 mg, 99%) as amorphous white solid.

R_f = 0.4 (20% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.40-7.31 (m, 6H, CH_{Ar}), 7.22 (d, *J* = 6.4 Hz, 2H, CH_{Ar}), 7.17 (d, *J* = 7.1 Hz, 2H, CH_{Ar}), 5.62 (br s, 1H, NH), 4.64 (s, 2H, CH_2-4), 4.41 (s, 2H, CH_2-4), 4.11 (d, *J* = 4.2 Hz, 2H, CH_2-3), 1.47 (s, 9H, CH_3-1). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 169.0 (C=O), 155.7 (C=O), 136.6 (C_{Ar}), 135.4 (C_{Ar}), 129.1 (CH_{Ar}), 128.7 (CH_{Ar}), 128.3 (CH_{Ar}), 127.9 (CH_{Ar}), 127.7 (CH_{Ar}), 126.5 (CH_{Ar}), 79.6 (C2), 49.0 (C4), 48.6 (C4), 42.4 (C3), 28.4 (C1). **IR**: ν [cm^{-1}] 3412, 2982, 2933, 1701, 1648, 1504, 1494, 1448, 1363, 1289, 1221, 1157, 949, 751, 702, 624. **LRMS** (ESI+) *m/z*, (%): 299 (50, $[M+H–isobutylene]^+$), 355 (20, $[M+H]^+$), 377 (100, $[M+Na]^+$), 731 (70, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{21}H_{26}N_2O_3Na$: 377.1836; found: 377.1839.

_N_-Benzyl-_N'_-(_tert_-butyloxycarbonyl)-_N_-propargylglycinamide (4d)

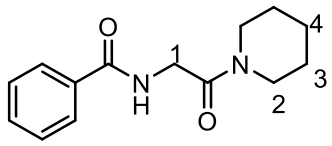


A suspension of *N*-(*tert*-butyloxycarbonyl)glycine (300 mg, 1.712 mmol), DCC (424 mg, 2.055 mmol) and 1-hydroxybenzotriazole (277.7 mg, 2.055 mmol) in CH_2Cl_2 (6 mL) was cooled to 0 ºC for 30 min and a solution of *N*-propargylbenzylamine[146] (298.4 mg, 2.055 mmol) in DCM (1 mL) was added dropwise. The reaction mixture was slowly warmed to rt and stirred overnight. After completion, the white powder precipitate was filtered off, the filtrate was diluted with EtOAc, washed with sat. $NaHCO_3$ solution and the solvent was removed to obtain a yellow liquid, which was purified by column chromatography (40% EtOAc/PE) to give **4d** (495 mg, 96%) as 1.2:1 rotameric mixture as colorless solid.

R_f = 0.25 (20% EtOAc/PE). **m.p.** 79-81 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.51-7.16 (m, 10H, CH_{Ar}, A+B), 5.52 (br s, 2H, NH, A+B), 4.73 (s, 2H, CH_2-9, B), 4.63 (s, 2H, CH_2-9, A), 4.25 (d, *J* = 2.5 Hz, 2H, CH_2-6, A), 4.15 (d, *J* = 4.0 Hz, 2H, CH_2-2, B), 4.08 (d, *J* = 4.4 Hz, 2H, CH_2-2, A), 3.90 (d, *J* = 2.5 Hz, 2H, CH_2-6, B), 2.32 (t, *J* = 2.5 Hz, 1H, CH-8, B), 2.25 (t, *J* = 2.5 Hz, 1H, CH-8, A), 1.49 (s, 9H, CH_3-5, B), 1.46 (s, 9H, CH_3-5, A). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 168.6 (C=O, A+B), 155.7 (C=O, A+B), 136.0 (C_{Ar}, A), 135.0 (C_{Ar}, B), 129.1 (CH_{Ar}, A), 128.8 (CH_{Ar}, A), 128.4 (CH_{Ar}, B), 128.1 (CH_{Ar}, B), 127.9 (CH_{Ar}, B), 126.9 (CH_{Ar}, A), 79.7 (C4, A+B), 78.1 (C7, A+B), 73.4 (C8, B), 72.5 (C8, A), 49.1 (C9, A), 48.7 (C9, B), 42.5 (C2, A), 42.4 (C2, B), 35.3 (C6, B), 34.4 (C6, A), 28.4 (C5, A+B). **IR**: ν [cm^{-1}] 3422, 3295, 3259, 2977, 2931, 2133, 1711, 1659, 1497, 1453, 1366, 1252, 1219, 1168, 1057, 734, 699. **LRMS**

(ESI+) *m/z*, (%): 247 (10, [M+H– isobutylene]$^+$), 325 (100, [M+Na]$^+$), 627 (10, [2M+Na]$^+$). **HRMS** (ESI+) *m/z*: ([M+Na]$^+$): calculated for $C_{17}H_{22}N_2O_3Na$: 325.1523, found: 325.1523.

***N'*-(Benzoyl)-*N*-pentamethyleneglycinamide (4e)**

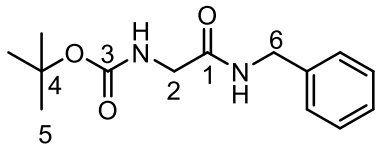


A solution of *N*-(benzoyl)glycine (1200 mg, 6.69 mmol), piperidine (1.3 mL, 13.39 mmol) in CH_2Cl_2 (30 mL) was cooled to 0 °C for and triethylamine (1.1 mL, 8.04 mmol) followed by HBTU (2794 mg, 7.37 mmol) were added. The reaction mixture was slowly warmed to rt and stirred overnight. After completion, the reaction mixture was diluted with EtOAc, washed with 1N HCl, followed by sat. $NaHCO_3$ solution, the organic layer was dried over $MgSO_4$ and the solvent was removed to obtain a yellow liquid, which was purified by column chromatography (30% EtOAc/PE) to give **4e** (798 mg, 48%) as colorless solid.

R_f = 0.25 (20% EtOAc/PE). **m.p.** 85-86 °C **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.93-7.79 (m, 2H), 7.57-7.41 (m, 3H), 7.44 (br s, 1H), 4.26 (d, *J* = 3.8 Hz, 2H), 3.70-3.57 (m, 2H), 3.51-3.31 (m, 2H), 1.86-1.54 (m, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 167.2 (C=O), 166.1 (C=O), 134.0 (C_{Ar}), 131.6 (CH_{Ar}), 128.5 (CH_{Ar}), 127.1 (CH_{Ar}), 45.5 (C2), 43.3 (C2), 41.7 (C1), 26.2 (C3), 25.5 (C3), 24.4 (C4).

The analytical data according to the literature.[147]

***N*-Benzyl-*N'*-(*tert*-butyloxycarbonyl)glycinamide (4f)**

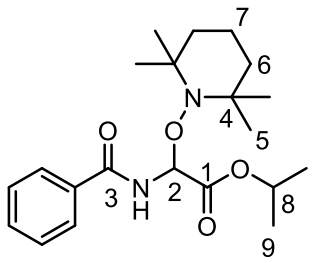


HATU (1.194 g, 3.14 mmol) was added to a stirred solution of *N*-(*tert*-butyloxycarbonyl) glycine (500 mg, 2.854 mmol), benzylamine (0.31 mL, 2.854 mmol) and DIPEA (2 mL, 11.417 mmol) in DMF (5 mL) and the mixture was stirred at rt for 24 h. After completion, the reaction mixture was diluted with EtOAc (30 mL), washed with H_2O (20 mL), the organic layer was dried over $MgSO_4$ and the solvent was evaporated in vacuum. The residue was purified by column chromatography (50% EtOAc/cyclohexane) to obtain **4f** (634 mg, 84%) as yellow oil.

R_f = 0.35 (50% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.42-7.16 (m, 5H, CH_{Ar}), 6.63 (br s, 1H, NH), 5.28 (s, 1H, NH), 4.46 (d, *J* = 5.8 Hz, 2H, CH_2-6), 3.84 (s, 2H, CH_2-2), 1.44 (s,

9H, CH_3-5). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 169.4 (C=O), 162.2 (C=O), 137.9 (C_{Ar}), 128.7 (CH_{Ar}), 127.7 (CH_{Ar}), 127.5 (CH_{Ar}), 84.1 (C4), 44.5 (C2), 43.4 (C6), 28.3 (C5). **IR**: ν [cm^{-1}] 3298, 2976, 2931, 1713, 1663, 1498, 1454, 1382, 1365, 1247, 1165, 1092, 1063, 1027, 737, 699, 660. **LRMS** (ESI+) *m/z*, (%): 209 (10, $[M+H–isobutylene]^+$), 265 (5, $[M+H]^+$), 287 (100, $[M+Na]^+$), 551 (15, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{14}H_{20}N_2O_3Na$: 287.1366, found: 287.1367.

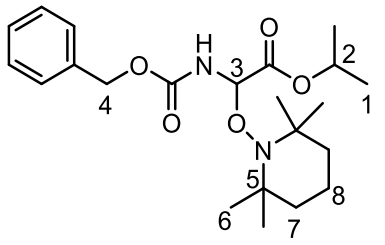
Isopropyl *N*-benzoyl-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)glycinate (5a)



Prepared according to general procedure **C** starting from isopropyl *N*-benzoylglycinate (**4a**), obtaining **5a** (600 mg, 70%) as colorless oil.

R_f = 0.75 (20% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.89-7.81 (m, 2H, CH_{Ar}), 7.59-7.53 (m, 1H, CH_{Ar}), 7.51-7.44 (m, 2H, CH_{Ar}), 7.05 (d, *J* = 9.8 Hz, 1H, NH), 6.21 (d, *J* = 9.7 Hz, 1H, CH-2), 5.14 (sept, *J* = 6.2 Hz, 1H, CH-8), 1.63-1.21 (m, 6H, CH_2-6, CH_2-7), 1.38 (s, 3H, CH_3-5), 1.35 (d, *J* = 6.2 Hz, 3H, CH_3-9), 1.32 (d, *J* = 6.3 Hz, 3H, CH_3-9), 1.18 (s, 3H, CH_3-5), 1.15 (s, 3H, CH_3-5), 1.09 (s, 3H, CH_3-5). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 167.9 (C=O), 166.4 (C=O), 133.8 (C_{Ar}), 132.0 (CH_{Ar}), 128.7 (CH_{Ar}), 127.2 (CH_{Ar}), 82.3 (C2), 69.7 (C8), 61.1 (C4), 59.5 (C4), 40.14 (C6), 40.06 (C6), 33.9 (C5), 32.9 (C5), 21.73 (C9), 21.69 (C9), 20.2 (C5), 20.0 (C5), 17.1 (C7). **IR**: ν [cm^{-1}] 3338, 2982, 2937, 1744, 1655, 1525, 1488, 1375, 1212, 1105, 712, 694. **LRMS** (ESI+) *m/z*, (%): 377 (50, $[M+H]^+$), 399 (100, $[M+Na]^+$), 775 (25, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{21}H_{32}N_2O_4Na$: 399.2254, found: 399.2254.

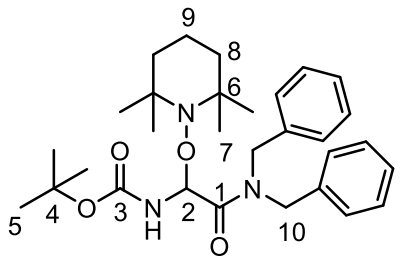
Isopropyl *N*-benzyloxycarbonyl-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)glycinate (5b)



Isopropyl *N*-(benzyloxycarbonyl)glycinate (**4b**) was subjected to general procedure **C** to obtain the product **5b** (1.0 g, 62%) as colorless solid.

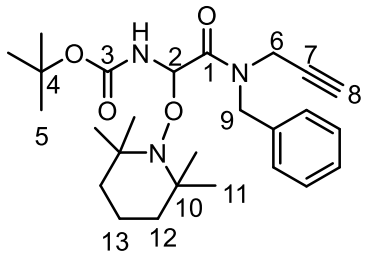
R_f = 0.6 (20% EtOAc/PE**). m.p.** 59 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.42-7.32 (m, 5H, CH_{Ar}), 5.77 (s, 2H, NH, CH-3), 5.22-5.02 (m, 3H, CH_2-4, CH-2), 1.59-1.31 (m, 6H, CH_2-7, CH_2-8), 1.33 (s, 3H, CH_3-6), 1.30 (d, *J* = 6.4 Hz, 6H, CH_3-1), 1.14 (s, 3H, CH_3-6), 1.12 (s, 3H, CH_3-6), 1.06 (s, 3H, CH_3-6). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 167.2 (C=O), 155.2 (C=O), 136.2 (C_{Ar}), 128.5 (CH_{Ar}), 128.2 ($2CH_{Ar}$), 84.0 (C3), 69.5 (C2), 67.1 (C4), 61.1 (C5), 59.3 (C5), 40.1 (C7), 33.6 (C6), 32.9 (C6), 21.7 (C1), 20.2 (C6), 20.0 (C6), 17.1 (C8). **IR**: v [cm^{-1}] 3359, 2978, 2933, 1733, 1509, 1467, 1455, 1377, 1363, 1319, 1260, 1233, 1200, 1133, 1106, 1059, 1025, 984, 957, 697. **LRMS** (ESI+) *m/z*, (%): 407 (30, $[M+H]^+$), 429 (100, $[M+Na]^+$), 835 ($[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{22}H_{34}N_2O_5Na$: 429.2360, found: 429.2359.

***N'*-(*tert*-Butyloxycarbonyl)-*N*,*N*-dibenzyl-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy) glycinamide (5c)**



Prepared following general procedure **C** starting from *N'*-(*tert*-butyloxycarbonyl)-*N*,*N*-dibenzyl glycinamide (**4c**), affording **5c** (210 mg, 73%) as colorless crystalline solid.
R_f = 0.55 (10% EtOAc/PE). **m.p.** 132-134 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.39-7.30 (m, 6H, CH_{Ar}), 7.24-7.20 (m, 4H, CH_{Ar}), 6.11 (d, *J* = 10.6 Hz, 1H, CH-2), 5.95 (d, *J* = 10.6 Hz, 1H, NH), 4.81 (d, *J* = 16.4 Hz, 1H, CH_2-10), 4.75 (d, *J* = 14.9 Hz, 1H, CH_2-10), 4.50 (d, *J* = 16.3 Hz, 1H, CH_2-10), 4.40 (d, *J* = 14.8 Hz, 1H, CH_2-10), 1.56-1.30 (m, 6H, CH_2-8, CH_2-9), 1.47 (s, 9H, CH_3-5), 1.22 (s, 3H, CH_3-7), 1.20 (s, 3H, CH_3-7), 1.12 (s, 3H, CH_3-7), 1.05 (s, 3H, CH_3-7). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 168.1 (C=O), 154.5 (C=O), 136.3 (C_{Ar}), 135.8 (C_{Ar}), 128.8 (CH_{Ar}), 128.6 (CH_{Ar}), 128.2 (CH_{Ar}), 127.8 (CH_{Ar}), 127.5 (CH_{Ar}), 127.3 (CH_{Ar}), 80.3 (C2), 79.7 (C4), 60.7 (C6), 59.4 (C6), 49.6 (C10), 47.4 (C10), 40.3 (C8), 33.5 (C7), 32.9 (C7), 28.3 (C5), 20.6 (C7), 20.5 (C7), 17.2 (C9). **IR**: v [cm^{-1}] 3320, 3001, 2977, 2930, 2869, 1706, 1649, 1510, 1497, 1453, 1364, 1250, 1219, 1171, 1014, 990, 959, 749, 734, 712, 698, 669, 644, 616. **LRMS** (ESI+) *m/z*, (%): 510 (100, $[M+H]^+$), 532 (100, $[M+Na]^+$), 1041 (5, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{30}H_{43}N_3O_4Na$: 532.3146, found: 532.3143.

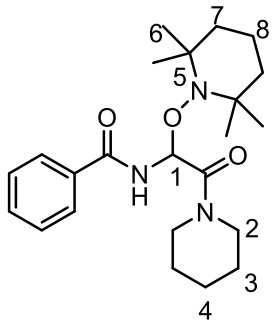
***N*-Benzyl-*N'*-(*tert*-butyloxycarbonyl)-*N*-propargyl-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)glycinamide (5d)**



Prepared following general procedure **C** starting from *N*-benzyl-*N'*-(*tert*-butyloxycarbonyl)-*N*-propargylglycinamide (**4d**), affording **5d** (237 mg, 78%) as pale yellow oil as 1:1 rotameric mixture.

R_f = 0.8 (20% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.38-7.30 (m, 10H, CH_{Ar}, A+B), 6.09 (d, *J* = 10.6 Hz, 1H, CH-2, A), 6.05 (d, *J* = 10.6 Hz, 1H, CH-2, B), 5.86 (d, *J* = 10.4 Hz, 1H, NH, A), 5.83 (d, *J* = 10.2 Hz, 1H, NH, B), 5.03 (d, *J* = 16.1 Hz, 1H, CH_2-9, A), 4.84 (d, *J* = 14.9 Hz, 1H, CH_2-9, B), 4.77 (d, *J* = 16.0 Hz, 1H, CH_2-9, A), 4.68 (d, *J* = 14.9 Hz, 1H, CH_2-9, B), 4.37 (dd, *J* = 18.3, 2.4 Hz, 1H, CH_2-6, A), 4.24 (dd, *J* = 17.3, 2.5 Hz, 1H, CH_2-6, B), 4.15-4.04 (m, 2H, CH_2-6, A+B), 2.34 (t, *J* = 2.4 Hz, 1H, CH-8, A), 2.23 (t, *J* = 2.5 Hz, 1H, CH-8, B), 1.61-1.32 (m, 12H, CH_2-12, CH_2-13, A+B) 1.49 (s, 9H, CH_3-5, A), 1.46 (s, 9H, CH_3-5, B), 1.26 (s, 3H, CH_3-11, A), 1.23 (s, 6H, CH_3-11, A), 1.20 (s, 3H, CH_3-11, A), 1.16 (s, 3H, CH_3-11, B), 1.11 (s, 6H, CH_3-11, B), 1.06 (s, 3H, CH_3-11, B). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 168.0 (C=O, A), 167.6 (C=O, B), 154.4 (C=O, A), 154.3 (C=O, B), 136.0 (C_{Ar}, A), 135.3 (C_{Ar}, B), 128.8 (CH_{Ar}, A), 128.7 (CH_{Ar}, B), 128.3 (CH_{Ar}, A), 127.9 (CH_{Ar}, B), 127.7 (CH_{Ar}, A), 127.5 (CH_{Ar}, B), 81.1 (CH-2, A), 80.3 (CH-2, B), 80.0 (C4, A), 79.9 (C4, B), 77.9 (C7, A+B), 73.4 (C8, A), 72.4 (C8, B), 60.6 (C10, A), 60.5 (C10, B), 59.8 (C10, A), 59.6 (C10, B), 49.7 (C9, A), 48.0 (C9, B), 40.3 (C12, A), 40.2 (C12, B), 35.9 (C6, A), 33.6 (C6, B), 33.3 (C11, A+B), 32.9 (C11, A+B), 28.3 (C5, A), 28.2 (C5, B), 20.62 (C11, A), 20.57 (C11, B), 20.5 (C11, A), 20.4 (C11, B), 17.2 (C13, A+B). **IR**: ν [cm^{-1}] 3308, 2975, 2931, 1716, 1656, 1497, 1442, 1365, 1242, 1214, 1162, 1014, 954, 928, 753, 713, 698, 665, 627. **LRMS** (ESI+) *m/z*, (%): 458 (20, $[M+H]^+$), 480 (100, $[M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{26}H_{39}N_3O_4Na$: 480.2833, found: 480.2829.

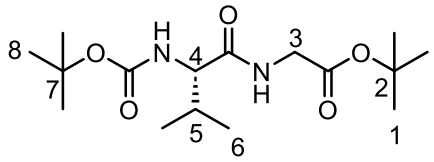
***N'*-(Benzoyl)-*N*-pentamethylene-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)glycinamide (5e)**



Prepared following general procedure **C** starting from *N'*-(Benzoyl)-*N*-pentamethylene-glycinamide (**4e**), affording **5e** (237 mg, 78%) as pale yellow oil.

R_f = 0.8 (20% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.91-7.83 (m, 2H, CH_{Ar}), 7.59-7.51 (m, 1H, CH_{Ar}), 7.49-7.43 (m, 3H, CH_{Ar}, NH), 6.48 (d, *J* = 9.8 Hz, 1H, CH-1), 3.81-3.57 (m, 3H, CH_2-2), 3.57-3.43 (m, 1H, CH_2-2), 1.74-1.38 (m, 12H, CH_2-3, CH_2-4, CH_2-7, CH_2-8), 1.30 (s, 3H, CH_3-6), 1.22 (s, 3H, CH_3-6), 1.19 (s, 3H, CH_3-6), 1.12 (s, 3H, CH_3-6). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 166.4 (C=O), 166.1 (C=O), 133.9 (C_{Ar}), 131.9 (CH_{Ar}), 128.6 (CH_{Ar}), 127.2 (CH_{Ar}), 78.7 (C1), 60.5 (C5), 59.8 (C5), 46.8 (C2), 43.3 (C2), 40.2 (C7), 40.1 (C7), 33.6 (C6), 33.2 (C6), 26.6 (C3), 25.5 (C3), 24.5 (C4), 20.5 (C6), 20.4 (C6), 17.2 (C8). **IR**: v [cm^{-1}] 3281, 3009, 2974, 2932, 2856, 1635, 1603, 1579, 1531, 1490, 1463, 1443, 1375, 1362, 1311, 1272, 1261, 1243, 1226, 1134, 1083, 1035, 1027, 1013, 990, 958, 928, 698, 666. **LRMS** (ESI+) *m/z*, (%): 402 (20, $[M+H]^+$), 424 (100, $[M+Na]^+$) 825 (20, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{23}H_{35}N_3O_3Na$: 424.2571, found: 424.2568.

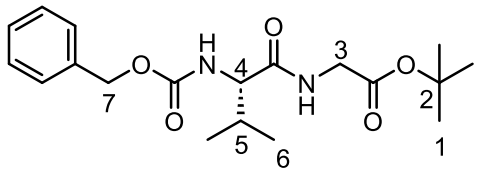
***tert*-Butyl *N*-(*N*-(*tert*-butyloxycarbonyl)-L-valyl)glycinate (4h)**



Prepared according to general procedure **A** from *N*-(*tert*-butyloxycarbonyl)-L-valine and *tert*-butyl glycinate•HCl, giving **4h** (505 mg, 83%) as colorless oil.

R_f = 0.4 (20% EtOAc/PE). $[\alpha]^{20}_{589}$ = –8.5 (c 1, $CHCl_3$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.45 (br s, 1H, NH), 5.07 (br s, 1H, NH), 4.02-3.89 (m, 3H, CH-4, CH_2-3), 2.24-2.16 (m, 1H, CH-5), 1.48 (s, 9H, CH_3-1), 1.46 (s, 9H, CH_3-8), 0.99 (d, *J* = 6.8 Hz, 3H, CH_3-6), 0.94 (d, *J* = 6.8 Hz, 3H, CH_3-6). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 171.7 (C=O), 168.8 (C=O), 155.9 (C=O), 82.4 (C2), 80.0 (C7), 59.9 (C4), 42.0 (C3), 30.9 (C5), 28.4 (C1), 28.1 (C8), 19.4 (C6), 17.7 (C6). **IR**: v [cm^{-1}] 3277, 2968, 2934, 1746, 1709, 1689, 1664, 1643, 1531, 1390, 1365, 1242, 1224, 1156, 1079, 1005, 847, 781, 751. **LRMS** (ESI+) *m/z*, (%): 219 (20, $[M+H–2isobutylene]^+$), 275 (5, $[M+H–isobutylene]^+$), 297 (15, $[M+Na–isobutylene]^+$), 331 (10, $[M+H]^+$), 353 (100, $[M+Na]^+$), 683 (5, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{16}H_{30}N_2O_5Na$: 353.2047, found: 353.2044.

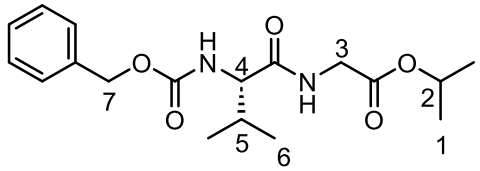
***tert*-Butyl *N*-(*N*-(benzyloxycarbonyl)-L-valyl)glycinate (4i)**



Prepared according to general procedure **A** from *N*-(benzyloxycarbonyl)-L-valine and *tert*-butyl glycinate•HCl, affording **4i** (286 mg, 99%) as colorless solid.

R_f = 0.4 (20% EtOAc/PE). **m.p.** 147-149 °C. $[\alpha]^{20}_{589}$ = –7.1 (c 0.406, $CHCl_3$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.39-7.34 (m, 5H, CH_{Ar}), 6.30 (br s, 1H, NH), 5.32 (d, *J* = 8.5 Hz, 1H, NH), 5.14 (s, 2H, CH_2-7), 4.11-3.82 (m, 3H, CH-4, CH_2-3), 2.24-2.16 (m, 1H, CH-5), 1.50 (s, 9H, CH_3-1), 1.01 (d, *J* = 6.8 Hz, 3H, CH_3-6), 0.96 (d, *J* = 6.8 Hz, 3H, CH_3-6). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 171.1 (C=O), 168.6 (C=O), 156.6 (C=O), 135.8 (C_{Ar}), 128.6 (CH_{Ar}), 128.2 (CH_{Ar}), 128.1 (CH_{Ar}), 82.5 (C2), 67.1 (C7), 60.3 (C4), 42.0 (C3), 31.0 (C5), 28.0 (C1), 19.2 (C6), 17.6 (C6). **IR**: v [cm^{-1}] 3288, 2961, 1741, 1694, 1650, 1531, 1391, 1369, 1291, 1238, 1157, 1042, 837, 749, 739, 717, 694. **LRMS** (ESI+) *m/z*, (%): 309 (80, $[M+H–isobutylene]^+$), 365 (40, $[M+H]^+$), 387 (100, $[M+Na]^+$), 729 (20, $[2M+H]^+$), 751 (60, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{19}H_{28}N_2O_5Na$: 387.1890, found: 387.1886.

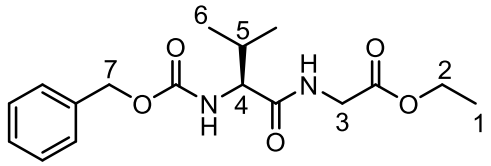
Isopropyl *N*-(*N*-(benzyloxycarbonyl)-L-valyl)glycinate (4j)



Prepared according to general procedure **A** from *N*-(benzyloxycarbonyl)-L-valine and isopropyl glycinate•HCl, providing **4j** (250 mg, 90%) as amorphous white solid.

R_f = 0.4 (20% EtOAc/PE). $[\alpha]^{20}_{589}$ = –16.2 (c 0.266, CH_3OH). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.39-7.34 (m, 5H, CH_{Ar}), 6.34 (br s, 1H, NH), 5.32 (d, *J* = 8.8 Hz, 1H, NH), 5.14-5.11 (m, 2H, CH_2-7), 5.10 (sept, *J* = 6.4 Hz, 1H, CH-2), 4.11-3.95 (m, 3H, CH-4, CH_2-3), 2.26-2.15 (m, 1H, CH-5), 1.28 (d, *J* = 6.3 Hz, 6H, CH_3-1), 1.01 (d, *J* = 6.8 Hz, 3H, CH_3-6), 0.97 (d, *J* = 6.8 Hz, 3H, CH_3-6). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 171.3 (C=O), 169.0 (C=O), 157.4 (C=O), 136.1 (C_{Ar}), 128.6 (CH_{Ar}), 128.2 (CH_{Ar}), 128.1 (CH_{Ar}), 69.5 (C2), 67.1 (C7), 60.3 (C4), 41.5 (C3), 30.9 (C5), 21.8 (C1), 19.2 (C6), 17.7 (C6). **IR**: ν [cm^{-1}] 3290, 2976, 2938, 2872, 1724, 1688, 1645, 1536, 1385, 1374, 1337, 1289, 1247, 1227, 1105, 1039, 972, 834, 782, 744, 697, 667, 600. **LRMS** (ESI+) *m/z*, (%): 351 (80, $[M+H]^+$), 373 (100, $[M+Na]^+$), 701 (10, $[2M+H]^+$), 723 (40, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+H]^+$): calculated for $C_{18}H_{27}N_2O_5$: 351.1914, found: 351.1909.

Ethyl-*N*-(*N*-(benzyloxycarbonyl)-L-valyl) glycinate (4k)

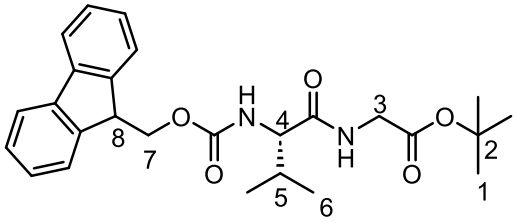


By following the general procedure **A**, using *N*-(benzyloxycarbonyl)-L-valine and glycine ethyl ester hydrochloride, the dipeptide **4k** (860 mg, 64%) was obtained as colorless liquid.

R_f = 0.5 (50% EtOAc/PE). $[\alpha]^{20}_{589}$ = +7.7 (c 1, $CHCl_3$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.75-7.31 (m, 5H, CH_{Ar}), 6.63 (d, *J* = 5.8 Hz, 1H, NH), 5.47 (d, *J* = 8.7 Hz, 1H, NH), 5.19-5.03 (m, 2H, CH_2-7), 4.21 (q, *J* = 7.1 Hz, 2H, CH_2-2), 4.13-3.92 (m, 3H, CH-4, CH_2-3), 2.26-2.09 (m, 1H, CH-5), 1.28 (t, *J* = 7.1 Hz, 3H, CH_3-1), 1.00 (d, *J* = 6.8 Hz, 3H, CH_3-6), 0.95 (d, *J* = 6.8 Hz, 3H, CH_3-6). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 171.6 (C=O), 169.7 (C=O), 156.5 (C=O), 136.2 (C_{Ar}), 128.5 (CH_{Ar}), 128.2 (CH_{Ar}), 128.0 (CH_{Ar}), 67.1 (C7), 61.6 (C2), 60.3 (C4), 41.3 (C3), 31.0 (C5), 19.2 (C6), 17.7 (C6), 14.1 (C1). **IR**: ν [cm^{-1}] 3287, 3092, 2969, 2871, 1746, 1688, 1650, 1560, 1535, 1388, 1372, 1292, 1245, 1205, 1136, 1039, 1025, 841, 744, 697, 678.

LRMS (ESI+) *m/z*, (%): 337 (20, $[M+H]^+$), 359 (100, $[M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{17}H_{24}N_2O_5Na$: 359.1577, found: 359.1579.

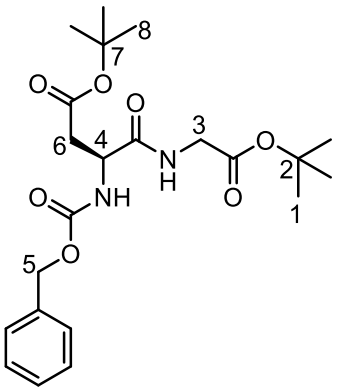
***tert*-Butyl *N*-(*N*-((9H-fluoren-9-yl)methoxycarbonyl)-L-valyl)glycinate (4l)**



Prepared following general procedure **A** from *N*-(9H-fluoren-9-yl-methoxycarbonyl)-L-valine and *tert*-butyl glycinate•HCl, providing **4l** (1.53 g, 68%) as colorless solid.

R_f = 0.4 (20% EtOAc/PE). **m.p.** 163-165 °C. $[\alpha]^{20}_{589}$ = –13.4 (c 0.319, $CHCl_3$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.79 (d, *J* = 7.6 Hz, 2H, CH_{Ar}), 7.62 (d, *J* = 7.3 Hz, 2H, CH_{Ar}), 7.44-7.41 (m, 2H, CH_{Ar}), 7.36-7.32 (m, 2H, CH_{Ar}), 6.32 (br s, 1H, NH_{Gly}), 5.40 (d, *J* = 9.0 Hz, 1H, NH_{Val}), 4.45 (dd, *J* = 10.6, 7.4 Hz, 1H, CH-7), 4.40 (dd, *J* = 10.7, 6.8 Hz, 1H, CH-7), 4.25 (t, *J* = 6.9 Hz, 1H, CH-8), 4.09 (dd, *J* = 8.7, 6.3 Hz, 1H, CH-4), 4.01 (dd, *J* = 18.3, 5.2 Hz, 1H, CH-3), 3.92 (dd, *J* = 18.2, 4.9 Hz, 1H, CH-3), 2.22-2.17 (m, 1H, CH-5), 1.49 (s, 9H, CH_3-1), 1.00 (d, *J* = 7.2 Hz, 3H, CH_3-6), 0.98 (d, *J* = 7.2 Hz, 3H, CH_3-6). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 171.3 (C=O), 168.6 (C=O), 156.4 (C=O), 143.9 (C_{Ar}), 141.3 (C_{Ar}), 127.7 (CH_{Ar}), 127.1 (CH_{Ar}), 125.1 (CH_{Ar}), 120.0 (CH_{Ar}), 82.5 (C2), 67.1 (C7), 60.3 (C4), 47.2 (C8), 42.0 (C3), 31.1 (C5), 28.0 (C1), 19.2 (C6), 17.8 (C6). **IR**: ν [cm^{-1}] 3288, 3067, 2972, 2872, 1730, 1690, 1650, 1537, 1369, 1295, 1249, 1158, 1032, 755, 739, 706, 665, 646. **LRMS** (ESI+) *m/z*, (%): 397 (20, $[M+H–isobutylene]^+$), 453 (5, $[M+H]^+$), 475 (80, $[M+Na]^+$), 927 (5, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{26}H_{32}N_2O_5Na$: 475.2203, found: 475.2200.

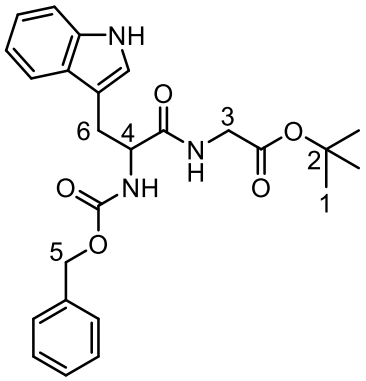
***tert*-Butyl-*N*-(*N*-(benzyloxycarbonyl)-O-(*tert*-butyl)-L-aspartyl glycinate (4m)**



By following the general procedure **A**, using *N*-(benzyloxycarbonyl)-O-(*tert*-butyl)-L-aspartic acid and glycine *tert*-butyl ester hydrochloride, the dipeptide **4m** (1200 mg, 92%) was obtained as colorless solid.

R_f = 0.6 (40% EtOAc/PE). **m.p.** 84-86 °C. $[\alpha]^{20}_{589}$ = +17.7 (c 1.04, $CHCl_3$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.46-7.32 (m, 5H, CH_{Ar}), 6.96 (s, 1H, NH_{Gly}), 5.98 (d, *J* = 8.6 Hz, 1H, NH_{Asp}), 5.17 (s, 2H, CH_2-5), 4.59 (ddd, *J* = 8.4, 6.6, 4.5 Hz, 1H, CH-4), 3.98-3.83 (m, 2H, CH_2-3), 2.95 (dd, *J* = 17.0, 4.5 Hz, 1H, CH_2-6), 2.66 (dd, *J* = 17.1, 6.4 Hz, 1H, CH_2-6), 1.49 (s, 9H, CH_3-1), 1.45 (s, 9H, CH_3-8). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 171.0 (C=O), 170.5 (C=O), 168.4 (C=O), 156.1 (C=O), 136.1 (C_{Ar}), 128.6 (CH_{Ar}), 128.3 (CH_{Ar}), 128.1 (CH_{Ar}), 82.3 (C2), 81.9 (C7), 67.3 (C5), 51.1 (C4), 42.2 (C3), 37.3 (C6), 28.0 (C1, C8). **IR**: v [cm^{-1}] 3361, 3329, 2981, 2935, 1732, 1719, 1652 1634, 1525, 1392, 1367, 1247, 1216, 1147, 1054, 843, 747, 699, 626, 604. **LRMS** (ESI+) *m/z*, (%): 437 (25, $[M+H]^+$), 459 (100, $[M+Na]^+$), 895 (25, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{22}H_{32}N_2O_7Na$: 459.2102, found: 459.2099.

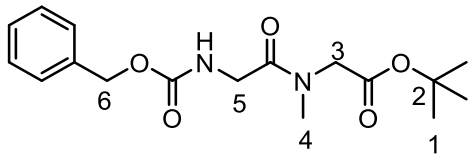
***tert*-Butyl-*N*-(*N*-(benzyloxycarbonyl)-DL-tryptophanyl) glycinate (4n)**



By following the general procedure **A**, using *N*-(benzyloxycarbonyl)-L-tryptophan) and glycine *tert*-butyl ester hydrochloride, the dipeptide **4n** (1300 mg, 96%) was obtained as colorless liquid as a racemic mixture.

R$_f$ = 0.6 (40% EtOAc/PE). $[\alpha]^{20}_{589}$ = 0 (c 0.87, $CHCl_3$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 8.39 (s, 1H, NH), 7.65 (d, *J* = 7.6 Hz, 1H, CH_{Ar}), 7.39-7.30 (m, 6H, CH_{Ar}), 7.24-7.16 (m, 1H, CH_{Ar}), 7.14-7.03 (m, 2H, CH_{Ar}), 6.32 (s, 1H, NH), 5.52 (s, 1H, NH), 5.11 (s, 2H, CH_2-5), 4.57 (d, *J* = 7.3 Hz, 1H, CH-4), 3.83 (d, *J* = 8.9 Hz, 1H, CH_2-3), 3.81 (d, *J* = 8.7 Hz, 1H, CH_2-3), 3.47-3.16 (m, 2H, CH_2-6), 1.44 (s, 9H, CH_3-1). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 171.4 (C=O), 168.4 (C=O), 156.1 (C=O), 136.2 ($2C_{Ar}$), 128.5 (CH_{Ar}), 128.2 (CH_{Ar}), 128.1 (CH_{Ar}), 127.4 (C_{Ar}), 123.3 (CH_{Ar}), 122.2 (CH_{Ar}), 119.7 (CH_{Ar}), 118.7 (CH_{Ar}), 111.3 (CH_{Ar}), 110.2 (C_{Ar}), 82.3 (C2), 67.0 (C5), 55.5 (C4), 42.1 (C3), 28.4 (C6), 28.0 (C1). **IR**: ν [cm^{-1}] 3303, 3058, 2978, 2933, 1712, 1665, 1621, 1510, 1456, 1368, 1223, 1150, 1050, 1026, 735, 697. **LRMS** (ESI+) *m/z*, (%): 396 (5, $[M+H–isobutylene]^+$), 418 (15, $[M+Na–isobutylene]^+$), 452 (5, $[M+H]^+$), 474 (100, $[M+Na]^+$), 925 (20, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{25}H_{29}N_3O_5Na$: 474.1999, found: 474.1999.

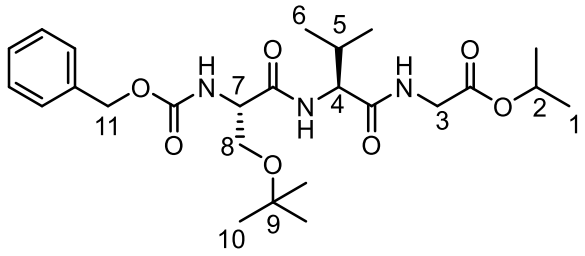
***tert*-Butyl-*N*-(*N*-(benzyloxycarbonyl)glycyl sarcosinate (4o)**



By following the general procedure **A**, using *N*-(benzyloxycarbonyl) glycine (518 mg, 2.48 mmol), sarcosine *tert*-butyl ester hydrochloride (450 mg, 2.48 mmol), the dipeptide **4o** (750 mg, 90%) was obtained as colorless liquid as 3:1 rotameric mixtures.

R_f = 0.6 (40% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.42-7.30 (m, 10H, CH_{Ar}, A+B), 5.88-5.65 (m, 2H, NH, A+B), 5.14 (s, 4H, CH_2-6, A+B), 4.10 (d, *J* = 4.3 Hz, 2H, CH_2-5, A), 4.05 (s, 2H, CH_2-3, A), 3.96 (d, *J* = 4.3 Hz, 2H, CH_2-5, B), 3.89 (s, 2H, CH_2-3, B), 3.04 (s, 3H, CH_3-4, A), 3.01 (s, 3H, CH_3-4, B), 1.49 (s, 9H, CH_3-1, B), 1.48 (s, 9H, CH_3-1, A). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 168.7 (C=O, A+B), 167.9 (C=O, A), 167.3 (C=O, B), 156.2 (C=O, A+B), 136.4 (C_{Ar}, A+B), 128.5 (CH_{Ar}, A+B), 128.12 (CH_{Ar}, B), 128.10 (CH_{Ar}, A), 128.0 (CH_{Ar}, A+B), 83.2 (C2, B), 82.2 (C2, A), 66.90 (C6, B), 66.88 (C6, A), 51.2 (C3, B), 50.3 (C3, A), 42.7 (C5, A), 42.4 (C5, B), 35.2 (C4, A), 35.1 (C4, B), 28.1 (C1, A), 28.0 (C1, B). **IR**: v [cm^{-1}] 3337, 2926, 1725, 1658, 1487, 1455, 1408, 1368, 1233, 1155, 1049, 841, 741, 699. **LRMS** (ESI+) *m/z*, (%): 359 (100, $[M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{17}H_{24}N_2O_5Na$: 359.1577, found: 359.1576.

Cbz-Ser(O*t*Bu)-Val-Gly-O*i*Pr (4p)

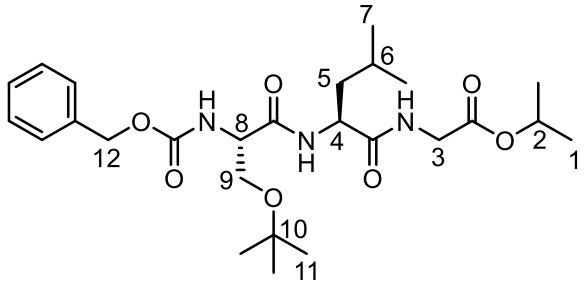


Pd/C (100 mg) was added to a solution of isopropyl *N*-(*N*-(benzyloxycarbonyl)-L-valyl) glycinate **4j** (500 mg, 1.42 mmol) in MeOH (8 mL) and the reaction mixture was stirred under a H_2 atmosphere (15 bar) at rt for 5 h. The reaction mixture was diluted with MeOH (10 mL), and filtered through Celite®. The solvent was removed under reduced pressure to obtain isopropyl *N*-(L-valyl)glycinate (320 mg, 1.48 mmol) as colorless oil and was directly used on the next step without further purification.

By following the general procedure **A**, using *N*-(benzyloxycarbonyl)-*O*-*tert*-butyl-L-serine (437 mg, 1.48 mmol) and isopropyl *N*-(L-valyl)glycinate (320 mg, 1.48 mmol), the tripeptide **4p** (420 mg, 58%) was obtained as colorless solid.

R_f = 0.6 (40% EtOAc/PE). **m.p.** 149-150 °C. $[\alpha]^{20}_{589}$ = −14.8 (c 0.31, $CHCl_3$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.42-7.31 (m, 5H, CH_{Ar}), 7.06 (d, *J* = 8.9 Hz, 1H, NH_{Val}), 6.74 (s, 1H, NH_{Ser}), 5.77 (d, *J* = 5.2 Hz, 1H, NH_{Gly}), 5.14 (s, 2H, CH_2-11), 5.07 (sept, *J* = 6.3 Hz, 1H, CH-2), 4.38 (dd, *J* = 8.9, 5.6 Hz, 1H, CH-4), 4.32-4.24 (m, 1H, CH-7), 3.99 (d, *J* = 5.4 Hz, 2H, CH_2-3), 3.86 (dd, *J* = 8.9, 3.8 Hz, 1H, CH_2-8), 3.47 (dd, *J* = 8.8, 7.2 Hz, 1H, CH_2-8), 2.37-2.23 (m, 1H, CH-5), 1.26 (d, *J* = 6.2 Hz, 3H, CH_3-1), 1.25 (d, *J* = 6.3 Hz, 1H, CH_3-1), 1.21 (s, 9H, CH_3-10), 0.99 (d, *J* = 6.8 Hz, 3H, CH_3-6), 0.95 (d, *J* = 6.8 Hz, 3H, CH_3-6). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 170.9 (C=O), 170.5 (C=O), 169.1 (C=O), 156.3 (C=O), 136.0 (C_{Ar}), 128.6 (CH_{Ar}), 128.32 (CH_{Ar}), 128.27 (CH_{Ar}), 74.4 (C9), 69.2 (C2), 67.3 (C11), 61.6 (C8), 58.4 (C4), 55.1 (C7), 41.4 (C3), 30.3 (C5), 27.4 (C10), 21.8 (C1), 19.3 (C6), 17.5 (C6). **IR**: v [cm^{-1}] 3284, 3079, 2973, 2933, 2873, 1723, 1692, 1638, 1537, 1465, 1455, 1400, 1364, 1342, 1301, 1267, 1239, 1226, 1196, 1148, 1107, 1094, 1052, 1037, 962, 880, 837, 738, 694, 675, 612. **LRMS** (ESI+) *m/z*, (%): 438 (5, $[M+H–isobutylene]^+$), 494 (10, $[M+H]^+$), 516 (100, $[M+Na]^+$), 1009 (15, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{25}H_{39}N_3O_7Na$: 516.2680, found: 516.2679.

Cbz-Ser(O*t*Bu)-Leu-Gly-O*i*Pr (4q)

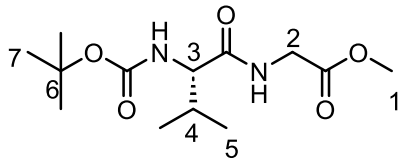


Pd/C (200 mg) was added to a solution of isopropyl *N*-(*N*-(benzyloxycarbonyl)-L-leucyl) glycinate (1000 mg, 2.74 mmol) in MeOH (15 mL) and the reaction mixture was stirred under H_2 atmosphere (15 bar) at rt for 5 h. The reaction mixture was diluted with MeOH (10 mL), filtered through Celite®, the solvent was removed at reduced pressure to obtain isopropyl-*N*-(L-leucyl)glycinate as colorless oil (500 mg, 2.17 mmol), which was directly used for next step without further purification.

By following the general procedure **A**, using *N*-benzyloxycarbonyl-*O*-*tert*-butyl-L-serine (641 mg, 2.17 mmol) and isopropyl-*N*-(L-leucyl) glycinate (500 mg, 2.17 mmol), the tripeptide **4q** (820 mg, 74%) was obtained as colorless solid.

R_f = 0.6 (40% EtOAc/PE). **m.p.** 109-110 °C. $[\alpha]^{20}_{589}$ = −12.8 (c 0.85, $CHCl_3$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.46-7.32 (m, 5H, CH_{Ar}), 6.90 (br s, 2H, NH_{Leu}, NH_{Gly}), 5.74 (s, 1H, NH_{Ser}), 5.14 (s, 2H, CH_2-12), 5.05 (sept, *J* = 6.4 Hz, 1H, CH-2), 4.65-4.49 (m, 1H, CH-4), 4.27 (td, *J* = 6.6, 3.7 Hz, 1H, CH-8), 4.09-3.72 (m, 3H, CH_2-3, CH_2-9), 3.47 (dd, *J* = 8.8, 6.8 Hz, 1H, CH_2-9), 1.81-1.73 (m, 1H, CH_2-5), 1.72-1.63 (m, 1H, CH-6), 1.62-1.51 (m, 1H, CH_2-5), 1.26 (d, *J* = 6.2 Hz, 3H, CH_3-1), 1.25 (d, *J* = 6.2 Hz, 3H, CH_3-1), 1.19 (s, 9H, CH_3-11), 0.94 (d, *J* = 8.5 Hz, 3H, CH_3-7), 0.93 (d, *J* = 8.2 Hz, 3H, CH_3-7). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 171.9 (C=O), 170.5 (C=O), 169.1 (C=O), 156.3 (C=O), 136.0 (C_{Ar}), 128.6 (CH_{Ar}), 128.32 (CH_{Ar}), 128.27 (CH_{Ar}), 74.3 (C10), 69.1 (C2), 67.3 (C12), 61.5 (C9), 55.1 (C8), 51.6 (C4), 41.4 (C3), 40.8 (C5), 27.3 (C11), 24.6 (C6), 23.1 (C7), 21.7 (C7, C1). **IR**: ν [cm^{-1}] 3320, 3257, 3070, 2973, 2870, 1744, 1725, 1703, 1664, 1643, 1538, 1469, 1400, 1368, 1264, 1236, 1199, 1147, 1103, 1064, 1040, 872, 746, 698, 622. **LRMS** (ESI+) *m/z*, (%): 452 (5, $[M+H–isobutylene]^+$), 508 (15, $[M+H]^+$), 530 (100, $[M+Na]^+$), 1037 (20, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{26}H_{41}N_3O_7Na$: 530.2837, found: 530.2833.

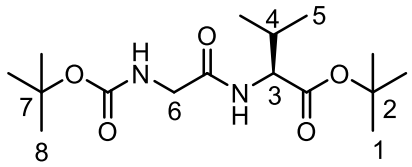
Methyl *N*-(*N*-(*tert*-butyloxycarbonyl)-L-valyl)glycinate (4r)



Prepared following general procedure **A** from *N*-(*tert*-butyloxycarbonyl)-L-valine and methyl glycinate•HCl, affording **4r** (656 mg, 76%) as colorless oil.

R_f = 0.45 (20% EtOAc/PE). $[\alpha]^{20}_{589}$ = +18.8 (c 0.883, $CHCl_3$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.47 (s, 1H, NH), 5.03 (d, *J* = 6.1 Hz, 1H, NH), 4.09-4.07 (m, 2H, CH_2-2), 4.01 (dd, *J* = 8.1, 6.4 Hz, 1H, CH-3), 3.78 (s, 3H, CH_3-1), 2.26-2.18 (m, 1H, CH-4), 1.47 (s, 9H, CH_3-7), 1.01 (d, *J* = 6.8 Hz, 3H, CH_3-5), 0.95 (d, *J* = 6.8 Hz, 3H, CH_3-5). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 171.9 (C=O), 170.1 (C=O), 155.9 (C=O), 80.1 (C6), 59.9 (C3), 52.4 (C1), 41.1 (C2), 30.7 (C4), 28.3 (C7), 19.2 (C5), 17.6 (C5). **IR**: ν [cm^{-1}] 3314, 2959, 2872, 1750, 1686, 1656, 1523, 1386, 1365, 1298, 1246, 1210, 1166, 1020, 914, 845, 704, 661. **LRMS** (ESI+) *m/z*, (%): 233 (10, $[M+H–isobutylene]^+$), 255 (10, $[M+Na–isobutylene]^+$), 289 (5, $[M+H]^+$), 311 (100, $[M+Na]^+$), 599 (25, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{13}H_{24}N_2O_5Na$: 311.1577, found: 311.1577.

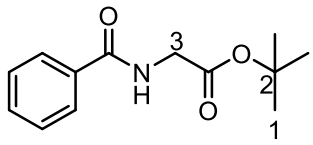
***tert*-Butyl *N*-(*N*-(*tert*-butyloxycarbonyl)-L-glycyl)valinate (4s)**



Prepared following general procedure **A** from *N*-(*tert*-butyloxycarbonyl)glycine and *tert*-butyl valinate•HCl, affording **4s** (1.1 g, 97%) as colorless oil.

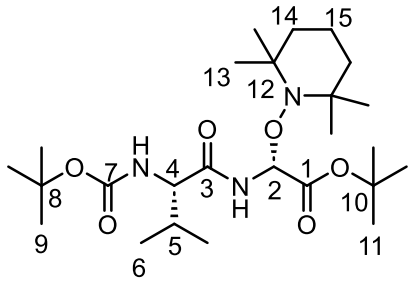
R_f = 0.5 (50% EtOAc/ Hex). $[\alpha]^{20}_{589}$ = +23.2 (c 1.01, $CHCl_3$). **^{1}H NMR** (400 MHz; $CDCl_3$) δ = 6.56 (d, *J* = 9.2 Hz, 1H, NH_{Val}), 5.15 (br s, 1H, NH_{Gly}), 4.47 (dd, *J* = 8.9, 4.5 Hz, 1H, CH-3), 3.89 (dd, *J* = 16.7, 5.6 Hz, 1H, CH_2-6), 3.79 (dd, *J* = 16.7, 5.6 Hz, 1H, CH_2-6), 2.24-2.12 (m, 1H, CH-4), 1.48 (s, 18H, CH_3-1, CH_3-8), 0.95 (d, *J* = 6.8 Hz, 3H, CH_3-5), 0.91 (d, *J* = 7.0 Hz, 3H, CH_3-5). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 170.9 (C=O), 169.2 (C=O), 82.1 (C7, C2), 57.2 (C3), 44.4 (C6), 31.5 (C4), 28.3 (C1), 28.0 (C8), 18.9 (C5), 17.5 (C5). **IR**: ν [cm^{-1}] 3319, 2973, 2933, 1721, 1665, 1514, 1457, 1392, 1367, 1313, 1278, 1249, 1144, 1051, 1029, 847, 785. **LRMS** (ESI+) *m/z*, (%): 331 (10, $[M+H]^+$), 353 (100, $[M+Na]^+$).**HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{16}H_{30}N_2O_5Na$: 353.2047, found: 353.2049.

***tert*-Butyl *N*-benzoylglycinate (4t)**



Sat. K_2CO_3 solution (20 mL) was added to a suspension of *tert*-butyl glycinate hydrochloride (4.02 g, 23.90 mmol) in Et_2O (15 mL) at 0 °C. Benzoyl chloride (4.20 mL, 35.80 mmol) was added dropwise and the mixture was stirred at 0 °C. After 5 min, a white clump formed, CH_2Cl_2 (15 mL) was added to dissolve it and the clear solution was stirred at 0 °C for 3 h. After completion, the reaction mixture was diluted with DCM (50 mL) and water (50 mL). The layers were separated and the aqueous was extracted with DCM (3x30 mL). The combined organic layers were washed with sat. $NaHCO_3$ solution (50 mL), water (50 mL) and brine (50 mL), dried over $MgSO_4$ and the solvent was removed at reduced pressure. Drying under high vacuum yielded **4t** (4.95 g, 88%) as an amorphous white solid that was used without further purification. **R_f** = 0.6 (50% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.85-7.82 (m, 2H, CH_{Ar}), 7.55-7.52 (m, 1H, CH_{Ar}), 7.48-7.44 (m, 2H, CH_{Ar}), 6.69 (br s, 1H, NH), 4.17 (d, *J* = 4.9 Hz, 2H, CH_2-3), 1.53 (s, 9H, CH_3-1). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 169.3 (C=O), 167.3 (C=O), 133.9 (C_{Ar}), 131.7 (CH_{Ar}), 128.6 (CH_{Ar}), 127.0 (CH_{Ar}), 82.6 (C2), 42.5 (C3), 28.1 (C1). **IR**: v [cm^{-1}] 3304, 3003, 2974, 2935, 1748, 1725, 1637, 1535, 1366, 1235, 1218, 1144, 833, 753, 730, 692, 635, 615. **LRMS** (ESI+) *m/z*, (%): 180 (20, $[M+H–isobutylene]^+$), 202 (30, $[M+Na–isobutylene]^+$), 258 (100, $[M+Na]^+$), 493 (20, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{13}H_{17}NO_3Na$: 258.1101, found: 258.1101.

***tert*-Butyl *N*-(*S*)-(*N*-(*tert*-butyloxycarbonyl)-L-valyl)-(2*R* and 2*S*)-((2,2,6,6-tetramethyl piperidin-1-yl)oxy)glycinate (5h)**



Prepared according to general procedure **C**, starting from *tert*-butyl *N*-(*N*-(*tert*-butyloxycarbonyl)-L-valyl)glycinate (**4h**) (100 mg, 0.303 mmol) and KHMDS (0.91 mL, 0.908 mmol, 1 M in THF) as base, affording **5h** (110 mg, 82%) as partially separable 2:1 diastereomeric mixture as amorphous white solid. The minor diastereomer was recrystallized in EtOAc/hexane (1:50) giving colorless needle-like crystals; the major diastereomer was obtained as colorless oil.

Major diastereomer (S^{Val},R^{Gly})

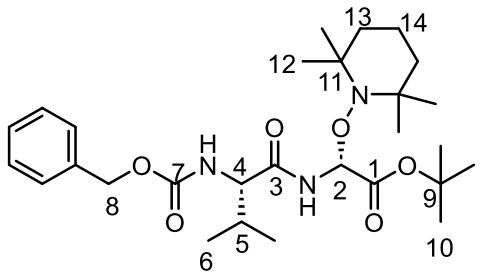
R$_f$ = 0.5 (10% EtOAc/PE). $[\alpha]^{20}_{589}$ = +9.5 (c 1, $CHCl_3$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.96 (d, *J* = 9.6 Hz, 1H, NH), 5.85 (d, *J* = 9.6 Hz, 1H, CH-2), 4.89 (d, *J* = 9.0 Hz, 1H, NH), 4.07 (dd, *J* = 9.1, 4.8 Hz, 1H, CH-4), 2.35-2.30 (m, 1H, CH-5), 1.54-1.27 (m, 6H, CH_2-14, CH_2-15), 1.50 (s, 9H, CH_3-11), 1.47 (s, 9H, CH_3-9), 1.28 (s, 3H, CH_3-13), 1.15 (s, 3H, CH_3-13), 1.12 (s, 3H, CH_3-13), 1.06 (s, 3H, CH_3-13), 1.01 (d, *J* = 6.8 Hz, 3H, CH_3-6), 0.90 (d, *J* = 6.7 Hz, 3H, CH_3-6). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 171.1 (C=O), 167.3 (C=O), 155.7 (C=O), 82.6 (C2), 82.5 (C10), 80.1 (C8), 60.9 (C12), 59.8 (C4), 59.4 (C12), 40.13 (C14), 40.06 (C14), 33.9 (C13), 32.9 (C13), 29.9 (C5), 28.3 (C11), 27.9 (C9), 20.1 (C13), 20.0 (C13), 19.5 (C6), 17.1 (C15), 17.0 (C6).

Minor diastereomer (S^{Val},S^{Gly})

R$_f$ = 0.5 (10% EtOAc/PE). **m.p.** 170-172 °C. $[\alpha]^{20}_{589}$ = –42.2 (c 0.071, $CHCl_3$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.62 (d, *J* = 10.0 Hz, 1H, NH), 5.87 (d, *J* = 9.9 Hz, 1H, CH-2), 5.11 (d, *J* = 9.1 Hz, 1H, NH), 3.96 (dd, *J* = 9.0, 6.1 Hz, 1H, CH-4), 2.14-2.09 (m, 1H, CH-5), 1.52-1.29 (m, 6H, CH_2-14, CH_2-15), 1.50 (s, 9H, CH_3-11), 1.45 (s, 9H, CH_3-9), 1.29 (s, 3H, CH_3-13), 1.15 (s, 3H, CH_3-13), 1.10 (s, 3H, CH_3-13), 1.06 (s, 3H, CH_3-13), 0.96 (d, *J* = 7.0 Hz, 3H, CH_3-6), 0.94 (d, *J* = 7.2 Hz, 3H, CH_3-6). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 170.8 (C=O), 167.1 (C=O), 155.6 (C=O), 82.6 (C2), 79.7 (C10, C8), 60.9 (C12), 59.9 (C4), 59.3 (C12), 40.1 (C14), 33.7 (C13), 32.8 (C13), 31.1 (C5), 28.3 (C11), 27.9 (C9), 20.2 (C13), 20.0 (C13), 19.1 (C6), 17.8 (C6), 17.1 (C15). **IR**: ν [cm^{-1}] 3366, 3318, 2975, 2932, 2870, 1743, 1712, 1664, 1518, 1364,

1232, 1155, 1133, 1073, 1002, 734, 703. **LRMS** (ESI+) *m/z*, (%): 486 (95, $[M+H]^+$), 508 (100, $[M+Na]^+$), 993 (15, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{25}H_{47}N_3O_6Na$: 508.3357, found: 508.3353.

***tert*-Butyl *N*-(*N*-(benzyloxycarbonyl)-L-valyl)-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy) glycinate (5i)**



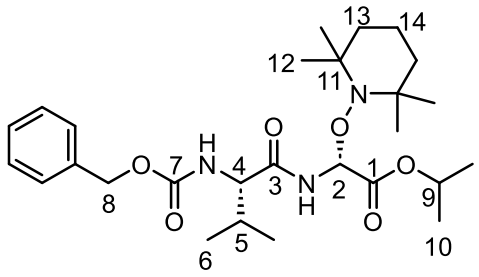
Prepared according to general procedure **C**, starting from *tert*-butyl *N*-(*N*-(benzyloxycarbonyl)-L-valyl)glycinate (**4i**) (280 mg, 0.768 mmol) and KHMDS (1 M in THF, 2.4 mL, 2.382 mmol) as the base, affording **5i** (328 mg, 82%) as inseparable 2:1 diastereomeric mixture as amorphous white solid.

R_f = 0.55 (10% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.42-7.31 (m, 10H, CH_{Ar}, CH_{Ar}*), 6.83 (d, *J* = 9.7 Hz, 1H, NH_{Gly}), 6.55 (d, *J* = 9.9 Hz, 1H, NH_{Gly}*), 5.89 (d, *J* = 9.7 Hz, 1H, CH-2*), 5.83 (d, *J* = 9.4 Hz, 1H, CH-2), 5.39 (d, *J* = 8.9 Hz, 1H, NH_{Val}*), 5.18 (d, *J* = 8.9 Hz, 1H, NH_{Val}), 5.14 (br s, 4H, CH_2-8, CH_2-8*), 4.14 (dd, *J* = 9.0, 4.9 Hz, 1H, CH-4), 4.05 (dd, *J* = 8.7, 6.1 Hz, 1H, CH-4*), 2.37-2.29 (m, 1H, CH-5), 2.18-2.09 (m, 1H, CH-5*), 1.58-1.34 (m, 12H, CH_2-13, CH_2-14, CH_2-13*, CH_2-14*), 1.51 (s, 9H, CH_3-10*), 1.50 (s, 9H, CH_3-10), 1.28 (br s, 6H, CH_3-12), 1.16 (s, 6H, CH_3-12*), 1.11 (br s, 6H, CH_3-12*), 1.05 (br s, 6H, CH_3-12), 1.01 (d, *J* = 6.9 Hz, 3H, CH_3-6), 0.98 (d, *J* = 6.8 Hz, 3H, CH_3-6*), 0.95 (d, *J* = 6.8 Hz, 3H, CH_3-6*), 0.91 (d, *J* = 6.8 Hz, 3H, CH_3-6). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 170.6 (C=O), 170.3 (C=O*), 167.2 (C=O), 167.0 (C=O*), 156.2 (C=O, C=O*), 136.7 (C_{Ar}, C_{Ar}*), 128.6 (CH_{Ar}), 128.5 (CH_{Ar}*), 128.3 (CH_{Ar}), 128.2 (CH_{Ar}, CH_{Ar}*), 128.1 (CH_{Ar}*), 82.9 (C2), 82.8 (C9*), 82.7 (C9), 82.5 (C2*), 67.2 (C8), 67.0 (C8*), 60.9 (C11, C11*), 60.4 (C4*), 60.3 (C4), 59.3 (C11, C11*), 40.12 (C13, C13*), 40.07 (C13, C13*), 33.7 (C12, C12*), 32.9 (C12, C12*), 31.3 (C5), 30.2 (C5*), 27.9 (C10, C10*), 20.2 (C12, C12*), 20.0 (C12, C12*), 19.6 (C6), 19.1 (C6*), 17.8 (C6), 17.10 (C14), 17.09 (C14*), 17.0 (C6*). **IR**: ν [cm^{-1}] 3303, 2974, 2934, 1737, 1692, 1670, 1526, 1371, 1233, 1156, 1043, 846, 736, 698, 634, 608. **LRMS** (ESI+) *m/z*, (%): 520 (100, $[M+H]^+$), 542 (45, $[M+Na]^+$), 1061 (10, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{28}H_{45}N_3O_6Na$: 542.3201, found: 542.3198.

α-Oxygenation of the zinc enolate: KHMDS solution (0.76 mL, 0.76 mmol, 1 M in THF) or freshly prepared LiHMDS solution (*n*-BuLi (0.5 mL, 0.76 mmol, 1.6 M in hexane) and HMDS (0.16 mL, 0.76 mmol) in THF (1 mL) at −78 °C) was added dropwise at –78 °C to a solution of glycine derivative **4i** (84 mg, 0.23 mmol) and $ZnCl_2$ (0.43 mL, 0.3 mmol, 0.7 M in THF) in THF (1.5 mL) and the mixture was stirred for 1 h. TEMPO (80 mg, 0.51 mmol) was added, the solution was warmed to 0 °C and stirred for 3 h. The reaction was quenched by addition of a few drops of sat. NH_4Cl solution and the mixture was filtered through a short pad of Celite®, which was washed with EtOAc. The solvent was removed at reduced pressure and the crude product was purified by column chromatography (10% EtOAc/cyclohexane) to obtain the α-oxygenated glycinate **5i** (50 mg, 42%) as inseparable 2:1 diastereomeric mixture as amorphous white solid and unreacted starting material was recovered (30 mg, 35%).

α-Oxygenation of the titanium enolate: KHMDS solution (1.8 mL, 1.8 mmol, 1 M in THF) was added dropwise to a solution of glycine derivative **4i** (200 mg, 0.55 mmol) in THF (3 mL) at –78 °C and the mixture was stirred for 15 min. $(O^iPr)_3Ti\,Cl$ (0.6 mL, 0.60 mmol, 1 M in hexane) was added dropwise to obtain a reddish brown solution and the mixture was stirred for 1 h. TEMPO (189 mg, 1.21 mmol) was added, the solution was warmed to 0 °C and stirred for 3 h. The reaction was quenched by addition of a few drops of sat. NH_4Cl solution and the mixture was filtered through a short pad of Celite®, which was washed with EtOAc. The solvent was removed at reduced pressure and the crude product was purified by column chromatography (10% EtOAc/cyclohexane) to obtain the α-oxygenated glycinate **5i** (100 mg, 35%) as inseparable 2:1 diastereomeric mixture as amorphous white solid and unreacted starting material was recovered (19 mg, 10%).

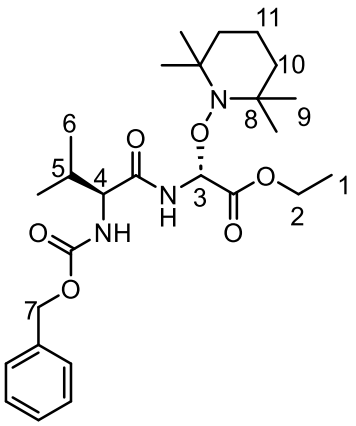
Isopropyl *N*-(*N*-(benzyloxycarbonyl)-L-valyl)-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy) glycinate (5j)



Prepared according to general procedure **C**, starting from isopropyl *N*-(*N*-(benzyloxycarbonyl)-L-valyl)glycinate (**4j**) (310 mg, 0.885 mmol) and KHMDS (1 M in THF, 1.4 mL, 1.413 mmol) as base, providing **5j** (304 mg, 68%) as inseparable 2:1 diastereomeric mixture as amorphous white solid.

R_f = 0.55 (10% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.41-7.33 (m, 10H, CH_{Ar}, CH_{Ar}*), 6.85 (d, *J* = 9.6 Hz, 1H, NH), 6.63 (d, *J* = 9.9 Hz, 1H, NH*), 5.96 (d, *J* = 9.8 Hz, 1H, CH-2*), 5.89 (d, *J* = 9.5 Hz, 1H, CH-2), 5.38 (d, *J* = 9.0 Hz, 1H, NH), 5.20 (d, *J* = 9.5 Hz, 1H, NH), 5.14 (s, 4H, CH_2-8, CH_2-8*), 5.11-5.05 (m, 2H, CH-9, CH-9*), 4.14 (dd, *J* = 9.4, 5.0 Hz, 1H, CH-4), 4.06 (dd, *J* = 9.0, 6.1 Hz, 1H, CH-4*), 2.35-2.27 (m, 1H, CH-5), 2.18-2.09 (m, 1H, CH-5*), 1.58-1.33 (m, 12H, CH_2-13, CH_2-14, CH_2-13*, CH_2-14*), 1.31 (d, *J* = 6.4 Hz, 6H, CH_3-10), 1.28 (d, *J* = 6.3 Hz, 6H, CH_3-10*), 1.28 (s, 3H, CH_3-12*), 1.24 (s, 6H, CH_3-12*), 1.15 (s, 9H, CH_3-12, CH_3-12*), 1.07 (s, 3H, CH_3-12), 1.05 (s, 3H, CH_3-12), 1.02 (d, *J* = 6.8 Hz, 3H, CH_3-6), 0.98 (d, *J* = 6.8 Hz, 3H, CH_3-6*), 0.95 (d, *J* = 6.8 Hz, 3H, CH_3-6*), 0.91 (d, *J* = 6.9 Hz, 3H, CH_3-6). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 170.6 (C=O), 170.4 (C=O*), 166.4 (C=O, C=O*), 155.9 (C=O*), 155.8 (C=O), 136.3 (C_{Ar}), 136.1 (C_{Ar}*), 128.6 (CH_{Ar}, CH_{Ar}*), 128.5 (CH_{Ar}*), 128.3 (CH_{Ar}), 128.2 (CH_{Ar}), 128.1 (CH_{Ar}*), 82.6 (C2), 82.3 (C2*), 69.6 (C9*), 69.5 (C9), 67.3 (C8*), 67.0 (C8), 60.9 (C11), 60.8 (C11*), 60.4 (C4), 60.3 (C4*), 59.4 (C11, C11*), 40.1 (C13, C13*), 33.7 (C12), 33.6 (C12), 32.9 (C12*), 32.8 (C12*), 31.3 (C5*), 30.3 (C5), 21.7 (C10), 21.6 (C10*), 20.2 (C12), 20.03 (C12), 19.98 (C12*) 19.95 (C12*), 19.5 (C6), 19.1 (C6*), 17.8 (C6, C6*), 17.09 (C14), 17.08 (C14*). **IR**: v [cm^{-1}] 3357, 3314, 2972, 2936, 2874, 1736, 1661, 1647, 1552, 1517, 1227, 1211, 1100, 1065, 1025, 1011, 992, 828, 736, 698, 653. **LRMS** (ESI+) *m/z*, (%): 506 (100, $[M+H]^+$), 528 (85, $[M+Na]^+$), 1033 (5, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{27}H_{49}N_3O_6Na$: 528.3044, found: 528.3041.

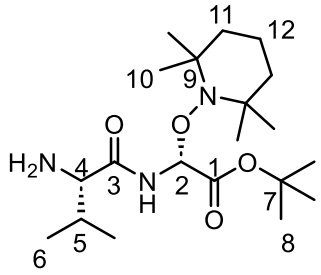
Ethyl *N*-(*N*-(benzyloxycarbonyl)-L-valyl)-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy) glycinate (5k)



Ethyl-*N*-(*N*-(benzyloxycarbonyl)-L-valyl) glycinate (**4k**) (550 mg, 1.63 mmol) was subjected to general procedure **C** with LHMDS as base (3 equiv.) to obtain the product **5k** (500 mg, 62%) as colorless oil as inseparable 2:1 diastereomeric mixture.

R_f = 0.6 (40% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.45-7.32 (m, 10H, $CH_{Ar,}$ CH_{Ar}*), 6.89 (d, *J* = 9.1 Hz, 1H, NH_{Gly}), 6.74 (d, *J* = 8.9 Hz, 1H, NH^*_{Gly}), 5.99 (d, *J* = 9.1 Hz, 1H, CH-3*), 5.91 (d, *J* = 8.9 Hz, 1H, CH-3), 5.38 (d, *J* = 9.0 Hz, 1H, NH^*_{Val}), 5.24 (d, *J* = 8.9 Hz, 1H, NH_{Val}), 5.14 (s, 4H, CH_2-7, CH_2-7*), 4.29-4.20 (m, 4H, CH_2-2, CH_2-2*), 4.18-4.05 (m, 2H, CH-4, CH-4*), 2.35-2.23 (m, 1H, CH-5), 2.19-2.10 (m, 1H, CH-5*), 1.53-125 (m, 12H, CH_2-10, CH_2-11, CH_2-10*, CH_2-11*), 1.31 (t, *J* = 7.1 Hz, 6H, CH_3-1, CH_3-1*), 1.25 (s, 3H, CH_3-9*), 1.21 (s, 3H, CH_3-9), 1.16 (s, 6H, CH_3-9*), 1.15 (s, 6H, CH_3-9), 1.07 (s, 6H, CH_3-9, CH_3-9*), 1.02 (d, *J* = 6.7 Hz, 3H, CH_3-6), 0.99-0.95 (m, 6H, CH_3-6*), 0.92 (d, *J* = 6.9 Hz, 3H, CH_3-6). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 170.6 (C=O), 170.4 (C=O*), 168.2 (C=O), 168.0 (C=O*), 156.4 (C=O, C=O*), 136.1 ($C_{Ar,}$ C_{Ar}*), 128.6 (CH_{Ar}), 128.5 (CH_{Ar}*), 128.3 (CH_{Ar}), 128.2 (CH_{Ar}*), 128.1 (CH_{Ar}, CH_{Ar}*), 82.6 (C3), 82.3 (C3*), 67.2 (C7), 67.1 (C7*), 61.7 (C2*), 61.6 (C2), 60.8 (C8, C8*), 60.3 (C4*), 60.2 (C4), 59.4 (C8, C8*), 40.1 (C10, C10*), 33.7 (C9, C9*), 32.9 (C9, C9*), 31.2 (C5*), 30.5 (C5), 20.2 (C9, C9*), 20.0 (C9, C9*), 19.5 (C6), 19.1 (C6*), 17.7 (C6, C6*), 17.1 (C11, C11*), 14.1 (C1*), 14.0 (C1). **IR**: v [cm^{-1}] 3303, 2969, 2933, 2873, 1746, 1670, 1524, 1466, 1456, 1375, 1363, 1259, 1213, 1195, 1132, 1093, 1025, 990, 958, 732, 696. **LRMS** (ESI+) *m/z*, (%): 357 (15, $[M+Na–TEMPOH]^+$), 492 ($[M+H]^+$), 514 ($[M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{26}H_{41}N_3O_6Na$: 514.2888, found: 514.2885.

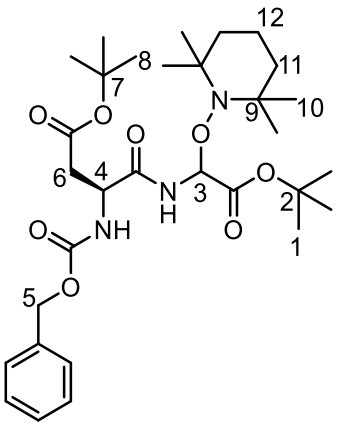
***tert*-Butyl 2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)-*N*-(L-valyl)glycinate (5l)**



Prepared according to general procedure C from tert-butyl N-(N-(9H-fluoren-9-yl-methoxycarbonyl)-L-valyl)glycinate (4l) (100 mg, 0.22 mmol) and KHMDS (1M in THF, 0.67 mL, 0.663 mmol) as base. The crude residue was purified by column chromatography (50% EtOAc/PE gradient to 100% EtOAc) to obtain 5l (63 mg, 74%) as inseparable 2:1 diastereomeric mixture as yellow oil.

R_f = 0.25 (100% EtOAc). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 8.27 (d, *J* = 10.4 Hz, 1H, NH), 7.99 (d, *J* = 9.9 Hz, 1H, NH*), 5.91 (d, *J* = 10.4 Hz, 1H, CH-2), 5.82 (d, *J* = 10.0 Hz, 1H, CH-2*), 3.31 (d, *J* = 3.5 Hz, 1H, CH-4), 3.20 (d, *J* = 4.1 Hz, 1H, CH-4*), 2.42-2.30 (m, 2H, CH-5, CH-5*), 1.57-1.02 (m, 40H, CH_3-10, CH_2-11, CH_2-12, NH_2, CH_3-10*, CH_2-11*, CH_2-12*, NH_2*), 1.51 (s, 9H, CH_3-8), 1.50 (s, 9H, CH_3-8*), 1.01 (d, *J* = 7.0 Hz, 3H, CH_3-6), 0.96 (d, *J* = 6.8 Hz, 3H, CH_3-6*), 0.89 (d, *J* = 6.8 Hz, 3H, CH_3-6*), 0.84 (d, *J* = 6.9 Hz, 3H, CH_3-6). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 173.8 (C=O, C=O*), 167.6 (C=O, C=O*), 83.0 (C2*), 82.5 (C2), 82.3 (C7*), 82.2 (C7), 60.92 (C9), 60.88 (C9*), 60.5 (C4*), 60.0 (C4), 59.24 (C9*), 59.22 (C9), 40.1 (C11, C11*), 34.0 (C10*), 33.7 (C10), 33.0 (C10*), 32.9 (C10), 30.8 (C5), 30.4 (C5*), 28.0 (C8, C8*), 20.2 (C10, C10*), 20.0 (C10, C10*), 19.7 (C6), 19.3 (C6*), 17.1 (C12, C12*), 16.4 (C6*), 15.9 (C6). **IR**: ν [cm^{-1}] 3349, 3330, 3314, 2973, 2931, 2872, 1741, 1686, 1498, 1368, 1157, 1134, 1049, 958, 911, 844, 730, 646. **LRMS** (ESI+) *m/z*, (%): 386 (100, $[M+H]^+$), 408 (10, $[M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{20}H_{39}N_3O_4Na$: 408.2833, found: 408.2834.

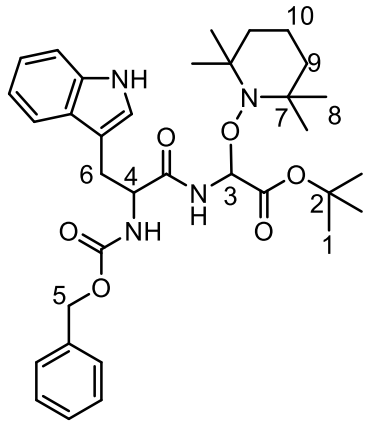
***tert*-Butyl *N*-(*N*-(benzyloxycarbonyl)-O-*tert*-butyl-L-aspartyl)-2-((2,2,6,6-tetramethyl piperidin-1-yl)oxy)glycinate (5m)**



tert-Butyl *N*-(*N*-(benzyloxycarbonyl)-O-*tert*-butyl-L-aspartyl)glycinate (**4m**) (160 mg, 0.37 mmol) was subjected to general procedure **C** to obtain the product **5m** (154 mg, 71%) as colorless liquid as inseparable 1.8:1 diastereomeric mixture.

R_f = 0.6 (20% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.47-7.32 (m, 12H, CH_{Ar}, CH_{Ar}*, NH, NH*), 6.00 (s, 2H, NH, NH*), 5.85 (d, *J* = 9.8 Hz, 1H, CH-3), 5.83 (d, *J* = 9.7 Hz, 1H, CH-3*), 5.16 (s, 4H, CH_2-5, CH_2-5*), 4.62-4.49 (m, 2H, CH-4, CH-4*), 2.99 (dd, *J* = 17.2, 4.3 Hz, 1H, CH_2-6), 2.88 (dd, *J* = 17.1, 4.5 Hz, 1H, CH_2-6*), 2.72-2.53 (m, 2H, CH_2-6, CH_2-6*), 1.58-1.30 (12H, CH_2-11, CH_2-12, CH_2-11*, CH_2-12*), 1.51 (s, 9H, CH_3-8), 1.50 (s, 9H, CH_3-8*), 1.46 (s, 9H, CH_3-1*), 1.45 (s, 9H, CH_3-1), 1.30 (s, 9H, CH_3-10, $2CH_3$-10*), 1.16 (s, 6H, CH_3-10), 1.09 (s, 3H, CH_3-10), 1.05 (s, 6H, CH_3-10*). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 171.1 (C=O), 171.0 (C=O*), 169.94 (C=O), 169.87 (C=O*), 166.9 (C=O), 166.8 (C=O*), 156.2 (C=O, C=O*), 136.2 (C_{Ar*}), 136.1 (C_{Ar}), 128.7 (CH_{Ar}, CH_{Ar}*), 128.40 (CH_{Ar}), 128.37 (CH_{Ar}*), 128.3 ($CH_{Ar,}$ CH_{Ar}*), 83.1 (C3*), 82.8 (C3), 82.63 (C7), 82.59 (C7*), 81.9 (C2, C2*), 67.4 (C5), 67.3 (C5*), 61.1 (C9, C9*), 59.4 (C9, C9*), 51.4 (C4, C4*), 40.2 (C11*), 40.1 (C11), 37.4 (C6*), 37.1 (C6), 33.7 (C10, C10*), 32.9 (C10, C10*), 28.2 (C8, C8*), 28.0 (C1, C1*), 20.2 (C10, C10*), 20.0 (C10, C10*), 17.2 (C12, C12*). **IR**: ν [cm^{-1}] 3321, 2976, 2934, 1732, 1500, 1367, 1241, 1153, 1044, 733, 698. **LRMS** (ESI+) *m/z*, (%): 353 (5, $[M+H–isobutylene]^+$), 375 (20, $[M+Na–isobutylene]^+$), 409 ($[M+H]^+$), 431 ($[M+Na]^+$), 839 ($[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{21}H_{32}N_2O_6Na$: 431.2153, found: 431.2154.

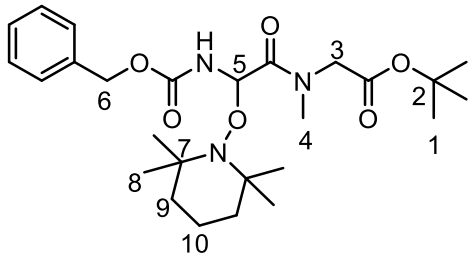
***tert*-Butyl *N*-(*N*-(benzyloxycarbonyl)-DL-tryptophanyl)-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)glycinate (5n)**



tert-Butyl-*N*-(*N*-(benzyloxycarbonyl)-DL-tryptophanyl)glycinate (**4n**) (170 mg, 0.38 mmol) was subjected to general procedure **C** to obtain the product **5n** (169 mg, 74%) as amorphous solid as inseparable racemic mixture.

R_f = 0.6 (20% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 8.07 (s, 2H, NH, NH*), 7.72 (d, *J* = 7.9 Hz, 1H, CH_{Ar}*), 7.63 (d, *J* = 7.9 Hz, 1H, CH_{Ar}), 7.40-7.30 (m, 12H, CH_{Ar}, CH_{Ar}*), 7.24-7.18 (m, 2H, CH_{Ar}, CH_{Ar}*), 7.16-7.10 (m, 2H, CH_{Ar}, CH_{Ar}*), 7.08-7.03 (m, 2H, CH_{Ar}, CH_{Ar}*), 6.97 (d, *J* = 9.7 Hz, 1H, NH*), 6.66 (d, *J* = 9.8 Hz, 1H, NH), 5.84 (d, *J* = 9.9 Hz, 1H, CH-3*), 5.83 (d, *J* = 9.6 Hz, 1H, CH-3), 5.48 (d, *J* = 7.5 Hz, 1H, NH), 5.20 (d, *J* = 8.3 Hz, 1H, NH*), 5.13 (s, 2H, CH_2-5), 5.08 (s, 2H, CH_2-5*), 4.69-4.52 (m, 2H, CH-4, CH-4*), 3.51-3.15 (m, 4H, CH_2-6, CH_2-6*), 1.61-1.29 (m, 12H, CH_2-9, CH_2-10, CH_2-9*, CH_2-10*), 1.47 (s, 9H, CH_3-1*), 1.46 (s, 9H, CH_3-1), 1.29 (s, 6H, CH_3-8, CH_3-8*), 1.15 (s, 3H, CH_3-8*), 1.11 (s, 3H, CH_3-8), 1.03 (s, 6H, CH_3-8, CH_3-8*), 0.97 (s, 3H, CH_3-8), 0.88 (s, 3H, CH_3-8*). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 170.8 (C=O), 170.5 (C=O*), 166.9 (C=O), 166.7 (C=O*), 156.0 (C=O, C=O*), 136.22 (C_{Ar}, C_{Ar}*), 136.16 (C_{Ar}, C_{Ar}*), 128.52 (CH_{Ar}), 128.51 (CH_{Ar}*), 128.2 (CH_{Ar}), 128.14 (CH_{Ar}*), 128.09 (CH_{Ar}, CH_{Ar}*), 127.4 (C_{Ar}, C_{Ar}*), 123.3 (CH_{Ar}, CH_{Ar}*), 122.3 (CH_{Ar}, CH_{Ar}*), 119.8 (CH_{Ar}, CH_{Ar}*), 118.9 (CH_{Ar}*), 118.7 (CH_{Ar}), 111.2 (CH_{Ar}, CH_{Ar}*), 110.4 (C_{Ar}), 110.2 (C_{Ar}*), 82.8 (C3), 82.6 (C2, C2*), 82.5 (C3*), 67.1 (C5), 67.0 (C5*), 61.0 (C7, C7*), 59.3 (C7*), 59.2 (C7), 55.4 (C4, C4*), 40.1 (C9, C9*), 40.0 (C9, C9*), 33.6 (C8), 33.5 (C8*), 32.9 (C8), 32.8 (C8*), 28.3 (C6, C6*), 27.9 (C1, C1*), 20.14 (C8), 20.08 (C8*), 20.0 (C8), 19.9 (C8*), 17.09 (C10*), 17.06 (C10). **IR**: ν [cm^{-1}] 3311, 2975, 2932, 1679, 1621, 1508, 1457, 1368, 1244, 1153, 1101, 1059, 1028, 988, 740, 697. **LRMS** (ESI+) *m/z*, (%): 607 (45, $[M+H]^+$), 629 (100, $[M+Na]^+$), 1235 (25, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{34}H_{46}N_4O_6Na$: 629.3310, found: 629.3308.

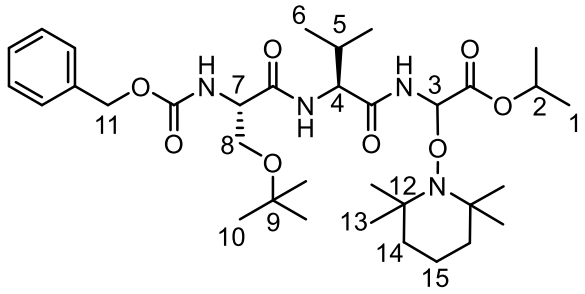
***tert*-Butyl *N*-(*N*-(benzyloxycarbonyl)-L-glycyl)-5-((2,2,6,6-tetramethylpiperidin-1-yl)oxy) sarcosinate (5o)**



Prepared according to general procedure **C**, starting from isopropyl *tert*-butyl-*N*-(*N*-(benzyloxycarbonyl)glycyl sarcosinate (**4o**) (200 mg, 0.59 mmol) and KHMDS (2 mL, 1.96 mmol, 1 M in THF) as base, providing **5o** (160 mg, 55%) as colorless oil as 2:1 rotameric mixture.

R_f = 0.6 (20% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.45-7.31 (m, 10H, CH_{Ar}, A+B), 6.21 (d, *J* = 10.0 Hz, 1H, NH, B), 6.09 (d, *J* = 10.4 Hz, 1H, CH-5, A), 6.00 (d, *J* = 10.4 Hz, 1H, NH, A), 5.88 (d, *J* = 10.0 Hz, 1H, CH-5, B), 5.25-5.07 (m, 4H, CH_2-6, A+B), 4.73 (d, *J* = 18.1 Hz, 1H, CH_2-3, B), 4.17 (d, *J* = 17.0 Hz, 1H, CH_2-3, A), 3.97 (d, *J* = 18.0 Hz, 1H, CH_2-3, B), 3.93 (d, *J* = 17.0 Hz, 1H, CH_2-3, A), 3.24 (s, 3H, CH_3-4, A), 3.01 (s, 3H, CH_3-4, B), 1.60-1.28 (m, 12H, CH_2-9, CH_2-10, A+B), 1.48 (s, 9H, CH_3-1, B), 1.47 (s, 9H, CH_3-1, A) 1.26 (s, 3H, CH_3-8, A), 1.22 (s, 3H, CH_3-8, B), 1.18 (s, 3H, CH_3-8, B), 1.16 (s, 6H, CH_3-8, A), 1.14 (s, 3H, CH_3-8, B), 1.08 (s, 6H, CH_3-8, A+B). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 168.2 (C=O, A+B), 167.6 (C=O, A+B), 155.3 (C=O, A+B), 136.4 (C_{Ar}, A+B), 128.5 (CH_{Ar}, A), 128.4 (CH_{Ar}, B), 128.09 (CH_{Ar}, B), 128.07 (CH_{Ar}, A), 128.0 (CH_{Ar}, A+B), 82.9 (C2, B), 82.0 (C2, A), 80.5 (C5, A+B), 66.9 (C6, A), 66.8 (C6, B), 60.6 (C7, A+B), 59.6 (C7, A+B), 52.1 (C3, B), 50.5 (C3, A), 40.2 (C9, A+B), 40.0 (C9, A+B), 36.3 (C4, A), 35.2 (C4, B), 33.4 (C8, A), 33.1 (C8, B), 32.8 (C8, A), 32.6 (C8, B), 28.1 (C1, A), 28.0 (C1, B), 20.6 (C8, B), 20.5 (C8, A), 20.4 (C8, A+B), 17.2 (C10, A), 17.1 (C10, B). **IR**: v [cm^{-1}] 3308, 2975, 2933, 1737, 1659, 1496, 1466, 1455, 1406, 1394, 1367, 1339, 1227, 1156, 1132, 1047, 1025, 975, 955, 918, 844, 774, 743, 698. **LRMS** (ESI+) *m/z*, (%): 492 (35, $[M+H]^+$), 514 (100, $[M+Na]^+$), 1005 (15, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{26}H_{41}N_3O_6Na$: 514.2888, found: 514.2886.

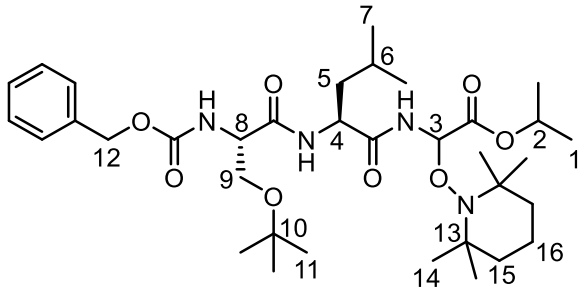
Cbz-Ser(O*t*Bu)-Val-Gly(OTMP)-O*i*Pr (5p)



Cbz-Ser(O*t*Bu)-Val-Gly-O*i*Pr (**4p**) (200 mg, 0.41 mmol) was subjected to general procedure **C** with LHMDS as base (4 equiv.) to obtain the product **5p** (239 mg, 91%) as amorphous solid as inseparable 2:1 diastereomeric mixture.

R_f = 0.6 (40% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.48-7.31 (m, 10H, CH_{Ar}), 7.03-6.89 (br s, 3H, NH_{Val}, NH_{Val}*, NH_{Gly}), 6.80 (d, *J* = 9.7 Hz, 1H, NH_{Gly}*), 5.92 (d, *J* = 9.8 Hz, 1H, CH-3*) , 5.89 (d, *J* = 9.5 Hz, 1H, CH-3), 5.74 (br s, 2H, NH_{Ser}, NH_{Ser}*), 5.15 (s, 4H, CH_2-11, CH_2-11*), 5.10-4.97 (m, 2H, CH-2, CH-2*), 4.40 (dd, *J* = 9.2, 5.1 Hz, 1H, CH-4), 4.34 (dd, *J* = 8.9, 6.0 Hz, 1H, CH-4*), 4.33-4.24 (m, 2H, CH-7, CH-7*), 3.89-3.86 (m, 2H, CH_2-8, CH_2-8*), 3.42 (dd, *J* = 15.9, 8.0 Hz, 2H, CH_2-8, CH_2-8*), 2.45-2.31 (m, 1H, CH-5), 2.20-2.18 (m, 1H, CH-5*), 1.59-1.28 (m, 12H, CH_2-14, CH_2-15, CH_2-14*, CH_2-15*), 1.30-1.26 (m, 12H, CH_3-1, CH_3-1*), 1.24 (s, 9H, CH_3-13, CH_3-13*), 1.22 (s, 18H, $CH_3$10, CH_3-10*), 1.14 (s, 9H, CH_3-13, CH_3-13*), 1.07 (6H, CH_3-13*), 0.99 (d, *J* = 6.8 Hz, 3H, CH_3-6), 0.95 (d, *J* = 6.8 Hz, 6H, CH_3-6*), 0.91 (d, *J* = 6.9 Hz, 3H, CH_3-6). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 170.5 (C=O), 170.2 (C=O, C=O*), 169.8 (C=O*), 167.7 (C=O), 167.6 (C=O*), 156.2 (C=O, C=O*), 136.13 (C_{Ar}*), 136.08 (C_{Ar}), 128.6 (CH_{Ar}, CH_{Ar}*), 128.3 (CH_{Ar}, CH_{Ar}*), 128.2 (CH_{Ar}, CH_{Ar}*), 82.8 (C3), 82.5 (C3*), 74.6 (C9), 74.4 (C9*), 69.5 (C2*), 69.4 (C2), 67.3 (C11), 67.2 (C11*), 61.7 (C8*), 61.6 (C8), 60.8 (C12, C12*), 59.5 (C12), 59.4 (C12*), 58.6 (C4*), 58.3 (C4), 54.9 (C7), 54.6 (C7*), 40.1 (C14, C14*), 33.8 (C13), 33.7 (C13*), 32.9 (C13), 32.8 (C13*), 30.9 (C5*), 29.8 (C5), 27.4 (C10, C10*), 21.6 (C1, C1*), 20.2 (C13, C13*), 20.0 (C13, C13*), 19.6 (C6), 19.1 (C6*), 17.9 (C6*), 17.2 (C6), 17.1 (C15, C15*). **IR**: ν [cm^{-1}] 3288, 2973, 2931, 2872, 1744, 1707, 1648, 1533, 1467, 1375, 1363, 1263, 1229, 1197, 1105, 1092, 1062, 1027, 910, 733, 696. **LRMS** (ESI+) *m/z*, (%): 514 (5, $[M+Na–TEMPOH]^+$), 649 ($[M+H]^+$), 671 ($[M+Na]^+$), 1319 ($[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{34}H_{56}N_4O_8Na$: 671.3990, found: 671.3989.

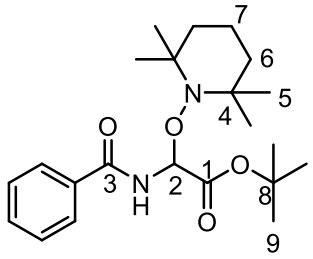
Cbz-Ser(*t*Bu)-Leu-Gly(OTMP)-O*i*Pr (5q)



Cbz-Ser(O*t*Bu)-Leu-Gly-O*i*Pr (**4q**) (200 mg, 0.39 mmol) was subjected to general procedure **C** to obtain the product **5q** (218 mg, 83%) as colorless solid as inseparable 2:1 diastereomeric mixture.

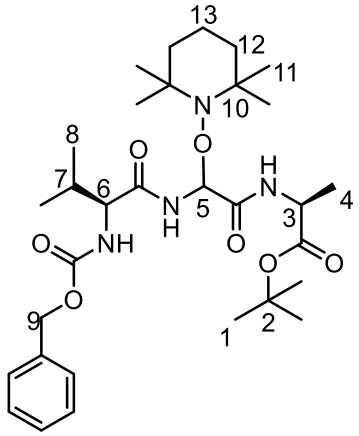
R_f = 0.6 (20% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.43-7.31 (m, 10H, CH_{Ar}, CH_{Ar}*), 7.13 (d, *J* = 9.7 Hz, 1H, NH_{Gly}), 7.01 (d, *J* = 9.6 Hz, 1H, NH_{Gly}*), 6.97 (d, *J* = 8.0 Hz, 1H, NH_{Leu}*), 6.87 (d, *J* = 8.7 Hz, 1H, NH_{Leu}), 5.91 (d, *J* = 9.8 Hz, 2H, CH-3, CH-3*), 5.69 (s, 2H, NH_{Ser}, NH_{Ser}*), 5.16 (s, 4H, CH_2-12, CH_2-12*), 5.11-4.98 (m, 2H, CH-2, CH-2*), 4.63-4.46 (m, 2H, CH-4, CH-4*), 4.29-4.25 (m, 2H, CH-8, CH-8*), 3.91-3.72 (m, 2H, CH_2-9, CH_2-9*), 3.56-3.25 (m, 2H, CH_2-9, CH_2-9*), 1.84-1.73 (m, 2H, CH_2-5, CH_2-5*), 1.70-1.63 (m, 2H, CH-6, CH-6*), 1.57-1.50 (m, 2H, CH_2-5, CH_2-5*), 1.48-1.33 (m, 12H, CH_2-15, CH_2-16, CH_2-15*, CH_2-16*), 1.31 (s, 3H, CH_3-14*), 1.29 (s, 3H, CH_3-14), 1.28 (d, *J* = 6.4 Hz, 12H, CH_3-1, CH_3-1*), 1.24 (s, 9H, CH_3-11*), 1.19 (s, 9H, CH_3-11), 1.14 (s, 12H, CH_3-14, CH_3-14*), 1.07 (s, 3H, CH_3-14), 1.06 (s, 3H, CH_3-14*), 0.97-0.90 (m, 12H, CH_3-7, CH_3-7*). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 171.0 (C=O), 170.7 (C=O*), 170.5 (C=O), 170.1 (C=O*), 167.5 (C=O, C=O*), 156.1 (C=O, C=O*), 136.1 (C_{Ar}, C_{Ar}*), 128.6 (CH_{Ar}, CH_{Ar}*), 128.32 (CH_{Ar}, CH_{Ar}*), 128.26 (CH_{Ar}, CH_{Ar}*), 82.5 (C3, C3*), 74.3 (C10, C10*), 69.41 (C2*), 69.37 (C2), 67.3 (C12), 67.2 (C12*), 61.5 (C9, C9*), 60.92 (C13), 60.87 (C13*), 59.4 (C13), 59.3 (C13*), 54.8 (C8, C8*), 52.0 (C4*), 51.6 (C4), 41.0 (C5*), 40.3 (C5), 40.1 (C15, C15*), 33.8 (C14, C14*), 32.9 (C14, C14*), 27.4 (C11*), 27.3 (C11), 24.7 (C7*), 24.5 (C7), 23.1 (C7), 23.0 (C7*), 21.72 (C1*), 21.69 (C1), 21.66 (C1), 21.60 (C1*), 20.2 (C14, C14*), 20.0 (C14, C14*), 17.11 (C16), 17.09 (C16*). **IR**: v [cm^{-1}] 3297, 2974, 2934, 2871, 1733, 1650, 1525, 1468, 1455, 1375, 1364, 1195, 1147, 1103, 1060, 1027, 1027, 985, 697. **LRMS** (ESI+) *m/z*, (%): 528 (5, [M+Na–TEMPOH]$^+$), 663 (100, [M+H]$^+$), 685 (40, [M+Na]$^+$), 1347 ([2M+Na]$^+$). **HRMS** (ESI+) *m/z*: ([M+Na]$^+$): calculated for $C_{35}H_{58}N_4O_8Na$: 685.4146, found: 685.4141.

***tert*-Butyl *N*-benzoyl-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)glycinate (5t)**[125]



Prepared according to general procedure C starting from *tert*-butyl *N*-benzoylglycinate (**4t)** (500 mg, 2.12 mmoles), obtaining **5t** (640 mg, 78%) as amorphous white solid.
R_f = 0.75 (20% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.91-7.79 (m, 2H, CH_{Ar}), 7.60-7.50 (m, 1H, CH_{Ar}), 7.50-7.44 (m, 2H, CH_{Ar}), 7.05 (d, *J* = 9.9 Hz, 1H, NH), 6.16 (d, *J* = 9.9 Hz, 1H, CH-2), 1.64-1.33 (m, 6H, CH_2-6, CH_2-7), 1.54 (s, 9H, CH_3-9), 1.42 (s, 3H, CH_3-5), 1.19 (s, 3H, CH_3-5), 1.13 (s, 3H, CH_3-5), 1.09 (s, 3H, CH_3-5). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 167.4 (C=O), 166.4 (C=O), 134.0 (C_{Ar}), 131.9 (CH_{Ar}), 128.7 (CH_{Ar}), 127.2 (CH_{Ar}), 82.8 (C8), 82.5 (C2), 61.1 (C4), 59.3 (C4), 40.2 (C6), 33.9 (C5), 32.9 (C5), 28.0 (C9), 20.2 (C5), 20.0 (C5), 17.1 (C7). **IR**: v [cm^{-1}] 3350, 2975, 2932, 2873, 1737, 1674, 1522, 1487, 1364, 1259, 1214, 1137, 1088, 1032, 973, 957, 913, 836, 812, 801, 778, 710, 698, 689, 664, 622. **LRMS** (ESI+) *m/z*, (%): 391 (85, $[M+H]^+$), 413 (100, $[M+Na]^+$), 803 (15, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{22}H_{34}N_2O_4Na$: 413.2411, found: 413.2410.

Cbz-Val-Gly(OTMP)-Ala-O*t*Bu (10)



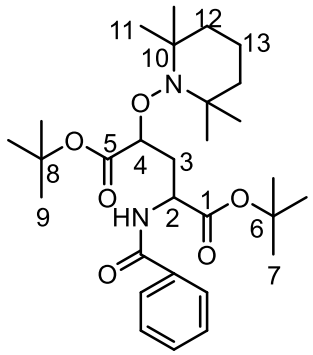
1 N LiOH in H_2O (81 μL, 0.08) was added dropwise to a solution of a diastereomeric mixture of ethyl *N*-(*N*-(benzyloxycarbonyl)-L-valyl)-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)glycinate (**5k)** (40 mg, 0.09 mmol) in THF (1 mL) and the mixture was stirred at rt for 2 h. After completion, the reaction mixture was treated with AmberChrom® 50WX4 hydrogen form to neutralize to pH~6, the residue was filtered, and the filtrate was evaporated to dryness

to obtain the carboxylic acid Cbz-Val-Gly(OTMP)-OH (38 mg, 0.08 mmol) as amorphous solid which was used directly for the peptide coupling.

Triethylamine (25 µL) was added to a mixture of Cbz-Val-Gly(OTMP)-OH (38 mg, 0.08 mmol) and L-alanine *tert*-butyl ester hydrochloride (15 mg, 0.08 mmol) in CH_2Cl_2 (1 mL) followed by the addition of HBTU (34 mg, 0.09 mmol). The reaction mixture was stirred for 3 h, brine was added, and the mixture was extracted with CH_2Cl_2 (2x3 mL). The combined organic extracts were washed with HCl solution (1 N, 1 mL), followed by sat. $NaHCO_3$ solution (1 mL). The organic layer was dried over $MgSO_4$, the solvent was removed at reduced pressure and the residue was purified by flash column chromatography (20% EtOAc/cyclohexane) to give the coupling product **10** (25 mg, 52%) as 2:1 diastereomeric mixture as colorless oil.

R_f = 0.6 (40% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.39-7.30 (m, 11H, CH_{Ar}, CH_{Ar}*, NH_{Ala}*), 7.17 (d, *J* = 7.6 Hz, 1H, NH_{Ala}), 6.97 (d, *J* = 8.3 Hz, 1H, NH_{Gly}), 6.72 (d, *J* = 9.4 Hz, 1H, NH_{Gly}*), 5.89 (d, *J* = 9.4 Hz, 1H, CH-5*), 5.54 (d, *J* = 8.1 Hz, 1H, CH-5), 5.46 (d, *J* = 9.2 Hz, 1H, NH_{Val}*), 5.36 (d, *J* = 9.2 Hz, 1H, NH_{Val}), 5.12 (s, 4H, CH_2-9, CH_2-9*), 4.52-4.40 (m, 2H, CH-3, CH-3*), 4.19-4.03 (m, 2H, CH-6, CH-6*), 2.30-2.19 (m, 1H, CH-7), 2.18-2.10 (m, 1H, CH-7*), 1.58-1.29 (m, 12H, CH_2-12, CH_2-13, CH_2-12*, CH_2-13*), 1.49 (s, 9H, CH_3-1*), 1.47 (s, 9H, CH_3-1), 1.43 (d, *J* = 7.1 Hz, 6H, CH_3-4, CH_3-4*), 1.22 (s, 6H, CH_3-11, CH_3-11*), 1.19 (s, 6H, CH_3-11, CH_3-11*), 1.09 (s, 12H, CH_3-11, CH_3-11*), 1.00 (d, *J* = 6.8 Hz, 3H, CH_3-8), 0.99 (d, *J* = 6.7 Hz, 3H, CH_3-8*), 0.94 (d, *J* = 6.7 Hz, 3H, CH_3-8*), 0.92 (d, *J* = 6.8 Hz, 3H, CH_3-8). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 171.52 (C=O), 171.46 (C=O*), 171.3 (C=O), 171.2 (C=O*), 167.0 (C=O*), 166.9 (C=O), 156.4 (C=O), 156.3 (C=O*), 136.3 (C_{Ar}), 136.2 (C_{Ar}*), 128.51 (CH_{Ar}), 128.50 (CH_{Ar}*), 128.2 (CH_{Ar}), 128.09 (CH_{Ar}*), 128.08 (CH_{Ar}), 128.0 (CH_{Ar}*), 84.1 (C5*), 83.1 (C5), 82.3 (C2*), 82.0 (C2), 67.1 (C9), 66.9 (C9*), 61.0 (C10, C10*), 60.3 (C6), 60.1 (C6*), 59.5 (C10, C10*), 48.9 (C3), 48.8 (C3*), 40.1 (C12*), 40.0 (C12), 33.8 (C11), 33.6 (C11*), 32.9 (C11), 32.8 (C11*), 31.3 (C7*), 30.7 (C7), 28.0 (C1, C1*), 20.6 (C11, C11*), 20.4 (C11), 20.3 (C11*), 19.4 (C8), 19.1 (C8*), 18.6 (C4), 18.4 (C4*), 17.7 (C8*), 17.3 (C8), 17.0 (C13, C13*). **IR**: ν [cm^{-1}] 3290, 2971, 2932, 2873, 1730, 1702, 1651, 1531, 1455, 1368, 1287, 1240, 1147, 1039, 734, 696. **LRMS** (ESI+) *m/z*, (%): 591 (20, $[M+H]^+$), 613 (100, $[M+Na]^+$), 1203 (10, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{31}H_{50}N_4O_7Na$: 613.3572, found: 613.3569.

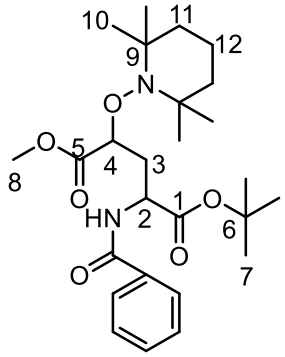
Di-*tert*-butyl *N*-benzoyl-4-(2,2,6,6-tetramethylpiperidin-1-yl)oxy)glutamate (11a)



Prepared according to general procedure **D**, starting from *tert*-butyl *N*-benzoyl-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)glycinate (**5t**) (100 mg, 0.26 mmol) and *tert*-butyl acrylate (0.38 mL, 2.56 mmol) providing **11a** (105 mg, 79%) as inseparable 1:1 diastereomeric mixture as colorless oil.

R_f = 0.65 (20% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.91-7.81 (m, 4H, CH_{Ar}, CH_{Ar}*), 7.54-7.48 (m, 2H, CH_{Ar}, CH_{Ar}*), 7.47-7.38 (m, 4H, CH_{Ar}, CH_{Ar}*), 7.08 (d, *J* = 6.1 Hz, 1H, NH), 6.76 (d, *J* = 8.0 Hz, 1H, NH*), 4.69 (ddd, *J* = 10.3, 8.0, 3.3 Hz, 1H, CH-2*), 4.53-4.49 (m, 1H, CH-2), 4.47 (dd, *J* = 6.5, 4.7 Hz, 1H, CH-4), 4.32 (dd, *J* = 10.0, 3.5 Hz, 1H, CH-4*), 2.50-2.42 (m, 2H, CH_2-3), 2.41-2.29 (m, 2H, CH_2-3*), 1.54-1.23 (m, 12H, CH_2-12, CH_2-13, CH_2-12*, CH_2-13*), 1.51 (s, 9H, CH_3-7), 1.50 (s, 9H, CH_3-7*), 1.49 (s, 9H, CH_3-9), 1.48 (s, 9H, CH_3-9*), 1.23 (s, 3H, CH_3-11), 1.18 (s, 3H, CH_3-11*), 1.15 (s, 9H, CH_3-11), 1.13 (s, 9H, CH_3-11*). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 172.4 (C=O), 171.1 (C=O*), 171.0 (C=O), 170.9 (C=O*), 167.2 (C=O), 167.1 (C=O*), 134.2 (C_{Ar}), 134.1 (C_{Ar}*), 131.5 (CH_{Ar}), 131.4 (CH_{Ar}*), 128.5 (CH_{Ar}), 128.4 (CH_{Ar}*), 127.2 (CH_{Ar}), 127.1 (CH_{Ar}*), 83.9 (C4), 82.8 (C4*), 82.2 (C8), 82.0 (C6, C6*), 81.9 (C8*), 60.1 (C10, C10*), 51.8 (C2), 50.2 (C2*), 40.4 (C12, C12*), 34.8 (C3), 33.9 (C3*), 33.5 (C11, C11*), 28.0 (C7, C7*), 27.9 (C9, C9*), 20.7 (C11, C11*), 20.2 (C11, C11*), 17.09 (C13), 17.06 (C13*). **IR**: ν [cm^{-1}] 3350, 2976, 2932, 1735, 1668, 1525, 1367, 1256, 1231, 1150, 845, 711. **LRMS** (ESI+) *m/z*, (%): 519 (55, $[M+H]^+$), 541 (100, $[M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{29}H_{46}N_2O_6Na$: 541.3248, found: 541.3245.

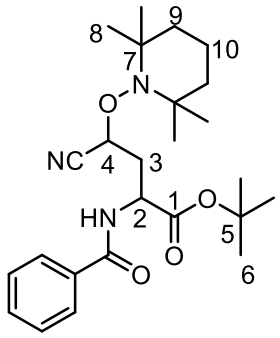
1-*tert*-Butyl 5-methyl *N*-benzoyl-4-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)glutamate (11b)



Prepared according to general procedure **D**, starting from *tert*-butyl *N*-benzoyl-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)glycinate (**5t**) (100 mg, 0.26 mmol) and methyl acrylate (0.12 mL, 1.28 mmol), providing **11b** (52 mg, 43%) as inseparable 1:1 diastereomeric mixture as colorless solid.

R_f = 0.65 (20% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.84-7.81 (m, 4H, CH_{Ar}, CH_{Ar}*), 7.53-7.49 (m, 2H, CH_{Ar}, CH_{Ar}*), 7.48-7.41 (m, 4H, CH_{Ar}, CH_{Ar}*), 6.79 (d, *J* = 7.9 Hz, 1H, NH*), 6.73 (d, *J* = 7.5 Hz, 1H, NH), 4.74 (dt, *J* = 8.1, 4.0 Hz, 1H, CH-2*), 4.68 (td, *J* = 7.6, 5.9 Hz, 1H, CH-2), 4.47 (dd, *J* = 9.3, 4.4 Hz, 1H, CH-4), 4.42 (dd, *J* = 9.6, 3.8 Hz, 1H, CH-4*), 3.64 (s, 6H, CH_3-8, CH_3-8*), 2.65-2.54 (m, 2H, CH_2-3, CH_2-3*), 2.42-2.32 (m, 2H, CH_2-3, CH_2-3*), 1.61-1.22 (m, 12H, CH_2-11, CH_2-11*, CH_2-12, CH_2-12*), 1.51 (s, 18H, CH_3-7, CH_3-7*), 1.22 (s, 6H, CH_3-10, CH_3-10*), 1.14 (s, 3H, CH_3-10), 1.11 (s, 3H, CH_3-10*), 1.09 (br s, 6H, CH_3-10, CH_3-10*), 1.02 (s, 6H, CH_3-10, CH_3-10*). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 173.5 (C=O), 173.0 (C=O*), 170.8 (C=O*), 170.7 (C=O), 167.7 (C=O), 166.8 (C=O*), 134.0 (C_{Ar}), 133.9 (C_{Ar}*), 131.7 (CH_{Ar}), 131.6 (CH_{Ar}*), 128.5 (CH_{Ar}, CH_{Ar}*), 127.1 (CH_{Ar}), 127.0 (CH_{Ar}*), 82.54 (C6), 82.50 (C6*), 82.48 (C4), 82.3 (C4*), 60.4 (C9, C9*), 51.8 (C8), 51.7 (C8*), 50.7 (C2), 50.0 (C2*), 40.2 (C11, C11*), 34.1 (C3), 34.0 (C3*), 33.0 (C10, C10*), 32.8 (C10, C10*), 28.0 (C7, C7*), 20.3 (C10, C10*), 20.2 (C10, C10*), 17.0 (C12, C12*). **IR**: v [cm^{-1}] 3335, 2948, 2926, 1739, 1644, 1538, 1367, 1336, 1259, 1177, 1149, 1111, 1026, 845, 796, 717, 694, 668, 601. **LRMS** (ESI+) *m/z*, (%): 477 (40, $[M+H]^+$), 499 (100, $[M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{26}H_{40}N_2O_6Na$: 499.2779, found: 499.2775.

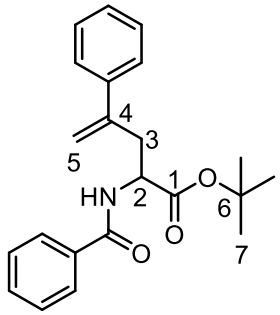
***tert*-Butyl 2-benzamido-4-cyano-4-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)butanoate (11c)**



Prepared according to general procedure **D**, starting from *tert*-butyl *N*-benzoyl-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)glycinate (**5t**) (100 mg, 0.26 mmol) and acrylonitrile (0.2 mL, 2.56 mmol), providing **11c** (60 mg, 53%) as inseparable 1:1 diastereomeric mixture as colorless oil.

R_f = 0.65 (20% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.86-7.82 (m, 4H, CH_{Ar}, CH_{Ar}*), 7.55-7.51 (m, 2H, CH_{Ar}, CH_{Ar}*), 7.49-7.43 (m, 4H, CH_{Ar}, CH_{Ar}*), 6.92-6.89 (m, 2H, NH, NH*), 4.98-4.79 (m, 4H, CH-2, CH-2*, CH-4, CH-4*), 2.63-2.56 (m, 2H, CH_2-3, CH_2-3*), 2.47-2.37 (m, 2H, CH_2-3, CH_2-3*), 1.60-1.28 (m, 12H, CH_2-9, CH_2-9*, CH_2-10, CH_2-10*), 1.54 (s, 18H, CH_3-6, CH_3-6*), 1.35 (s, 3H, CH_3-8*), 1.33 (s, 3H, CH_3-8), 1.20 (s, 3H, CH_3-8), 1.17 (s, 3H, CH_3-8), 1.15 (s, 3H, CH_3-8*), 1.12 (s, 3H, CH_3-8*), 1.10 (s, 3H, CH_3-8*), 1.06 (s, 3H, CH_3-8). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 170.2 (C=O*), 170.1 (C=O), 167.3 (C=O), 167.2 (C=O*), 133.8 (C_{Ar}), 133.7 (C_{Ar}*), 131.9 (CH_{Ar}*), 131.8 (CH_{Ar}), 128.64 (CH_{Ar}*), 128.58 (CH_{Ar}), 127.10 (CH_{Ar}*), 127.08 (CH_{Ar}), 119.3 (C≡N*), 118.7 (C≡N), 83.4 (C5), 83.3 (C5*), 71.33 (C4*), 71.31 (C4), 61.1 (C7), 61.0 (C7*), 60.04 (C7), 59.98 (C7*), 50.5 (C2), 50.0 (C2*), 40.0 (C9), 39.9 (C9*), 39.83 (C9*), 39.77 (C9), 36.1 (C3), 35.6 (C3*), 33.9 (C8*), 33.7 (C8), 33.6 (C8, C8*), 28.0 (C6, C6*), 20.7 (C8), 20.6 (C8, C8*), 20.3 (C8*), 17.0 (C10), 16.9 (C10*). **IR**: ν [cm^{-1}] 3335, 3058, 2977, 2928, 2851, 1729, 1645, 1530, 1486, 1451, 1367, 1297, 1257, 1151, 844, 710, 692. **LRMS** (ESI+) *m/z*, (%): 388 (20, $[M+H–isobutylene]^+$), 444 (50, $[M+H]^+$), 466 (100, $[M+Na]^+$), 909 (45, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{25}H_{37}N_3O_4Na$: 466.2676, found: 466.2677.

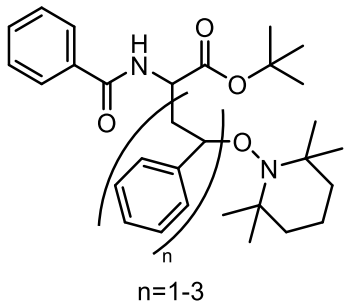
***tert*-Butyl 2-benzamido-4-phenylpent-4-eneoate (11d)**



Prepared according to general procedure **D**, starting from *tert*-butyl *N*-benzoyl-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)glycinate (**5t**) (118 mg, 0.30 mmol) and α–methylstyrene (0.4 mL, 3.02 mmol), providing **11d** (70 mg, 66%) as colorless oil.

R_f = 0.5 (20% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.54-7.25 (m, 10H, CH_{Ar}), 6.50 (d, *J* = 7.5 Hz, 1H, NH), 5.37 (d, *J* = 1.5 Hz, 1H, CH_2-5), 5.19 (d, *J* = 1.3 Hz, 1H, CH_2-5), 4.80 (dt, *J* = 7.5, 5.9 Hz, 1H, CH-2), 3.20 (dd, *J* = 14.2, 6.1 Hz, 1H, CH_2-3), 3.11 (dd, *J* = 14.2, 5.9 Hz, 1H, CH_2-3), 1.45 (s, 9H, CH_3-7). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 170.8 (C=O), 166.5 (C=O), 144.3 (C4), 140.8 (C_{Ar}), 134.0 (C_{Ar}), 131.5 (CH_{Ar}), 128.6 (CH_{Ar}), 128.4 (CH_{Ar}), 127.7 (CH_{Ar}), 126.9 (CH_{Ar}), 126.4 (CH_{Ar}), 116.6 (C5), 82.5 (C6), 52.6 (C2), 38.0 (C3), 28.0 (C7). **IR**: v [cm^{-1}] 3335, 3058, 2977, 2931, 1731, 1642, 1523, 1486, 1446, 1366, 1297, 1254, 1224, 1149, 903, 845, 777, 694. **LRMS** (ESI+) *m/z*, (%): 296 (60, $[M+H–isobutylene]^+$), 318 (40, $[M+Na–isobutylene]^+$), 352 (5, $[M+H]^+$), 374 (100, $[M+Na]^+$), 725 (5, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{22}H_{25}NO_3Na$: 374.1727, found: 374.1729.

Multiple addition of styrene to alkoxyamine (11e)

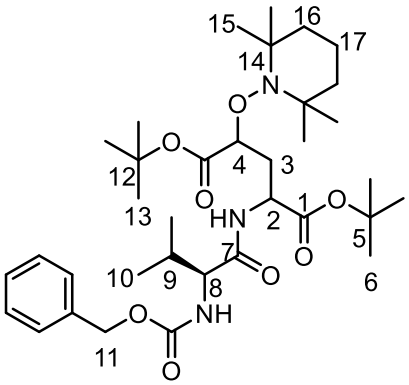


n=1-3

Prepared according to general procedure **D**, starting from *tert*-butyl *N*-benzoyl-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)glycinate (**5t**) (100 mg, 0.26 mmol) and styrene (0.29 mL, 2.56 mmol), providing **11e** (100 mg) as mixture of multiple addition products (mono/di/tri) in 7:10:3 ratio as colorless oil.

LCMS (ESI) *m/z*: 495.4 (70, $[C_{30}H_{42}N_2O_4 + H]^+$), 599.4 (100, $[C_{38}H_{50}N_2O_4 + H]^+$), 703.5 (30, $[C_{46}H_{58}N_2O_4 + H]^+$).

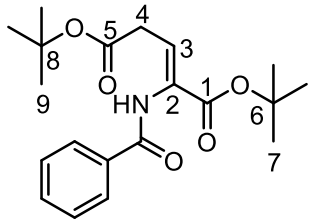
Di-*tert*-butyl *N*-(*N*-(benzyloxycarbonyl)-L-valyl)-4-(2,2,6,6-tetramethylpiperidin-1-yl)oxy) glutamate (11f)



According to general procedure D, starting from a 2:1 diastereomeric mixture of *tert*-butyl *N*-(*N*-(benzyloxycarbonyl)-L-valyl)-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)glycinate (**5i**) (300mg, 0.58 mmol) and *tert*-butyl acrylate (1.3 mL, 8.66 mmol), **11f** (225 mg, 60%) was obtained as white gummy solid as inseparable mixture of four diastereomers, which was used in the next step for cleavage of TEMPO.

R_f = 0.65 (20% EtOAc/PE). **IR**: v [cm^{-1}] 3326, 2974, 2932, 1731, 1668, 1506, 1456, 1367, 1232, 1151, 1026, 912, 844, 732, 697. **LRMS** (ESI+) *m/z*, (%): 648 (45, $[M+H]^+$), 670 (100, $[M+Na]^+$), 1317 (10, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{35}H_{57}N_3O_8Na$: 670.4038, found: 670.4034.

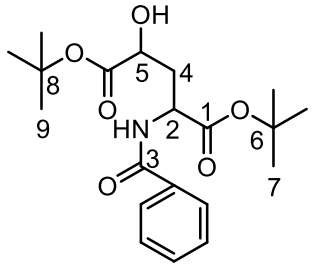
Di-*tert*-butyl 2-benzamidopent-2-enedioate (11g)



tert-Butyl acrylate (0.5 mL, 3.33 mmol) was added to a solution of *tert*-butyl *N*-benzoyl-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)glycinate (**5t**) (130 mg, 0.33 mmol) in degassed α,α,α-trifluorotoluene (2.5 mL) and the mixture was heated in a microwave reactor at 150 °C for 2 h. After completion, the solvent was removed in vacuum and the residue was purified by column chromatography (10% EtOAc/cyclohexane gradient to 20% EtOAc/cyclohexane) to obtain **11g** (63 mg, 53%) as single (Z)-diastereomer as colorless oil.

R_f = 0.45 (20% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 8.00 (s, 1H, NH), 7.91-7.83 (m, 2H, CH_{Ar}), 7.61-7.54 (m, 1H, CH_{Ar}), 7.52-7.46 (m, 2H, CH_{Ar}), 6.87 (t, *J* = 6.8 Hz, 1H, CH-3), 3.27 (d, *J* = 6.8 Hz, 2H, CH_2-4), 1.54 (s, 9H, CH_3-7), 1.48 (s, 9H, CH_3-9). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 170.2 (C=O), 165.2 (C=O), 163.6 (C=O), 133.9 (C_{Ar}), 132.1 (CH_{Ar}), 128.7 (CH_{Ar}), 127.9 (C2), 127.4 (CH_{Ar}), 126.1 (C3), 82.5 (C6), 81.3 (C8), 35.8 (C4), 28.1 (C7), 28.0 (C9). **IR**: ν [cm^{-1}] 3331, 2977, 2931, 1727, 1670, 1512, 1480, 1367, 1254, 1145, 844, 708. **LRMS** (ESI+) *m/z*, (%): 272 (30, $[M+Na–2isobutylene]^+$), 328 (10, $[M+Na–isobutylene]^+$), 384 (100, $[M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{20}H_{27}NO_5Na$: 384.1781, found: 384.1784.

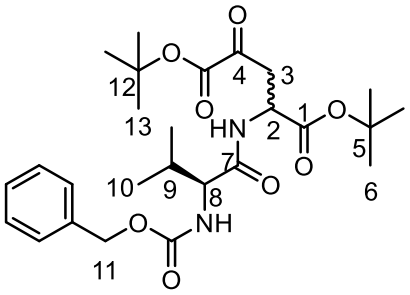
Di-*tert*-butyl *N*-benzoyl-4-hydroxyglutamate (12)



Zn dust (50.4 mg, 0.77 mmol) and AcOH (1 mL, 17.40 mmol) were added to a 1:1 diastereomeric mixture of di-*tert*-butyl *N*-benzyl-4-(2,2,6,6-tetramethylpiperidin-1-yl)oxy)glutamate (**11a**) (50 mg, 0.10 mmol) in THF/H_2O (1.2 mL, 3:1) and the reaction mixture was heated to 70-75 ºC with vigorous stirring for 3 h. After cooling to rt, the mixture was diluted with Et_2O (10 mL) and filtered through a plug of silica gel, which was washed with Et_2O (5 mL). After evaporation of the solvents, the resulting crude mixture was purified by column chromatography (20% EtOAc/PE) to obtain alcohol **12** (22 mg, 65%) as inseparable 1:1 diastereomeric mixture as colorless oil.

R_f = 0.6 (50% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.89-7.81 (m, 4H, CH_{Ar}, CH_{Ar}*), 7.53-7.50 (m, 2H, CH_{Ar}, CH_{Ar}*), 7.47-7.43 (m, 4H, CH_{Ar}, CH_{Ar}*), 7.34 (d, *J* = 7.9 Hz, 1H, NH), 7.10 (d, *J* = 6.8 Hz, 1H, NH*), 4.88 (dt, *J* = 7.9, 4.0 Hz, 1H, CH-2), 4.85-4.81 (m, 1H, CH-2*), 4.23 (dd, *J* = 9.8, 4.0 Hz, 1H, CH-5), 4.18 (dd, *J* = 9.9, 3.1 Hz, 1H, CH-5*), 3.90 (br s, 1H, OH), 3.38 (br s, 1H, OH*), 2.55-2.10 (m, 4H, CH_2-4, CH_2-4*), 1.51 (s, 18H, CH_3-9, CH_3-9*), 1.48 (s, 9H, CH_3-7), 1.44 (s, 9H, CH_3-7*). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 173.6 (C=O), 172.8 (C=O*), 171.3 (C=O), 171.0 (C=O*), 167.6 (C=O), 167.2 (C=O*), 133.8 (C_{Ar}), 133.6 (C_{Ar}*), 131.9 (CH_{Ar}), 131.8 (CH_{Ar}*), 128.58 (CH_{Ar}), 128.56 (CH_{Ar}*), 127.17 (CH_{Ar}), 127.14 (CH_{Ar}*), 82.9 (C6), 82.7 (C6*), 82.61 (C8), 82.55 (C8*), 68.3 (C5), 68.0 (C5*), 51.0 (C2), 50.9 (C2*), 36.7 (C4), 36.2 (C4*), 28.01 (C7), 27.98 (C9, C9*), 27.9 (C7*). **IR**: ν [cm^{-1}] 3376, 2978, 2926, 2854, 1728, 1650, 1530, 1488, 1368, 1252, 1155, 1115, 846, 713. **LRMS** (ESI+) *m/z*, (%): 268 (10, $[M+H-2isobutylene]^+$), 324 (5, $[M+H-isobutylene]^+$), 380 (5, $[M+H]^+$), 402 (100, $[M+Na]^+$), 781 (10, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{20}H_{29}NO_6Na$: 402.1887, found: 402.1887.

Di-*tert*-butyl *N*-(*N*-(benzyloxycarbonyl)-L-valyl)-4-oxoglutamate (13)



Zn dust (191.7 mg, 2.93 mmol) and AcOH (3.8 mL, 65.98 mmol) were added to a mixture of **11f** (225 mg, 0.37 mmol) in THF/H_2O (4.4 mL, 3:1) and the reaction mixture was heated to 75-80 ºC with vigorous stirring overnight. After cooling to rt, the mixture was diluted with Et_2O (20 mL), K_2CO_3 was added and the mixture was filtered through a plug of silica gel, which was washed with Et_2O (10 mL). After evaporation of the solvents, the resulting crude mixture was purified by column chromatography (20% EtOAc/cyclohexane) to obtain the alcohol derivative (92 mg, 53%) as inseparable mixture of diastereomers as white gummy solid which was used directly in the next step.

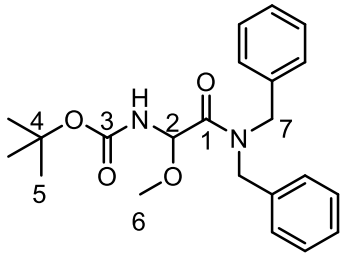
R_f = 0.6 (50% EtOAc/PE). **LRMS** (ESI+) *m/z*, (%): 397 (100, $[M+H–2isobutylene]^+$), 453 (40, $[M+H–isobutylene]^+$), 509 (65, $[M+H]^+$), 531 (45, $[M+Na]^+$), 1039 (10, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{26}H_{40}N_2O_8Na$: 531.2677, found: 531.2673.

DMP (96.5 mg, 0.23 mmol) was added to a solution of the alcohol derivative (90 mg, 0.19 mmol) in CH_2Cl_2 (3 mL) at 0 ºC, the turbid solution was warmed to rt and stirred for 2 h. After completion, sat. $Na_2S_2O_3$ solution (4 mL) was added to the reaction mixture, which was extracted with CH_2Cl_2 (25 mL). After separation, the organic layer was washed with sat. $NaHCO_3$ solution (3x10 mL), dried over $MgSO_4$ and the solvent was removed under reduced pressure. The residue was purified by column chromatography (10% EtOAc/cyclohexane gradient to 20% EtOAc/cyclohexane) to obtain **13** (40 mg, 45%) as 2:1 diastereomeric mixture as colorless oil.

R_f = 0.5 (20% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.41-7.30 (m, 10H, CH_{Ar}), 6.75 (d, *J* = 7.3 Hz, 1H, NH*), 6.65 (d, *J* = 7.9 Hz, 1H, NH), 5.38 (d, *J* = 8.8 Hz, 1H, NH), 5.30 (d, *J* = 7.8 Hz, 1H, NH*), 5.12 (br s, 4H, CH_2-11, CH_2-11*), 4.89-4.76 (m, 2H, CH-2, CH-2*), 4.14-3.99 (m, 2H, CH-8, CH-8*), 3.49-3.22 (m, 4H, CH_2-3, CH_2-3*), 2.24-2.09 (m, 2H, CH-9, CH-9*), 1.57 (s, 9H, CH_3-6), 1.56 (s, 9H, CH_3-6*), 1.46 (s, 18H, CH_3-13, CH_3-13*), 1.01 (d, *J* = 6.7 Hz, 3H, CH_3-10), 0.96 (d, *J* = 6.8 Hz, 6H, CH_3-10, CH_3-10*), 0.91 (d, *J* = 6.8 Hz, 3H, CH_3-10*). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 192.9 (C4), 192.7 (C4*), 170.8 (C=O*), 170.7 (C=O), 169.0 (C=O, C=O*), 159.3 (C=O*), 159.2 (C=O), 156.3 (C=O, C=O*), 136.22 (C_{Ar}),

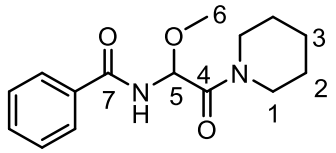
136.16 (C_{Ar}*), 128.6 (CH_{Ar}*), 128.5 (CH_{Ar}), 128.20 (CH_{Ar}*), 128.17 (CH_{Ar}), 128.1 (CH_{Ar}, CH_{Ar}*), 85.54 (C5*), 84.48 (C5), 83.21 (C12*), 83.16 (C12), 67.2 (C11*), 67.1 (C11), 60.2 (C8*), 60.0 (C8), 48.8 (C2*), 48.6 (C2), 41.6 (C3), 41.4 (C3*), 31.4 (C9), 31.0 (C9*), 27.9 (C6), 27.84 (C6*), 27.77 (C13, C13*), 19.1 (C10, C10*), 17.6 (C10), 17.5 (C10*). **IR**: v [cm^{-1}] 3295, 2962, 2925, 2852, 1721, 1687, 1654, 1529, 1456, 1393, 1368, 1292, 1247, 1154, 1096, 1067, 1028, 841, 739, 696. **LRMS** (ESI+) *m/z*, (%): 507 (10, $[M+H]^+$), 529 (100, $[M+Na]^+$), 1035 (10, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{296}H_{38}N_2O_8Na$: 529.2520, found: 529.2515.

***N'*-(*tert*-Butyloxycarbonyl)-*N*,*N*-dibenzyl-2-methoxyglycinamide (14a)**



Pd/C (5 mg, 10 w/w%) was added to a solution of *N'*-(*tert*-butyloxycarbonyl)-*N*,*N*-dibenzyl-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)glycinamide (**5c**) (50 mg, 0.10 mmol) in MeOH (1 mL) and the reaction mixture was stirred under a hydrogen atmosphere (15 bar) for 24 h. After completion, the reaction mixture was filtered through Celite®, which was washed with MeOH, the solvent was removed under reduced pressure and the residue was purified by column chromatography (10% EtOAc/cyclohexane) to afford **14a** (20 mg, 53%) as colorless solid.
R_f = 0.75 (20% EtOAc/PE). **m.p.** 90-92 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.41-7.30 (m, 6H, CH_{Ar}), 7.26-7.17 (m, 4H, CH_{Ar}), 6.33 (d, *J* = 8.7 Hz, 1H, NH), 5.70 (d, *J* = 8.7 Hz, 1H, CH-2), 4.68-4.37 (m, 4H, CH_2-7), 3.37 (s, 3H, CH_3-6), 1.50 (s, 9H, CH_3-5). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 167.2 (C=O), 155.3 (C=O), 136.3 (C_{Ar}), 135.7 (C_{Ar}), 128.9 (CH_{Ar}), 128.7 (CH_{Ar}), 128.2 (CH_{Ar}), 127.9 (CH_{Ar}), 127.6 (CH_{Ar}), 127.4 (CH_{Ar}), 80.3 (C4), 77.7 (C2), 54.2 (C6), 49.6 (C7), 47.7 (C7), 28.3 (C5). **IR**: v [cm^{-1}] 3416, 2979, 2917, 2838, 1719, 1652, 1494, 1476, 1460, 1446, 1421, 1364, 1328, 1218, 1156, 1074, 1055, 1017, 954, 763, 722, 704, 696, 663, 607. **LRMS** (ESI+) *m/z*, (%): 407 (100, $[M+Na]^+$), 791 (5, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{22}H_{28}N_2O_4Na$: 407.1941, found: 407.1937.

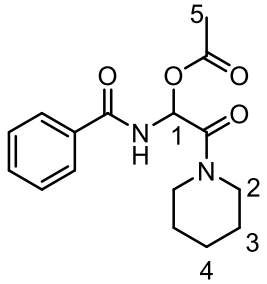
***N'*-(Benzoyl)-*N*-pentamethylene-2-methoxyglycinamide (14b)**



N'-(Benzoyl)-*N*-pentamethylene-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)glycinamide (**5e**) (100 mg, 0.25 mmol) was subjected to general procedure **C**, mediated by acetic acid in MeOH (3 mL) and the substituted product **14b** was obtained as colorless solid (55 mg, 80%).

R$_f$ = 0.4 (20% EtOAc/PE). **m.p.** 69-70 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.96 (d, *J* = 8.0 Hz, 1H, NH), 7.95-7.86 (m, 2H, CH_{Ar}), 7.62-7.53 (m, 1H, CH_{Ar}), 7.52-7.45 (m, 2H, CH_{Ar}), 6.01 (d, *J* = 8.1 Hz, 1H, CH-5), 3.76-3.50 (m, 4H, CH_2-1), 3.45 (s, 3H, CH_3-6), 1.75-1.48 (m, 6H, CH_2-2, CH_2-3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 167.8 (C=O), 164.7 (C=O), 133.5 (C_{Ar}), 132.1 (CH_{Ar}), 128.6 (CH_{Ar}), 127.3 (CH_{Ar}), 75.5 (C5), 54.6 (C6), 46.7 (C1), 43.7 (C1), 26.3 (C2), 25.6 (C2), 24.4 (C1). **IR**: ν [cm^{-1}] 3303, 2937, 2858, 1644, 1603, 1580, 1522, 1459, 1444, 1323, 1283, 1247, 1230, 1191, 1136, 1079, 712, 694, 609. **LRMS** (ESI+) *m/z*, (%): 245 (5, $[M+H–MeOH]^+$), 299 (100, $[M+Na]^+$), 575 ($[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{15}H_{20}N_2O_3Na$: 299.1366, found: 299.1367.

***N'*-(Benzoyl)-*N*-pentamethylene-2-acetoxyglycinamide (14c)**

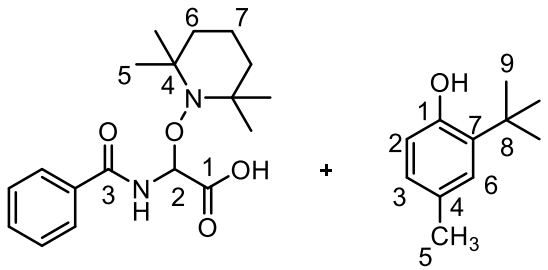


N'-(Benzoyl)-*N*-pentamethylene-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)glycinamide (**5e**) (50 mg, 0.12 mmol) was subjected to general procedure **C**, mediated by acetic acid in CH_3CN (1 mL) and the substituted product **14c** was obtained as colorless oil (23 mg, 60%).

R$_f$ = 0.4 (20% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 8.05 (d, *J* = 8.6 Hz, 1H, NH), 7.94-7.87 (m, 2H, CH_{Ar}), 7.63-7.53 (m, 1H, CH_{Ar}), 7.51-7.44 (m, 2H, CH_{Ar}), 7.24 (d, *J* = 8.9 Hz, 1H, CH-1), 3.76-3.52 (m, 4H, CH_2-2), 2.09 (s, 3H, CH_3-5), 1.75-1.57 (m, 6H, CH_2-3, CH_2-4). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 169.4 (C=O), 166.4 (C=O), 164.4 (C=O), 132.8 (C_{Ar}), 132.4 (CH_{Ar}), 128.7 (CH_{Ar}), 127.5 (CH_{Ar}), 69.7 (C1), 46.5 (C2), 44.1 (C2), 26.3 (C3), 25.5 (C3), 24.4 (C4), 21.7 (C5). **IR**: ν [cm^{-1}] 3273, 2937, 2857, 1743, 1644, 1533, 1468, 1446, 1413,

1370, 1316, 1237, 1164, 1139, 1038, 1014, 710, 669. **LRMS** (ESI+) *m/z*, (%): 327 (100, $[M+Na]^+$), 631 ($[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{16}H_{20}N_2O_4Na$: 327.1315, found: 327.1313.

Attempted Friedel-Crafts-type reaction of *tert*-butyl *N*-benzoyl-2-((2,2,6,6-tetramethyl piperidin-1-yl)oxy)glycinate



15a **15b**

$BF_3{\cdot}OEt_2$ (126 µL, 1.021 mmol) was added to a solution of **5t** (200 mg, 0.51 mmol) and *p*-cresol (81 µL, 0.77 mmol) in degassed DCM (6 mL) at 0 °C. The reaction mixture was warmed to rt and stirred overnight. $NaHCO_3$ solution (5 mL) was added, the aqueous layer was extracted with DCM (2x10 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed and the resulting yellow oil was purified by column chromatography furnishing **15b** (38 mg, 45%) (5% EtOAc/cyclohexane), recovered **5t** (25 mg, 13%) (5% EtOAc/cyclohexane gradient to 10% EtOAc/cyclohexane) and **15a** (48 mg, 28%) (40% EtOAc/cyclohexane) as pale yellow oil.

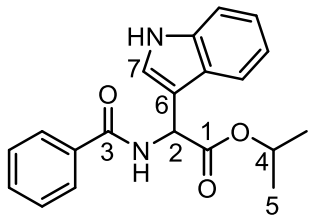
***N*-benzoyl-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)glycine (15a)**

R_f = 0.3 (50% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 13.39 (br s, 1H, OH), 7.87-7.85 (m, 2H, CH_{Ar}), 7.55-7.49 (m, 1H, CH_{Ar}), 7.48-7.43 (m, 2H, CH_{Ar}), 7.33 (d, *J* = 9.7 Hz, 1H, NH), 6.12 (d, *J* = 9.8 Hz, 1H, CH-2), 1.59-1.30 (m, 6H, CH_2-6, CH_2-7), 1.36 (s, 3H, CH_3-5), 1.16 (s, 6H, CH_3-5), 1.08 (s, 3H, CH_3-5). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 174.7 (C=O), 166.2 (C=O), 134.8 (C_{Ar}), 131.4 (CH_{Ar}), 128.5 (CH_{Ar}), 127.2 (CH_{Ar}), 84.7 (C2), 60.8 (C4), 59.0 (C4), 40.1 (C6), 33.7 (C5), 33.1 (C5), 20.3 (C5), 20.1 (C5), 17.2 (C6). **IR**: ν [cm^{-1}] 3366, 3195, 3060, 2941, 1726, 1666, 1613, 1577, 1509, 1479, 1448, 1389, 1527, 764, 622. **LRMS** (ESI+) *m/z*, (%): 335 (100, $[M+H]^+$), 357 (10, $[M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{18}H_{26}N_2O_4Na$: 357.1785, found: 357.1782.

2-*tert*-Butyl-4-methyl phenol (15b)

R_f = 0.85 (50% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 7.12 (d, *J* = 1.8 Hz, 1H, CH_{Ar}-6), 6.92 (dd, *J* = 7.9, 2.1 Hz, 1H, CH_{Ar}-3), 6.60 (d, *J* = 7.9 Hz, 1H, CH_{Ar}-2), 4.66 (s, 1H, OH), 2.32 (s, 3H, CH_3-5), 1.45 (s, 9H, CH_3-9). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 151.9 (C1), 135.8 (C7), 129.6 (C4), 127.8 (C6), 127.2 (C3), 116.4 (C2), 34.4 (C8), 29.7 (C9), 20.8 (C5).
The analytical data according to the literature.[148]

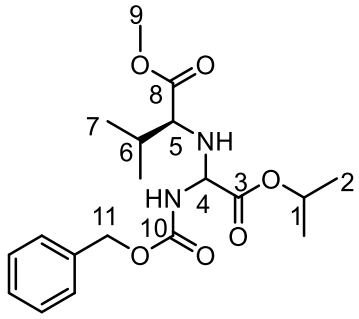
Isopropyl *N*-benzoyl-2-(1*H*-indol-3-yl)glycinate (15c)



Indole (32 mg, 0.27 mmol) was added to a solution of isopropyl *N*-benzoyl-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)glycinate (**5a**) (100 mg, 0.27 mmol) in CH_3CN (3 mL). TFA (20 µL, 0.26 mmol) was added dropwise to the reaction mixture at rt, which was stirred at 70 °C for 2 h. After completion, the reaction mixture was diluted with EtOAc (10 mL), washed with sat. $NaHCO_3$ solution (5 mL), the organic layer was dried over $MgSO_4$ and the solvent was removed in vacuum. The brown residue was purified by column chromatography (50% EtOAc/cyclohexane) to obtain **15c** (65 mg, 71%) as amorphous white solid.

R_f = 0.4 (80% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 8.61 (s, 1H, NH_{Indole}), 7.85-7.78 (m, 3H, CH_{Ar}), 7.55-7.48 (m, 1H, CH_{Ar}), 7.46-7.38 (m, 3H, CH_{Ar}), 7.27-7.14 (m, 3H, CH_{Ar}), 7.05 (d, *J* = 7.0 Hz, 1H, NH), 6.04 (d, *J* = 7.1 Hz, 1H, CH-2), 5.14 (sept, *J* = 6.3 Hz, 1H, CH-4), 1.32 (d, *J* = 6.2 Hz, 3H, CH_3-5), 1.15 (d, *J* = 6.3 Hz, 3H, CH_3-5). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 170.9 (C=O), 166.9 (C=O), 136.4 (C_{Ar}), 133.9 (C_{Ar}), 131.7 (CH_{Ar}), 128.6 (CH_{Ar}), 127.1 (CH_{Ar}), 125.5 (C_{Ar}), 123.7 (CH_{Ar}), 122.6 (CH_{Ar}), 120.2 (CH_{Ar}), 119.2 (CH_{Ar}), 111.6 (CH_{Ar}), 111.2 (C_{Ar}), 69.6 (C4), 50.6 (C2), 21.8 (C5), 21.5 (C5). **IR**: ν [cm^{-1}] 3418, 3357, 2981, 1724, 1628, 1577, 1528, 1371, 1324, 1291, 1198, 1105, 818, 772, 736, 715, 689. **LRMS** (ESI+) *m/z*, (%): 337 (10, $[M+H]^+$), 359 (100, $[M+Na]^+$), 695 (65, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{20}H_{20}N_2O_3Na$: 359.1366, found: 359.1365.

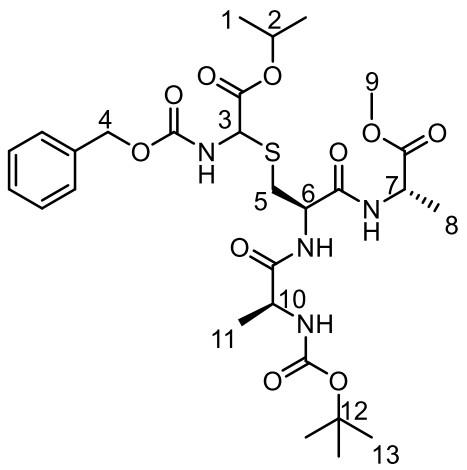
Cbz-Gly(*N*-Val-OMe)-O*i*Pr (16a)



Isopropyl *N*-benzyloxycarbonyl-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)glycinate (**5b**) (100 mg, 0.25 mmol) was subjected to general procedure **E**, catalyzed by citric acid (10 mg, 0.05 mmol) with L-valine methyl ester (**N2**) (32 mg, 0.25 mmol) in CH_3CN and the substituted product **16a** was obtained as colorless oil (82 mg, 88%) as 1:1 diastereomeric mixture.

R_f = 0.6 (40% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.41-7.33 (m, 10H, CH_{Ar}, CH_{Ar}*), 5.57 (d, *J* = 8.0 Hz, 1H, NH), 5.39 (d, *J* = 9.2 Hz, 1H, NH*), 5.23-4.94 (m, 8H, CH_2-11, CH_2-11*, CH-1, CH-1*, CH-4, CH-4*), 3.67 (s, 3H, CH_3-9), 3.61 (s, 3H, CH_3-9*), 3.23 (dd, *J* = 9.5, 5.5 Hz, 1H, CH-5), 3.07 (dd, *J* = 11.1, 5.8 Hz, 1H, CH-5*), 2.82 (dd, *J* = 11.5, 4.1 Hz, 1H, NH*), 2.44 (dd, *J* = 10.0, 9.5 Hz, 1H, NH), 1.99-1.79 (m, 2H, CH-6, CH-6*), 1.35-1.23 (m, 12H, CH_3-2, CH_3-2*), 1.03-0.87 (m, 12H, CH_3-7, CH_3-7*). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 176.1 (C=O), 175.3 (C=O*), 168.9 (C=O, C=O*), 155.6 (C=O, C=O*), 136.2 (C_{Ar}, C_{Ar}*), 128.5 (CH_{Ar}, CH_{Ar}*), 128.24 (CH_{Ar}, CH_{Ar}*), 128.17 (CH_{Ar}, CH_{Ar}*), 70.1 (C1), 69.7 (C1*), 67.2 (C4), 67.0 (C11, C11*), 66.0 (C4*), 63.6 (C5), 62.4 (C5*), 51.8 (C9, C9*), 31.9 (C6), 31.7 (C6*), 21.69 (C2), 21.65 (C2*), 21.62 (C2), 21.58 (C2*), 19.3 (C7), 19.1 (C7*), 18.2 (C7), 18.0 (C7*). **IR**: ν [cm^{-1}] 3344, 2961, 2874, 1716, 1509, 1467, 1455, 1374, 1323, 1304, 1235, 1200, 1180, 1146, 1104, 1024, 994, 774, 753, 738, 697. **LRMS** (ESI+) *m/z*, (%): 381 (10, $[M+H]^+$), 403 ($[M+Na]^+$), 783 ($[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{19}H_{28}N_2O_6Na$: 403.1840, found: 403.1841.

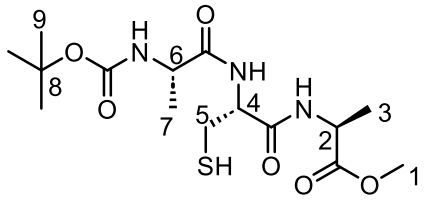
Cbz-Gly(Boc-Ala-Cys-Ala-OMe)-O*i*Pr (16b)



Isopropyl *N*-(benzyloxycarbonyl)-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)glycinate (**5b**) (100 mg, 0.25 mmol) was subjected to general procedure **E**, catalyzed by citric acid (10 mg, 0.05 mmol) with Boc-Ala-Cys-Ala-OMe (**N3**) (92 mg, 0.25 mmol) in CH_3CN and product **16b** was obtained as colorless oil (120 mg, 78%) as inseparable 1:1 diastereomeric mixture.

R_f = 0.6 (40% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 8.00 (d, *J* = 6.2 Hz, 1H, NH_{Cys}*), 7.43-7.33 (m, 11H, CH_{Ar}, CH_{Ar}*, NH_{Ala}*), 7.05-6.86 (m, 2H, NH_{Ala}, NH_{Cys}), 6.42 (d, *J* = 7.6 Hz, 1H, NH_{Gly}), 6.21 (d, *J* = 6.8 Hz, 1H, NH_{Gly}*), 5.43 (s, 1H, NH_{Ala}), 5.37 (d, *J* = 7.3 Hz, 1H, CH-3), 5.24-5.08 (m, 7H, CH_2-4, CH_2-4*, CH-2, CH-2*, CH-3*), 5.02 (s, 1H, NH_{Ala}*), 4.96-4.92 (m, 1H, CH-6), 4.86-4.79 (m, 1H, CH-6*), 4.61-4.46 (m, 2H, CH-7, CH-7*), 4.41-4.32 (m, 1H, CH-10*), 4.26-4.16 (m, 1H, CH-10), 3.75 (s, 3H, CH_3-9), 3.73 (s, 3H, CH_3-9*), 3.42 (dd, *J* = 14.8, 3.6 Hz, 1H, CH_2-5*), 3.08 (dd, *J* = 14.6, 6.4 Hz, 1H, CH_2-5), 2.98 (dd, *J* = 14.8, 4.8 Hz, 1H, CH_2-5*), 2.90 (dd, *J* = 14.6, 7.1 Hz, 1H, CH_2-5), 1.47 (s, 18H, CH_3-13, CH_3-13*), 1.45-1.36 (m, 12H, CH_3-8, CH_3-11, CH_3-8*, CH_3-11*), 1.31 (d, *J* = 6.4 Hz, 12H, CH_3-1, CH_3-1*). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 172.8 (C=O), 172.7 (C=O*), 172.6 (C=O, C=O*), 169.5 (C=O), 169.3 (C=O*), 168.8 (C=O), 168.3 (C=O*), 155.4 (C=O, C=O*), 155.3 (C=O, C=O*), 135.8 (C_{Ar}*), 135.6 (C_{Ar}), 128.64 (CH_{Ar}*), 128.61 (CH_{Ar}), 128.5 (CH_{Ar}), 128.4 (CH_{Ar}*), 128.2 (CH_{Ar}), 128.1 (CH_{Ar}*), 70.7 (C2, C2*), 67.8 (C4), 67.5 (C4*), 57.5 (C3), 55.6 (C3*), 53.0 (C6), 52.5 (C9*), 52.4 (C9), 51.5 (C6*), 50.5 (C10, C10*), 48.4 (C7*), 48.3 (C7), 34.5 (C5*), 33.5 (C5), 28.4 (C13*), 28.3 (C13), 21.68 (C1), 21.65 (C1*), 21.53 (C1), 21.46 (C1*), 18.0 (C8, C8*), 17.9 (C11, C11*). **IR**: v [cm^{-1}] 3302, 2979, 2936, 1716, 1658, 1515, 1454, 1412, 1366, 1320, 1223, 1167, 1105, 1051, 1028, 985, 753, 698, 667, 622. **LRMS** (ESI+) *m/z*, (%): 627 (55, $[M+H]^+$), 649 (100, $[M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{28}H_{42}N_4O_{10}NaS$: 649.2514, found: 649.2508.

Boc-Ala-Cys-Ala-OMe (N3)

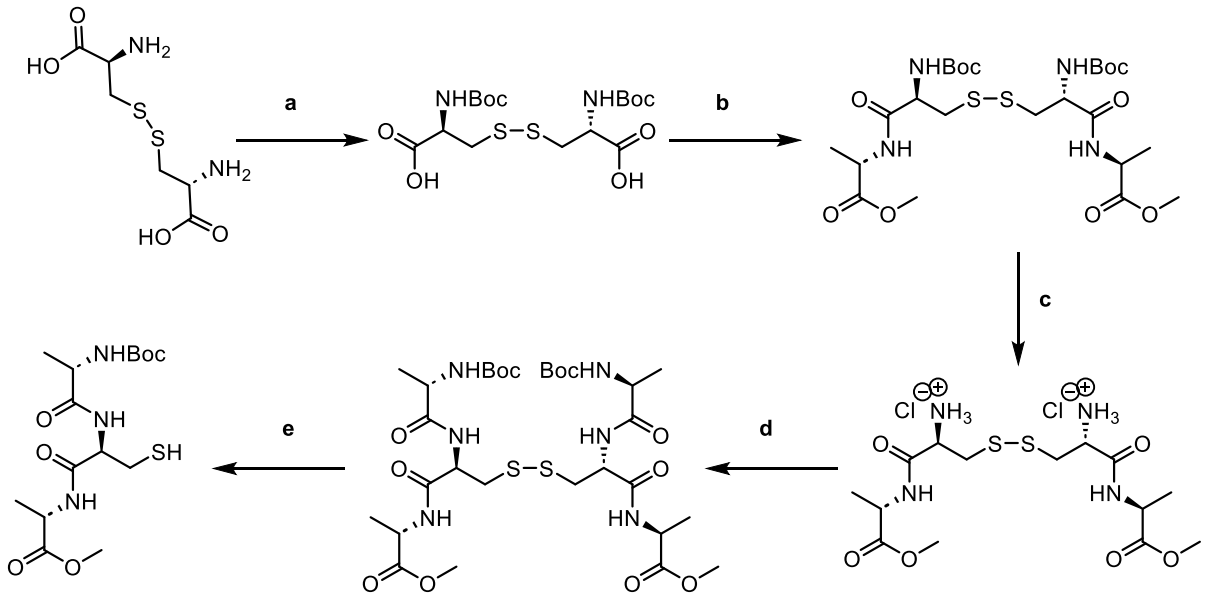


The compound was synthesized by Dr. Jan Picha at IOCB, Prague.

R$_f$ = 0.69 (EtOAc); $[\alpha]^{20}_{589}$ = −29.6 (c = 2; DMF).

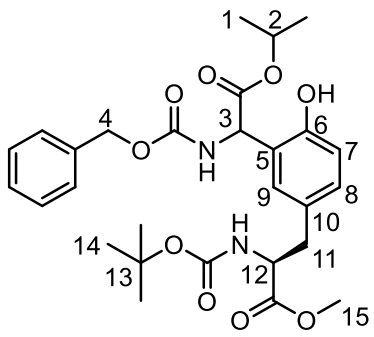
^{1}H NMR (600 MHz, $CDCl_3$) δ = 7.19 (br s, 2H, NH), 5.13 (br s, 1H, NH), 4.68 (ddd, *J* = 8.0, 5.8, 4.5 Hz, 1H, CH-4), 4.51 (q, 1H, *J* = 7.2 Hz, CH-6 or CH-2), 4.18 (m, 1H, CH-6 or CH-2), 3.73 (s, 3H, CH_3-1), 3.11 ddd, *J* = 13.5, 8.0, 4.5 Hz, 1H, CH_2-5), 2.73 (ddd, *J* = 13.5, 10.0, 5.8 Hz, 1H, CH_2-5), 1.69 (br s, 1H, SH), 1.43 (s, 9H, CH_3-9), 1.42 (d, *J* = 7.2 Hz, 3H, CH_3-7 or CH_3-3), 1.38 (d, *J* = 7.2 Hz, 3H, CH_3-7 or CH_3-3). **^{13}C NMR** (150.9 MHz, $CDCl_3$) δ = 172.8 (C=O), 172.7 (C=O), 169.2 (C=O), 155.7 (C=O), 80.5 (C8), 53.9 (C4), 52.5 (C1), 50.6 (C6 or C2), 48.4 (C6 or C2), 28.3 (C9), 26.8 (C5), 17.9 (C3 or C7), 17.75 (C3 or C7). **HRMS** (ESI+) *m/z*: ([M+Na]$^+$): calculated for $C_{15}H_{27}O_6N_3SNa$ 400.1513; found: 400.1510.

Reaction scheme for the synthesis:



Reagents, conditions, yields: (a) Boc_2O, K_2CO_3, water and dioxane, 1 h at 0°C then overnight at rt (65%); (b) Ala-OMe·HCl, HBTU, HOBt, DIPEA, DMF, overnight at rt (55%); (c) 4M HCl in dioxane, overnight at rt (73 %); (d) Boc-Ala-OH, HCTU, DIPEA, DMF, overnight at rt (77 %); (e) Tributylphosphine, THF, water, overnight at rt (75%).

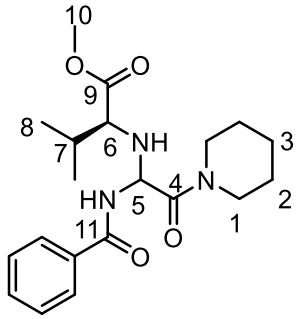
Cbz-Gly(Boc-Tyr-OMe)-O*i*Pr (16c)



Isopropyl *N*-(benzyloxycarbony)l-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)glycinate (**5b**) (100 mg, 0.25 mmol) was subjected to general procedure **E**, catalyzed by citric acid (10 mg, 0.05 mmol) with Boc-Tyr-OMe (**N4**) (116 mg, 0.39 mmol) in CH_3CN and the product **16c** was obtained as colorless oil (27 mg, 20%) as inseparable 1:1 diastereomeric mixture and unreacted starting materials were recovered.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.18 (s, 2H, OH, OH*), 7.40-7.24 (m, 10H, CH_{Ar}, CH_{Ar}*), 7.22-7.12 (m, 2H, CH_{Ar}-8, CH_{Ar}-8*), 7.02-6.92 (m, 2H, CH_{Ar}-9, CH_{Ar}-9*), 6.83-6.72 (m, 2H, CH_{Ar}-7, CH_{Ar}-7*), 6.29 (s, 1H, NH), 6.00 (s, 1H, NH*), 5.42 (d, *J* = 8.0 Hz, 1H, CH-3), 5.37 (d, *J* = 8.0 Hz, 1H, CH-3*), 5.22-5.07 (m, 6H, CH_2-4, CH_2-4*, CH-2, CH-2*), 4.99 (d, *J* = 8.3 Hz, 2H, NH, NH*), 4.60-4.47 (m, 2H, CH-12, CH-12*), 3.72 (s, 6H, CH_3-15, CH_3-15*), 3.10-2.96 (m, 4H, CH_2-11, CH_2-11*), 1.44 (s, 9H, CH_3-14*), 1.36 (d, *J* = 6.4 Hz, 12H, CH_3-1, CH_3-1*), 1.35 (s, 9H, CH_3-14). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 179.1 (C=O, C=O*), 174.7 (C=O, C=O*), 155.1 (C=O, C=O*, C6, C6*), 132.6 (C_{Ar}, C_{Ar}*), 131.1 (C10, C10*), 130.4 (C9, C9*), 129.0 (C8, C8*), 128.60 (CH_{Ar}), 128.58 (CH_{Ar}*), 128.4 ($CH_{Ar,}$), 128.3 (CH_{Ar}*), 128.23 (CH_{Ar}), 128.16 (CH_{Ar}*), 127.6 (C5, C5*), 115.5 (C7, C7*), 80.0 (C13, C13*), 70.6 (C3, C3*), 69.6 (C2, C2*), 66.2 (C4, C4*), 54.6 (C12, C12*), 52.2 (C15, C15*), 37.1 (C11, C11*), 28.3 (C14*), 27.6 (C14), 20.1 (C1, C1*). **IR**: v [cm^{-1}] 3342, 2979, 2931, 2851, 1711, 1614, 1515, 1446, 1390, 1367, 1307, 1218, 1166, 1104, 1056, 1027, 829, 779, 737, 699. **LRMS** (ESI+) *m/z*, (%): 567 (100, $[M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{28}H_{36}N_2O_9Na$: 567.2313, found: 567.2316.

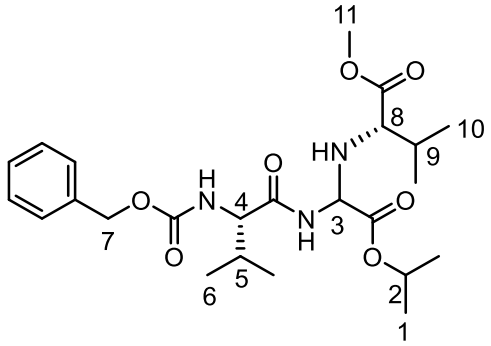
Bz-Gly(*N*-Val-OMe)-NPip (16d)



N'-(Benzoyl)-*N*-pentamethylene-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)glycinamide (**5e**) (100 mg, 0.25 mmol) was subjected to general procedure **C**, mediated by acetic acid with L-valine methyl ester (**N2**) in CH_3CN (3 mL) and the product **16d** was obtained as colorless solid (74 mg, 80%) as 1:1 diastereomeric mixture.

R_f = 0.6 (40% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.87-7.73 (m, 4H, CH_{Ar}, CH_{Ar}*), 7.55-7.50 (m, 2H, CH_{Ar}, CH_{Ar}*), 7.48-7.42 (m, 6H, CH_{Ar}, CH_{Ar}*, NH, NH*), 5.83-5.77 (m, 1H, CH-5), 5.74 (d, *J* = 6.1 Hz, 1H, CH-5*), 3.87-3.79 (m, 2H, CH_2-1, CH_2-1*), 3.73-3.68 (m, 2H, CH_2-1, CH_2-1*), 3.66-3.46 (m, 4H, CH_2-1, CH_2-1*), 3.39-3.34 (m, 1H, CH-6), 3.15-3.02 (m, 1H, CH-6*), 2.91 (s, 1H, NH), 2.57 (s, 1H, NH*), 1.95-1.87 (m, 1H, CH-7), 1.87-1.78 (m, 1H, CH-7*), 1.75-1.54 (m, 12H, CH_2-2, CH_2-3, CH_2-2*, CH_2-3*), 0.97 (d, *J* = 6.7 Hz, 3H, CH_3-8), 0.92 (d, *J* = 6.8 Hz, 3H, CH_3-8*), 0.92 (d, *J* = 6.8 Hz, 3H, CH_3-8*), 0.89 (d, *J* = 6.8 Hz, 3H, CH_3-8). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 176.7 (C=O), 175.3 (C=O*), 170.0 (C=O), 166.9 (C=O*), 166.9 (C=O), 166.6 (C=O*), 133.7 (C_{Ar}, C_{Ar}*), 131.8 (CH_{Ar}), 131.7 (CH_{Ar}*), 128.6 (CH_{Ar}), 128.5 (CH_{Ar}*), 127.08 (CH_{Ar}), 127.07 (CH_{Ar}*), 63.5 (C6), 62.1 (C5), 61.5 (C5*), 61.4 (C6*), 51.6 (C10), 51.5 (C10*), 46.8 (C1), 46.7 (C1*), 43.6 (C1), 43.4 (C1*), 32.1 (C7), 31.8 (C7*), 26.5 (C2), 26.3 (C2*), 25.6 (C2*), 25.5 (C2), 24.42 (C3), 24.40 (C3*), 19.4 (C8), 19.2 (C8*), 18.5 (C8*), 18.1 (C8). **IR**: ν [cm^{-1}] 3320, 2936, 2858, 1733, 1630, 1579, 1515, 1458, 1440, 1295, 1245, 1226, 1198, 1180, 1148, 1136, 1122, 1073, 1009, 998, 852, 802, 774, 710, 693, 665, 639, 604. **LRMS** (ESI+) *m/z*, (%): 398 (100, $[M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{20}H_{29}N_3O_4Na$: 398.2050, found: 398.2050.

Cbz-Val-Gly(*N*-L-Val-OMe)-O*i*Pr (17a)



A diastereomeric mixture of isopropyl *N*-(*N*-(benzyloxycarbonyl)-L-valyl)-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy) glycinate (**5j**) (d.r. = 2:1, 300 mg, 0.59 mmol) was subjected to general procedure **E**, catalyzed by citric acid (23 mg, 0.12 mmol) with L-valine methyl ester (**N2**) (78 mg, 0.59 mmol) as nucleophile in CH_3CN (10 mL) and the product **17a** was obtained as colorless solid (277 mg, 97%) as 1.3:1 diastereomeric mixture.

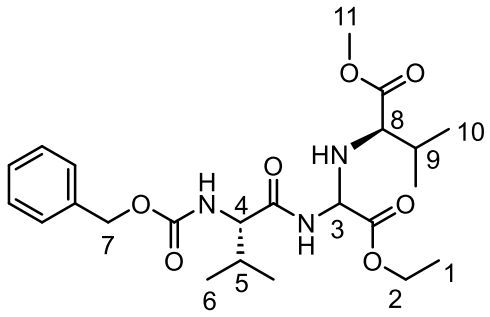
R_f = 0.6 (40% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.42-7.31 (m, 10H, CH_{Ar}, CH_{Ar}*), 6.56 (d, *J* = 8.1 Hz, 1H, NH), 6.31 (d, *J* = 8.7 Hz, 1H, NH*), 5.39 (d, *J* = 8.8 Hz, 1H, NH*), 5.24-5.01 (m, 9H, CH_2-7, CH_2-7*, NH, CH-3, CH-3*, CH-2, CH-2*), 4.07 (dd, *J* = 8.8, 5.0 Hz, 1H, CH-4), 4.01 (dd, *J* = 8.7, 5.9 Hz, 1H, CH-4*), 3.71 (s, 3H, CH_3-11*), 3.69 (s, 3H, CH_3-11), 3.27 (dd, *J* = 8.7, 4.5 Hz, 1H, CH-8), 3.11 (dd, *J* = 11.0, 5.4 Hz, 1H, CH-8*), 2.78 (dd, *J* = 11.0, 3.1 Hz, 1H, NH*), 2.43 (dd, *J* = 9.6, 3.4 Hz 1H, NH), 2.26-2.14 (m, 1H, CH-5), 2.18-2.06 (m, 1H, CH-5*), 2.07-2.00 (m, 1H, CH-9), 1.99-1.88 (m, 1H, CH-9*), 1.33-1.25 (m, 12H, CH_3-1, CH_3-1*), 1.02-0.81 (m, 24H, CH_3-6, CH_3-6*, CH_3-10, CH_3-10*). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 175.7 (C=O*), 174.9 (C=O), 171.3 (C=O), 171.2 (C=O*), 169.0 (C=O*), 168.9 (C=O), 156.3 (C=O, C=O*), 136.3 (C_{Ar}), 136.1 (C_{Ar}*), 128.55 (CH_{Ar}), 128.52 (CH_{Ar}*), 128.22 (CH_{Ar}), 128.20 (CH_{Ar}*), 128.1 (CH_{Ar}*), 128.0 (CH_{Ar}), 70.2 (C2), 69.7 (C2*), 67.2 (C7), 67.0 (C7*), 65.6 (C3*), 64.1 (C3), 63.7 (C8), 62.8 (C8*), 60.2 (C4), 60.1 (C4*), 52.0 (C11*), 51.8 (C11), 31.9 (C9*), 31.5 (C9), 31.4 (C5), 30.7 (C5*), 21.7 (C1*), 21.64 (C1), 21.61 (C1), 21.55 (C1*), 19.31 (C6), 19.28 (C6*), 19.14 (C6*), 19.06 (C6), 17.9 (C10), 17.8 (C10*), 17.4 (C10*), 17.0 (C10). **IR**: v [cm^{-1}] 3307, 2957, 1734, 1691, 1655, 1648, 1528, 1467, 1455, 1373, 1293, 1240, 1201, 1183, 1143, 1106, 1037, 995, 778, 747, 695, 657, 633. **LRMS** (ESI+) *m/z*, (%): 480 (90, $[M+H]^+$), 502 (100, $[M+Na]^+$), 981 ($[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{24}H_{37}N_3O_7Na$: 502.2524, found: 502.2521.

Reaction procedure for substitution reaction catalyzed by chiral acid:

A solution of L-valine methyl ester (**N2**) (16 mg, 0.12 mmol) in solvent (1 mL) was added to a solution of diastereomeric mixture of isopropyl *N*-(*N*-(benzyloxycarbonyl)-L-valyl)-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy) glycinate (**5j**) (d.r. = 2:1, 50 mg, 0.10 mmol) and a chiral acid ((*S*)-TRIP or (*R*)-TRIP or L-tartaric acid or D-tartaric acid) (0.02 mmol) in the mentioned solvent (CH_3CN or CH_2Cl_2 or toluene or 1:1 mixture of CH_3CN/toluene) at rt. The reaction mixture was heated to 37 °C and stirred overnight. After completion, the reaction mixture was evaporated to dryness and the residue was analyzed by ^{1}H NMR spectroscopy to obtain 1.8:1 diastereomeric mixture of **17a**.

The major diastereomer was partially separated. $[\alpha]^{20}_{589}$ = +2.2 (c = 0.19; $CHCl_3$).

Cbz-Val-Gly(*N*-D-Val-OMe)-OEt (**17b**)

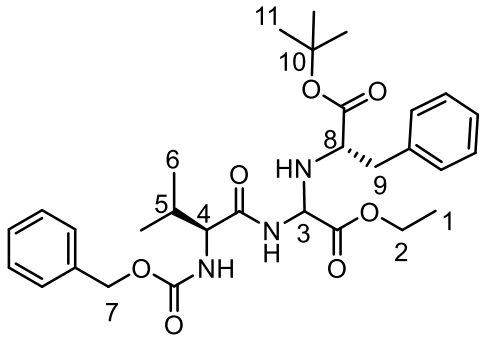


A diastereomeric mixture of ethyl *N*-(*N*-(benzyloxycarbonyl)-L-valyl)-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy) glycinate (**5k**) (d.r. = 2:1, 30 mg, 0.06 mmol) was subjected to general procedure **E**, catalyzed by citric acid (2.3 mg, 0.01 mmol) with D-valine methyl ester (**N7**) (8 mg, 0.06 mmol) as nucleophile in CH_3CN/toluene (1:1, 1.2 mL) and the product **17b** was obtained as partially separable 5:1 diastereomeric mixture as colorless solid (22 mg, 78%). **R_f** = 0.6 (40% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.42-7.30 (m, 10H, CH_{Ar}, CH_{Ar}*), 6.53 (d, *J* = 7.2 Hz, 1H, NH_{Gly}*), 6.43 (d, *J* = 6.7 Hz, 1H, NH_{Gly}), 5.37 (d, *J* = 9.2 Hz, 1H, NH_{Val}), 5.26 (d, *J* = 8.6 Hz, 2H, CH-3, NH_{Val}*), 5.20 (d, *J* = 8.3 Hz, 1H, CH-3*), 5.13 (s, 4H, CH_2-7, CH_2-7*), 4.37-4.16 (m, 4H, CH_2-2, CH_2-2*), 4.10 (dd, *J* = 8.4, 5.1 Hz, 1H, CH-4*), 4.01 (dd, *J* = 8.7, 5.5 Hz, 1H, CH-4), 3.74 (s, 3H, CH_3-11*), 3.64 (s, 3H, CH_3-11), 3.23 (br s, 1H, CH-8), 3.16 (br s, 1H, CH-8*), 2.72 (s, 1H, NH_{Aminal}*), 2.47 (s, 1H, NH_{Aminal}), 2.30-2.16 (m, 1H, CH-5*), 2.13-2.05 (m, 1H, CH-5), 2.04-1.91 (m, 2H, CH-9, CH-9*), 1.30 (t, *J* = 7.2 Hz, 6H, CH_3-1, CH_3-1*), 1.01 (d, *J* = 6.8 Hz, 3H, CH_3-6*), 0.96 (d, *J* = 6.7 Hz, 6H, CH_3-6, CH_3-10*), 0.94 (d, *J* = 6.8 Hz, 6H, CH_3-10, CH_3-6*), 0.91 (d, *J* = 6.8 Hz, 3H, CH_3-6), 0.88 (d, *J* = 6.8, Hz, 3H, CH_3-10*), 0.86 (d, *J* = 6.8 Hz, 3H, CH_3-10). **^{13}C NMR** (101 MHz, $CDCl_3$) δ

= 176.1 (C=O), 174.8 (C=O*), 171.4 (C=O*), 171.2 (C=O), 169.5 (C=O*), 169.4 (C=O), 156.2 (C=O, C=O*), 136.3 (C_{Ar}, C_{Ar}*), 128.6 (CH_{Ar}*), 128.5 (CH_{Ar}), 128.3 (CH_{Ar}*), 128.2 (CH_{Ar}), 128.1 (CH_{Ar}, CH_{Ar}*), 67.2 (C7*), 67.0 (C7), 65.2 (C3), 64.0 (C3*), 63.7 (C8), 62.9 (C8*), 62.2 (C2*), 61.9 (C2), 60.3 (C4*), 60.0 (C4), 52.0 (C11), 51.9 (C11*), 31.8 (C9), 31.6 (C9*), 31.5 (C5), 30.7 (C5*), 19.4 (C10, C10*), 19.1 (C10), 19.0 (C10), 17.7 (C6, C6*), 17.6 (C6), 17.2 (C6*), 14.0 (C1, C1*). **IR**: v [cm^{-1}] 3301, 3034, 2958, 2872, 1734, 1692, 1656, 1534, 1468, 1369, 1298, 1244, 1205, 1144, 1115, 1067, 1041, 999, 779, 741, 696. **LRMS** (ESI+) *m/z*, (%): 466 (15, $[M+H]^+$), 488 (100, $[M+Na]^+$), 954 ($[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{23}H_{35}N_3O_7Na$: 488.2367, found: 488.2363.

The major diastereomer was partially separated. $[\alpha]^{20}_{589} = -2.5$ (c = 0.17; $CHCl_3$).

Cbz-Val-Gly(*N*-Phe-O*t*Bu)-OEt (18)

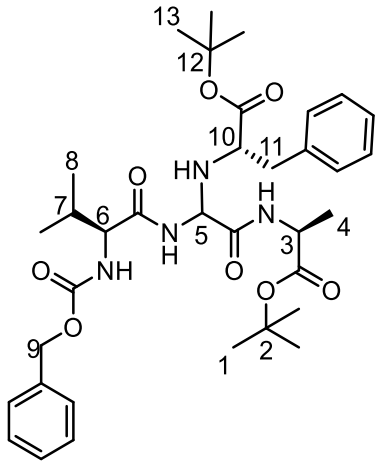


A diastereomeric mixture of ethyl *N*-(*N*-(benzyloxycarbonyl)-L-valyl)-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy) glycinate (**5k**) (d.r. = 2:1, 100 mg, 0.20 mmol) was subjected to general procedure **E**, catalyzed by citric acid (8 mg, 0.04 mmol) with phenylalanine *tert*-butyl ester (**N5**) (45 mg, 0.20 mmol) in CH_3CN (2 mL) and the product **18** was obtained as colorless oil (80 mg, 71%) as 1:1 diastereomeric mixture.

R_f = 0.6 (40% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.44-7.18 (m, 20H, CH_{Ar}, CH_{Ar}*), 6.48 (d, *J* = 7.7 Hz, 1H, NH_{Gly}), 5.85 (d, *J* = 8.3 Hz, 1H, NH_{Gly}*), 5.33 (d, *J* = 8.6 Hz, 1H, NH_{Val}), 5.24-5.22 (m, 2H, CH-3*, NH_{Val}*), 5.16-5.09 (m, 5H, CH_2-7, CH_2-7*, CH-3), 4.24-4.10 (m, 4H, CH_2-2, CH_2-2*), 4.05 (dd, *J* = 8.6, 5.5 Hz, 1H, CH-4), 3.93 (dd, *J* = 8.6, 5.4 Hz, 1H, CH-4*), 3.64 (dd, *J* = 9.8, 6.7 Hz, 1H, CH-8), 3.49 (dd, *J* = 8.5, 8.1 Hz, 1H, CH-8*), 3.08-2.81 (m, 4H, CH_2-9, CH_2-9*), 2.74 (dd, *J* = 9.6, 3.4 Hz, 1H, NH_{Phe}), 2.55 (dd, *J* = 8.5, 7.2 Hz, 1H, NH_{Phe}*), 2.20-2.13 (m, 1H, CH-5*), 2.10-1.99 (m, 1H, CH-5), 1.42 (s, 9H, CH_3-11), 1.39 (s, 9H, CH_3-11*), 1.28 (t, *J* = 7.2 Hz, 6H, CH_3-1, CH_3-1*), 0.97 (d, *J* = 6.8 Hz, 3H, CH_3-6), 0.96 (d, *J* = 6.8 Hz, 3H, CH_3-6*), 0.89 (d, *J* = 6.8 Hz, 6H, CH_3-6, CH_3-6*). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 175.7 (C=O, C=O*), 171.6 (C=O, C=O*), 161.4 (C=O, C=O*), 152.5 (C=O,

C=O*), 137.5 (C_{Ar}, C_{Ar}*), 137.1 (C_{Ar}, C_{Ar}*), 129.6 (CH_{Ar}, CH_{Ar}*), 128.4 (CH_{Ar}, CH_{Ar}*), 128.3 (CH_{Ar}, CH_{Ar}*), 128.2 (CH_{Ar}, CH_{Ar}*), 128.12 (CH_{Ar}), 128.08 (CH_{Ar}*), 126.73 (CH_{Ar}), 126.67 (CH_{Ar}*), 67.2 (C7*), 67.0 (C7), 63.9 (C3*), 63.8 (C3), 62.2 (C2), 61.9 (C2*), 60.0 (C4, C4*), 59.4 (C8*), 59.2 (C8), 39.9 (C9), 39.3 (C9*), 31.4 (C5*), 30.9 (C5), 28.0 (C11*), 27.9 (C11), 19.3 (C6), 18.9 (C6*), 17.2 (C6, C6*), 14.02 (C1), 14.00 (C1*). **IR**: v [cm^{-1}] 3318, 2962, 2927, 1726, 1662, 1523, 1499, 1456, 1368, 1281, 1229, 1153, 1028, 739, 699. **LRMS** (ESI+) *m/z*, (%): 556 (20, $[M+H]^+$), 578 (100, $[M+Na]^+$), 1133 (10, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{30}H_{41}N_3O_7Na$: 578.2837, found: 578.2835.

Cbz-Val-Gly(*N*-Phe-O*t*Bu)-Ala-O*t*Bu (19)



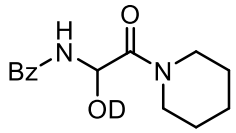
An aqueous LiOH solution (1 N, 100 µL, 0.09 mmol) was added dropwise to a 1:1 diastereomeric mixture Cbz-Val-Gly(*N*-Phe-O*t*Bu)-OEt **18a** (50 mg, 0.09 mmol) in THF (1 mL) and the mixture was stirred at rt for 2 h. After completion, the reaction mixture was treated with AmberChrom® 50WX4 hydrogen form to neutralize to pH~6, the residue was filtered, and the filtrate was evaporated to dryness to obtain the carboxylic acid Cbz-Val-Gly(*N*-Phe-O*t*Bu)-OH as amorphous solid which was used directly for peptide coupling.

Triethylamine (25 µL) was added to a mixture of Cbz-Val-Gly(*N*-Phe-O*t*Bu)-OH and L-alanine *tert*-butyl ester hydrochloride (14 mg, 0.08 mmol) in CH_2Cl_2 (1 mL) followed by addition of HBTU (32 mg, 0.08 mmol) and the reaction mixture was stirred at rt for 3 h. Brine was added, the mixture was extracted with CH_2Cl_2 (2x3 mL). The combined organic extracts were subsequently washed with HCl solution (1 N, 1 mL), and by sat. $NaHCO_3$ solution (1 mL). The organic layer was dried over $MgSO_4$, the solvent was removed at reduced pressure and the residue was purified by flash column chromatography (20% EtOAc/cyclohexane) to give the coupling product **19** (40 mg, 67%) as inseparable 1:1 diastereomeric mixture as colorless oil.

R_f = 0.6 (40% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.47 (d, *J* = 7.7 Hz, 1H, NH_{Ala}), 7.41-7.16 (m, 21H, CH_{Ar}, CH_{Ar}*, NH_{Ala}*), 6.84 (d, *J* = 6.5 Hz, 1H, NH), 6.49 (d, *J* = 5.9 Hz, 1H, NH*), 5.42 (d, *J* = 8.4 Hz, 1H, NH_{Val}), 5.33 (d, *J* = 8.0 Hz, 1H, NH_{Val}*), 5.21-5.01 (m, 5H, CH_2-9, CH_2-9*, CH-5), 4.83 (d, *J* = 6.0 Hz, 1H, CH-5*), 4.40-4.24 (m, 2H, CH-3, CH-3*), 4.11 (dd, *J* = 8.7, 5.0 Hz, 1H, CH-6), 3.99 (dd, *J* = 8.4, 5.0 Hz, 1H, CH-6*), 3.77-3.76 (m, 1H, CH-10), 3.65-3.54 (m, 1H, CH-10*), 3.13-2.84 (m, 4H, CH_2-11, CH_2-11*), 2.76 (s, 2H, NH_{Phe}, NH_{Phe}*), 2.29-2.14 (m, 1H, CH-7), 2.13-2.01 (m, 1H, CH-7*), 1.49 (s, 9H, CH_3-1), 1.46 (s, 9H, CH_3-1*), 1.41 (s, 9H, CH_3-13), 1.36 (s, 9H, CH_3-13*), 1.33 (d, *J* = 7.2 Hz, 3H, CH_3-4), 1.30 (d, *J* = 7.2 Hz, 3H, CH_3-4*), 0.98 (d, *J* = 6.8 Hz, 3H, CH_3-8), 0.94 (d, *J* = 6.8 Hz, 3H, CH_3-8*), 0.90 (d, *J* = 6.8 Hz, 3H, CH_3-8), 0.87 (d, *J* = 6.8 Hz, 3H, CH_3-8*). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 173.5 (C=O), 173.0 (C=O*), 172.5 (C=O), 172.0 (C=O*), 171.3 (C=O), 171.2 (C=O*), 168.6 (C=O*), 168.5 (C=O), 156.3 (C=O*), 156.0 (C=O), 137.5 (C_{Ar}), 137.3 (C_{Ar}*), 136.4 (C_{Ar}), 136.2 (C_{Ar}*), 129.7 (CH_{Ar}, CH_{Ar}*), 129.6 (CH_{Ar}, CH_{Ar}*), 128.51 (CH_{Ar}), 128.46 (CH_{Ar}*), 128.3 (CH_{Ar}, CH_{Ar}*), 128.13 (CH_{Ar}*), 128.09 (CH_{Ar}), 126.72 (CH_{Ar}), 126.67 (CH_{Ar}*), 81.9 (C2), 81.8 (C2*), 81.7 (C12), 81.6 (C12*), 67.1 (C9*), 66.9 (C9), 65.5 (C5*), 64.9 (C5), 60.1 (C6, C10), 59.7 (C6*), 59.4 (C10*), 49.00 (C3), 48.97 (C3*), 40.0 (C11, C11*), 31.9 (C7), 31.0 (C7*), 27.98 (C1), 27.96 (C1*), 27.94 (C13), 27.87 (C13*), 19.3 (C8), 19.0 (C8*), 18.1 (C4), 17.9 (C4*), 17.5 (C8), 17.3 (C8*). **IR**: ν [cm^{-1}] 3308, 2975, 2931, 1726, 1648, 1498, 1455, 1392, 1368, 1284, 1228, 1147, 1095, 1028, 846, 734, 698. **LRMS** (ESI+) *m/z*, (%): 655 (10, $[M+H]^+$), 677 (100, $[M+Na]^+$), 1331 (5, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{35}H_{50}N_4O_8Na$: 677.3521, found: 677.3522.

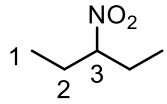
Stability of aminal derivative 16d in TFA

The following procedure outlines the methodology employed to assess the stability of an aminal in acidic conditions:



TFA (4 μL, 0.05 mmol) in D_2O (1 μL, 0.05 mmol) was added to a solution of glycine aminal compound **16d** (10 mg, 0.03 mmol) in CD_3CN (0.5 mL) in an NMR tube and fumaric acid (3.1 mg, 0.03 mmol) was added as internal standard. The reaction was stirred for 12 h and analyzed by ^{1}H NMR.

3-nitropentane (21)

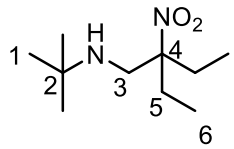


Sodium nitrite (3.66 g, 52.97 mmol) was added to a solution of 3-bromopentane (4.20 mL, 33.10 mmol) in DMSO (70 mL). The reaction mixture was stirred at rt for 18 h, cooled to 0 °C and cold water (20 mL) was added. The mixture was extracted with pentane (3×40 mL). The combined extracts were washed with water (2×5 mL), dried over $MgSO_4$ and the solvent was removed under reduced pressure. The product **21** was obtained as blue liquid and used without further purification (2.77 g, 71%).

^{1}H NMR (400 MHz; $CDCl_3$) δ = 4.38-4.31 (m, 1H, CH-3), 2.05-1.93 (m, 2H, CH_2-2), 1.85-1.75 (m, 2H, CH_2-2), 0.97 (t, *J* = 7.4 Hz, 6H, CH_3-1). **^{13}C NMR** (400 MHz; $CDCl_3$) δ = 91.9 (C3), 26.8 (C2), 10.3 (C1).

The analytical data according to the literature.[141]

***N-tert*-Butyl-(2-ethyl-2-nitrobutyl) amine (22)**

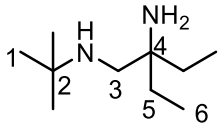


tert-Butylamine (2.11 mL, 20.06 mmol) was added to crude 3-nitropentane (2.35 g, 20.06 mmol). Aqueous formaldehyde (37%, 1.51 mL, 20.06 mmol) was added over 10 min while keeping the temperature between 20-30 °C, and the mixture was stirred at 50 °C for 18 h. After the reaction was completed, pentane (100 mL) was added to the cooled mixture, the organic layer was separated, washed with water (2×20 mL), and dried over $MgSO_4$. After filtration the solvent was evaporated under vacuum, and the crude product was purified by vacuum distillation (140 °C (bath temperature), 6.1 mbar) to obtain **22** as pale yellow oil (2.2 g, 54 %).

R_f = 0.8 (20% EtOAc/ Hex). **^{1}H NMR** (400 MHz; $CDCl_3$) δ = 2.95 (s, 2H, CH_2-3), 2.00 (q, *J* = 7.5 Hz, 4H, CH_2-5), 1.08 (s, 9H, CH_3-1), 0.87 (t, *J* = 7.5 Hz, 6H, CH_3-6). **^{13}C NMR** (400 MHz; $CDCl_3$) δ = 95.9 (C4), 50.3 (C2), 45.6 (C3), 29.1 (C1), 26.2 (C5), 8.0 (C6).

The analytical data according to the literature.[141]

N-*tert*-Butyl-2-ethylbutan-1,2-diamine (23)

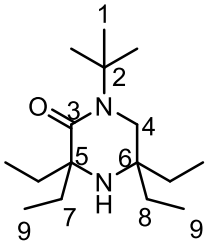


N-*tert*-Butyl-(2-ethyl-2-nitrobutyl) amine (100 mg, 0.49 mmol) was dissolved in a mixture of HOAc (0.72 mL) and H_2O (1 mL). While cooling in an ice bath, zinc powder (193.9 mg, 2.97 mmol) was added and the reaction mixture was subsequently stirred at rt for 2 h. The excess of zinc was filtered off and solid $NaHCO_3$ was added to the filtrate until the solution turned basic. The mixture was extracted with Et_2O (3×20 mL), and the extracts were dried over $MgSO_4$. The solvent was evaporated under vacuum and the product was obtained as a colorless liquid (80 mg, 94 %).

R_f = 0.1 (100 % EtOAc). **^{1}H NMR** (400 MHz; $CDCl_3$) δ = 2.64 (s, 2H, CH_2-3), 1.52-1.35 (m, 4H, CH_2-5), 1.15 (s, 9H, CH_3-1), 0.90-0.87 (m, 6H, CH_3-6). **^{13}C NMR** (400 MHz; $CDCl_3$) δ = 60.7 (C2), 52.9 (C4), 47.4 (C3), 28.7 (C1), 23.7 (C5), 7.4 (C6).

The analytical data according to the literature.[141]

1-*tert*-Butyl-3,3,5,5-tetraethyl-2-piperazinone (24A)

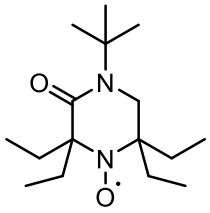


KOH powder (130 mg, 2.32 mmol) was added slowly at 10 °C to a mixture of N-*tert*-butyl-2-ethylbutan-1,2-diamine (80 mg, 0.464 mmol), 3-pentanone (0.6 mL, 5.57 mmol) and $CHCl_3$ (0.06 mL, 0.69 mmol) and the reaction mixture was stirred at rt for 18 h. After completion, the reaction mixture filtered, the filtrate was evaporated to dryness and the crude product was purified by flash column chromatography (1:10 Et_2O/ pentane) to obtain piperazinone **24A** as colourless oil (39 mg, 32%)

R_f = 0.5 (1:5 Et_2O/Pentane). **^{1}H NMR** (400 MHz; $CDCl_3$) δ = 3.17 (s, 2H, CH_2-4), 1.64 (q, *J* = 7.5 Hz, 4H, CH_2-7), 1.44 (s, 9H, CH_3-1), 1.40 (q, *J* = 7.2 Hz, 4H, CH_2-8), 0.90-0.84 (m, 12H, CH_3-9). **^{13}C NMR** (400 MHz; $CDCl_3$) δ = 174.6 (C3), 62.0 (C5), 57.1 (C2), 53.4 (C6), 51.0 (C4), 32.3 (C8), 28.9 (C7), 28.4 (C1), 8.1 (C9), 7.8 (C9).

The analytical data according to the literature.[141]

1-*tert*-Butyl-3,3,5,5-tetraethylpiperazin-2-one-4-oxyl radical (NO1)

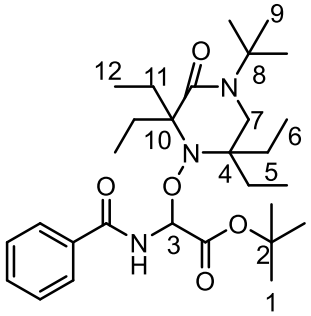


Peroxyacetic acid (39 wt.% in HOAc, 0.22 mL, 1.06 mmol) was added to a solution of piperazinone (190 mg, 0.71 mmol) in EtOAc (2.5 mL) at 0 °C. The reaction mixture was stirred at rt for 16 h. Pentane (10 mL) was added to the reaction mixture, which was washed with sat. $NaHCO_3$ solution (6 mL), the organic layer was dried over $MgSO_4$, the solvent was evaporated under reduced pressure and the residue was purified by column chromatography (8% EtOAc/pentane) to obtain the nitroxide **NO1** as yellowish red oil (150 mg, 75%).

R_f = 0.4 (10 % EtOAc/Pentane). **LRMS** (ESI+) *m/z*, (%): 283 (100, [M]), 284 (20, $[M+H]^+$). **HRMS** (ESI+) *m/z*: ([M]): calculated for $C_{16}H_{31}N_2O_2$: 283.2386, found: 283.2383.

The analytical data according to the literature.[141]

***tert*-Butyl *N*-benzoyl-2-((1-*tert*-butyl-3,3,5,5-tetraethyl-2-oxopiperazinyloxy)glycinate (25)**

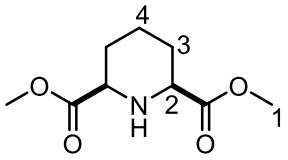


KHMDS (0.45 mL, 0.4437 mmol,1 M in THF) was added dropwise to a solution of *tert*-butyl *N*-benzoylglycinate (**4t)** (48 mg, 0.20 mmol) in THF (1 mL) at −78 °C. After 30 min., nitroxyl radical (**NO1**) (128 mg, 0.44 mmol) was added, and the reaction mixture was stirred at 0 °C for 1 h. After completion, 4-5 drops of saturated NH_4Cl solution were added, the reaction mixture was filtered through a Celite® pad. The solvents were evaporated under reduced pressure, and the residue was purified by flash chromatography (10 % EtOAc/hexane) to give oxygenated product **25** as white amorphous solid (40 mg, 40 %).

R_f = 0.6 (20% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.86 (d, *J* = 7.2 Hz, 2H, CH_{Ar}), 7.58-7.48 (m, 3H, CH_{Ar}), 7.09 (d, *J* = 9.8 Hz, 1H, NH), 6.09 (d, *J* = 10.0 Hz, 1H, CH-3), 3.15 (s, 2H, CH_2-7), 2.01-1.91 (m, 4H, CH_2-11), 1.70-1.65 (m, 4H, CH_2-5), 1.53 (s, 9H, CH_3-1),

1.41 (s, 9H, CH_3-9), 1.15 (t, *J* = 7.4 Hz, 3H, CH_3-12), 1.05 (t, *J* = 7.4 Hz, 6H, CH_3-12, CH_3-6), 0.78 (t, *J* = 7.3 Hz, 3H, CH_3-6).

2,6-Bis(methoxycarbonyl)piperidine (27)

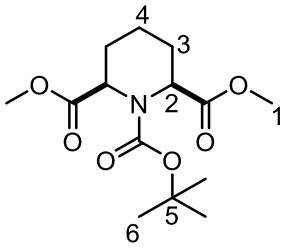


Dimethyl pyridine-2,6-dicarboxylate **26** (1.01 g, 5.16 mmol) was added to a suspension of palladium on charcoal (200 mg) in MeOH (12 mL). The mixture was stirred in an autoclave under a hydrogen atmosphere (18 bars) at 60 °C for 36 h. The mixture was filtered through Celite® and the solvent was evaporated at reduced pressure. The resulting white solid was dried to yield the product **27** (1.04 g, 95 %), which was used directly for the next step.

R_f = 0.1 (20% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 3.76 (s, 6H, CH_3-1), 3.41 (dd, *J* = 11.0, 2.5 Hz, 2H, CH-2), 2.45 (br s, 1H, NH), 1.96-1.56 (m, 6H, CH_2-3, CH_2-4). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 172.9 (C=O), 58.4 (C2), 52.1 (C1), 28.7 (C3), 24.1 (C4). **IR**: ν [cm^{-1}] 3342, 3019, 2982, 2954, 2931, 2869, 2807, 1730, 1438, 1332, 1317, 1212, 1183, 1150, 1131, 1099, 1061, 1046, 1000, 973, 922, 884, 774, 653. **LRMS** (ESI+) *m/z,* (%): 142 (100, $[M+H-CO_2Me]^+$), 202 (40, $[M+H]^+$), 224 (55, $[M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_9H_{15}O_4NNa$: 224.0893; found: 224.0895.

The analytical data according to the literature.[143]

2,6-Bis(methoxycarbonyl)-*N*-(*tert*-butyloxycarbonyl)piperidne (28)



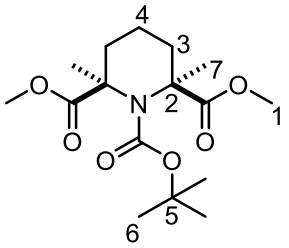
Boc_2O (2.3 mL, 10.14 mmol) was added to a solution of piperidine **27** (1.02 g, 5.07 mmol) in toluene (35 mL) and the mixture was heated to 95 °C for 16 h. The solvent was evaporated under reduced pressure and the residue was purified by flash column chromatography (5% EtOAc/ cyclohexane) to yield the Boc-protected piperidine **28** as colorless oil (1.48 g, 95%).

R_f = 0.4 (20% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 4.82 (br s, 2H, CH-2), 3.72 (s, 6H, CH_3-1), 1.90-1.63 (m, 6H, CH_2-3, CH_2-4), 1.50 (s, 9H, CH_3-6). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 172.1 (C=O), 155.4 (C=O), 80.9 (C5), 51.9 (C1, C2), 28.3 (C6), 25.6 (C3), 16.5

(C4). **IR**: ν [cm^{-1}] 2953, 2871, 1735, 1695, 1437, 1399, 1366, 1347, 1332, 1308, 1253, 1200, 1169, 1131, 1082, 1057, 1013, 1001, 921, 881, 856, 801, 772, 654. **LRMS** (ESI+) *m/z,* (%): 268 (35, $[M+Na\text{-isobutylene}]^+$), 324 (100, $[M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{14}H_{23}O_6NNa$: 324.1418; found: 324.1419.

The analytical data according to the literature.[143]

2,6-Bis(methoxycarbonyl)-*N*-(*tert*-butyloxycarbonyl) *cis*-2,6-dimethylpiperidine (29)

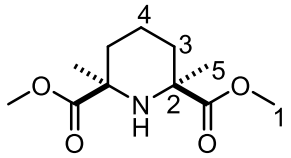


HMDS (12.3 mL, 58.8 mmol) was dissolved in THF (118 mL) and the reaction was cooled to −60 °C. *n*-BuLi (36.8 mL, 58.8 mmol, 1.6M in hexane) was added dropwise via syringe and the solution was stirred for 30 min. This solution was added dropwise to a solution of the N-protected piperidine **28** (7.09 g 23.5 mmol) in THF (23 mL) and stirring continued at −60 °C for another 60 min. After cooling to −78 °C, iodomethane (7.3 mL, 118.0 mmol) was slowly added, after stirring for 90 min the solution was warmed to room temperature and stirring was continued overnight. The reaction was quenched by the addition of sat. NH_4Cl solution (50 mL) and the aqueous layer was extracted with EtOAc (150 mL). The combined organic solutions were washed with brine, dried over $MgSO_4$ and the solvent was evaporated at reduced pressure. Purification by column chromatography (20% EtOAc/cyclohexane) yielded the product **29** as 10:1 diastereomeric mixture (*cis*/*trans*) as a pale yellow oil (6.86 g, 89%).

R_f = 0.3 (20% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 3.77 (s, 6H, CH_3-1), 1.89-1.63 (m, 6H, CH_2-3, CH_2-4), 1.55 (s, 6H, CH_3-7), 1.41 (s, 9H, CH_3-6). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 176.0 (C=O), 155.1 (C=O), 81.7 (C5), 60.6 (C2), 51.8 (C1), 32.5 (C3), 28.1 (C6, C7), 15.4 (C4). **IR**: ν [cm^{-1}] 2982, 2951, 1736, 1691, 1434, 1383, 1362, 1340, 1303, 1270, 1172, 1152, 1138, 1123, 1086, 1010, 976, 908, 856, 777, 734, 702. **LRMS** (ESI+) *m/z,* (%): 352 (100, $[M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{16}H_{27}O_6NNa$: 352.1731; found: 352.1732.

The analytical data according to the literature.[143]

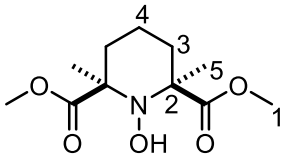
***cis*-2,6-Bis(methoxycarbonyl)-2,6-dimethylpiperidine (30)**



N-tert-Butoxycarbonylpiperidine **29** (1.97 g, 5.98 mmol) was dissolved in a mixture of 48% hydrobromic acid and conc. acetic acid (HBr/AcOH = 1/9, 50 mL) and stirred at room temperature for 4 h. After completion, the reaction mixture was evaporated to dryness, brine (10 mL) was added, neutralized with Na_2CO_3 powder, and the aqueous layer was extracted with EtOAc (60 mL). The combined organic layers were dried over $MgSO_4$, the solvent was evaporated under reduced pressure and the residue was obtained as colorless oil. (1.27 g, 92%). **R_f** = 0.2 (50% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 3.76 (s, 1H, NH), 3.67 (s, 6H, CH_3-1), 1.93-1.60 (m, 6H, CH_2-3, CH_2-4), 1.28 (s, 6H, CH_3-5). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 176.6 (C=O), 57.6 (C2), 51.6 (C1), 33.6 (C3), 30.1 (C5), 19.3 (C4). **IR**: ν [cm^{-1}] 3342, 3020, 2951, 1727, 1451, 1221, 1194, 1177, 1145, 1133, 1095, 1073, 978, 758, 714. **LRMS** (ESI+) *m/z,* (%): 170 (95, $[M-CO_2Me]^+$), 230 (10, $[M+H]^+$), 252 (100, $[M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{11}H_{19}O_4NNa$: 252.1212; found: 252.1209.

The analytical data according to the literature.[143]

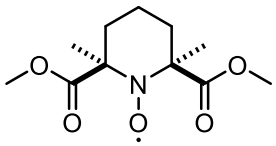
***cis*-2,6-Bis(methoxycarbonyl)-*cis*-2,6-dimethyl-1-hydroxypiperidine (31)**



*m*CPBA (1.04 g, 70-75%, 4.22 mmol) was added portion wise to a solution of the piperidine **30** (880 mg, 3.84 mmol) in CH_2Cl_2 (40 mL) under nitrogen at 0 °C. The reaction mixture was warmed to room temperature and stirred for 4 h and an orange-colored solution was formed. After the completion, the mixture was diluted with CH_2Cl_2 (40 mL), washed with 5% Na_2CO_3 and the organic layer was dried over $MgSO_4$, the solvent was evaporated under vacuum to obtain *N*-hydroxyamine **31** (5% EtOAc/cyclohexane) as colorless oil (470 mg, 50%) and nitroxide **NO2** (10% EtOAc/cyclohexane) as orange solid (74 mg, 24%)

R_f = 0.4 (20% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.34 (s, 1H, OH), 3.72 (s, 6H, CH_3-1), 2.18-2.13 (m, 2H, CH_2-3), 1.59-1.45 (m, 4H, CH_2-3, CH_2-4), 1.53 (s, 6H, CH_3-5. **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 175.2 (C=O), 65.6 (C2), 51.6 (C1), 36.2 (C3), 25.7 (C5), 18.3 (C4). **IR**: ν [cm^{-1}] 3436, 2952, 1723, 1448, 1433, 1287, 1239, 1193, 1174, 1146, 1131, 1074, 995, 974, 756, 726. **LRMS** (ESI+) *m/z,* (%): 268 (100, $[M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{11}H_{19}O_5NNa$: 268.1155; found: 268.1153.

***cis*-2,6-Bis(methoxycarbonyl)-2,6-dimethylpiperidine-1-oxyl (NO2)**

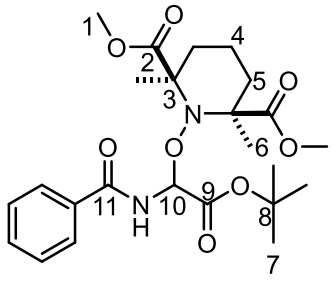


$CuBr_2$ (245 mg, 0.27 mmol) was added to a solution of *N*-hydroxy amine **31** (1000 mg, 4.08 mmol) in CH_2Cl_2 (40 mL) and oxygen was bubbled for 3 h. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with brine (20 mL). The organic layer was dried over $MgSO_4$, the solvent was evaporated under reduced pressure and the residue was purified by column chromatography (20% EtOAc/cyclohexane) to obtain nitroxide **NO2** (980 mg, 98%) as orange solid.

R_f = 0.3 (20% EtOAc/PE). **m.p.** 61-63 °C. **IR**: ν [cm^{-1}] 2952, 1723, 1448, 1433, 1287, 1239, 1193, 1174, 1146, 1131, 1074, 995, 974, 756, 726. **LRMS** (ESI+) *m/z,* (%): 186 (60, $[M+H-CO_2Me]^+$), 208 (80, $[M+Na-CO_2Me]^+$), 267 (100, $[M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{11}H_{18}O_5NNa$: 267.1077; found: 267.1080. **Anal. Calcd** for $C_{11}H_{18}NO_5$: C, 54.09; H, 7.43; N, 5.73. Found: C, 54.52; H, 7.36; N, 5.77.

The analytical data according to the literature.[143]

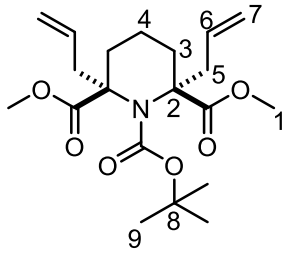
***tert*-Butyl *N*-(benzoyl)-(2*R* and 2*S*)-(*cis*-(2,6-bis(methoxycarbonyl)-2,6-dimethyl piperidine-1-yl)oxy)glycinate (32)**



A freshly prepared LiHMDS solution (*n*-BuLi (0.6 mL, 0.93 mmol, 1.6 M in hexane) and HMDS (0.2 mL, 0.93 mmol) in THF (1 mL) at −78 °C) was added dropwise to a solution of glycine derivative (**4q**) (100 mg, 0.42 mmol) in THF (1.5 mL) at −78 °C and the mixture was stirred for 30 min. Nitroxide **NO2** (230 mg, 0.94 mmol) was added and the reaction mixture was warmed to 0 °C over 1h. The reaction was quenched by a few drops of sat. NH_4Cl solution, diluted with EtOAc, and filtered through a short pad of Celite®. The solvent was evaporated at reduced pressure and the crude product was purified by column chromatography (10% EtOAc/cyclohexane) to give oxygenated product **32** as 1:1 diastereomeric mixture (110 mg, 54%).

R_f = 0.4 (40% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.89-7.82 (m, 4H, CH_{Ar}, CH_{Ar}*), 7.74 (br s, 1H, NH*), 7.57-7.43 (m, 6H, CH_{Ar}, CH_{Ar}*), 7.21 (br s, 1H, NH), 6.14 (d, *J* = 9.0 Hz, 1H, CH-10*), 6.06 (d, *J* = 7.6 Hz, 1H, CH-10), 3.76 (s, 3H, CH_3-1), 3.70 (s, 3H, CH_3-1*), 3.65 (s, 3H, CH_3-1*), 3.52 (s, 3H, CH_3-1), 1.93-1.44 (m, 12H, CH_2-4, CH_2-4*, CH_2-5, CH_2-5*), 1.68 (s, 9H, CH_3-7*), 1.55 (s, 12H, CH_3-6, CH_3-6*), 1.49 (s, 9H, CH_3-7). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 175.6 (C=O, C=O*), 167.0 (C=O, C=O*), 166.1 (C=O, C=O*), 133.8 (C_{Ar}, C_{Ar}*), 132.3 (CH_{Ar}*), 131.8 (CH_{Ar}), 128.7 (CH_{Ar}), 128.6 (CH_{Ar}*), 127.3 (CH_{Ar}), 127.2 (CH_{Ar}*), 84.2 (C10), 83.7 (C10*), 82.4 (C8, C8*), 68.7 (C3), 68.5 (C3*), 52.6 (C1), 52.5 (C1*), 35.7 (C5), 35.5 (C5*), 27.9 (C7, C7*), 17.6 (C6), 17.4 (C6*), 15.2 (C4, C4*). **IR**: ν [cm^{-1}] 3436, 2998, 2980, 2949, 1749, 1733, 1722, 1674, 1520, 1483, 1457, 1329, 1256, 1230, 1203, 1185, 1159, 1143, 1117, 1068, 1047, 1022, 977, 716, 692. **LRMS** (ESI+) *m/z,* (%): 479 (10, $[M+H]^+$), 501 (100, $[M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{24}H_{34}O_8N_2Na$: 501.2207; found: 501.2207.

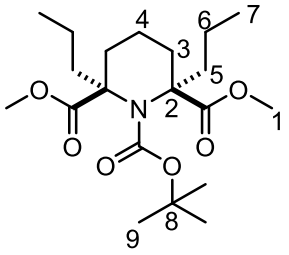
***cis*-2,6-Bis(methoxycarbonyl)-*N*-(*tert*-butyloxycarbonyl)-2,6-diallylpiperidine (33)**



NaHMDS (16 mL, 15.8 mmol, 1M in THF) was added dropwise to a solution of the *N*-protected piperidine **28** (1.9 g, 6.3 mmol) in THF (40 mL) at −78 °C and the solution was stirred for 60 min. Allyl bromide (2.7 mL, 31.5 mmol) was added dropwise, the solution was warmed to room temperature after 90 min and the reaction mixture was stirred overnight. After completion, the reaction was quenched by addition of sat. NH_4Cl solution (10 mL) and the aqueous layer was extracted with EtOAc (2x30 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and the solvent was evaporated at reduced pressure. Purification by column chromatography (10% EtOAc/cyclohexane) yielded the product **33** as a colorless oil as 5:1 diastereomeric mixture (1.68 g, 69%).

R_f = 0.6 (20% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.21-6.02 (m, 4H, CH-6, CH-6*), 5.24-4.94 (m, 8H, CH_2-7, CH_2-7*), 3.74 (s, 12H, CH_3-1, CH_3-1*), 3.07-2.92 (m, 4H, CH_2-5, CH_2-5*), 2.71-2.56 (m, 4H, CH_2-5, CH_2-5*), 2.00-1.88 (m, 4H, CH_2-3, CH_2-3*), 1.88-1.77 (m, 4H, CH_2-3, CH_2-3*), 1.73-1.48 (m, 4H, CH_2-4, CH_2-4*), 1.43 (s, 9H, CH_3-9*), 1.41 (s, 9H, CH_3-9). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 175.3 (C=O, C=O*), 154.4 (C=O, C=O*), 135.6 (C6*), 135.5 (C6), 117.9 (C7, C7*), 81.9 (C8), 81.7 (C8*), 63.4 (C2, C2*), 51.9 (C1, C1*), 42.0 (C5, C5*), 32.8 (C3, C3*), 28.1 (C9, C9*), 14.8 (C4, C4*). **IR**: ν [cm^{-1}] 2962, 2926, 2852, 1741, 1697, 1369, 1344, 1260, 1164, 1093, 1019, 799. **LRMS** (ESI+) *m/z,* (%): 404 (100, $[M+Na]^+$), 785 (25, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{20}H_{31}O_6NNa$: 404.2044; found: 404.2039.

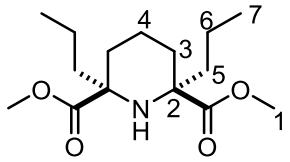
2,6-Bis(methoxycarbonyl)-*N*-(*tert*-butyloxycarbonyl)-*cis*-2,6-dipropylpiperidine (34)



Pd/C (100 mg, 10 wt.% loading) was added to a solution of diallylpiperidine **33** (950 mg, 2.49 mmol) in MeOH (12 mL) and the reaction mixture was stirred under a hydrogen atmosphere in an autoclave at 50 °C for 3 h. After completion, the reaction mixture was diluted with MeOH (20 mL) and filtered through Celite®. The solvent was removed under reduced pressure and purification by column chromatography (10% EtOAc/cyclohexane) yielded the propyl derivative **34** (860 mg, 90%) as colorless oil as 5:1 diastereomeric mixture.

R_f = 0.6 (20% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 3.71 (s, 12H, CH_3-1, CH_3-1*), 2.12-1.97 (m, 4H, CH_2-3, CH_2-3*), 1.96-1.79 (m, 12H, CH_2-3, CH_2-3*, CH_2-5, CH_2-5*), 1.73-1.45 (m, 12H, CH_2-6, CH_2-6*, CH_2-4, CH_2-4*), 1.42 (s, 9H, CH_3-9*), 1.39 (s, 9H, CH_3-9), 0.94 (t, *J* = 7.3 Hz, 12H, CH_3-7, CH_3-7*). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 175.5 (C=O), 174.1 (C=O*), 154.8 (C=O, C=O*), 81.3 (C8, C8*), 63.6 (C2, C2*), 51.9 (C1*), 51.8 (C1), 39.8 (C3, C3*), 33.1 (C5, C5*), 28.2 (C9*), 28.0 (C9), 18.4 (C6, C6*), 15.4 (C4, C4*), 14.8 (C7), 14.6 (C7*). **IR**: ν [cm^{-1}] 2955, 2874, 1736, 1691, 1456, 1433, 1367, 1342, 1322, 1272, 1241, 1168, 1135, 1108, 1068, 1033, 1018, 772, 734. **LRMS** (ESI+) *m/z,* (%): 408 (100, $[M+Na]^+$), 793 (10, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{20}H_{35}O_6NNa$: 408.2357; found: 408.2355.

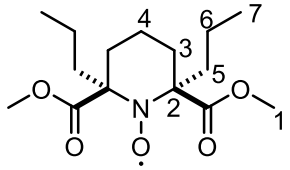
2,6-Bis(methoxycarbonyl)-*cis*-2,6-dipropylpiperidine (35)



N-*tert*-(Butoxycarbonyl)piperidine **34** (800 mg, 2.07 mmol) was dissolved in a mixture of 48% hydrobromic acid and conc. acetic acid (HBr/HOAc = 1/9, 40 mL) and the reaction mixture was stirred at room temperature overnight. After completion, the reaction mixture was evaporated to dryness, brine (10 mL) was added, neutralized with Na_2CO_3 powder, and the aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic layers were dried over $MgSO_4$, the solvent was evaporated under reduced pressure and the residue was obtained as colorless oil (500 mg, 84%) as 5:1 diastereomeric mixture, which was used in the next step without further purification.

R_f = 0.2 (50% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 3.66 (s, 6H, CH_3-1), 2.48 (s, 1H, NH), 2.27-2.09 (m, 2H, CH_2-5), 1.89-1.69 (m, 1H, CH_2-6), 1.68-1.55 (m, 3H, CH_2-6, 2CH_2-3), 1.51-1.38 (m, 2H, CH_2-3), 1.37-1.24 (m, 2H, CH_2-6), 1.23-1.13 (m, 2H, CH_2-5), 1.12-1.02 (m, 2H, CH_2-4), 0.88 (t, *J* = 7.3 Hz, 6H, CH_3-7). **LRMS** (ESI+) *m/z,* (%): 226 (55, [M-CO_2Me]$^+$), 286 (100, [M+H]$^+$), 308 (30, [M+Na]$^+$). **HRMS** (ESI+) *m/z*: ([M+Na]$^+$): calculated for $C_{15}H_{27}O_4NNa$: 308.1832; found: 308.1830.

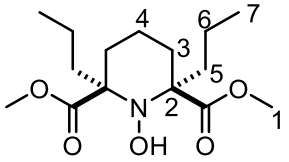
***cis*-2,6-Bis(methoxycarbonyl)-2,6-dipropylpiperidine-N-oxyl (NO3)**



*m*CPBA (475 mg, 70–75%, 1.92 mmol) was added portionwise to a solution of piperidine **35** (500 mg, 1.75 mmol) in CH_2Cl_2 (40 mL) under nitrogen at 0 °C. The reaction mixture was warmed to room temperature and stirred for 4 h and an orange-colored solution was formed. After completion, the mixture was diluted with CH_2Cl_2 (40 mL) and washed with an aqueous Na_2CO_3 (5% solution). The organic layer was dried over $MgSO_4$ and the solvent was evaporated under vacuum. Purification by column chromatography (10% EtOAc/cyclohexane) yielded nitroxide **NO3** as orange solids (280 mg, 53%) and *N*-hydroxy amine **36** as colorless oil (110 mg, 21%)

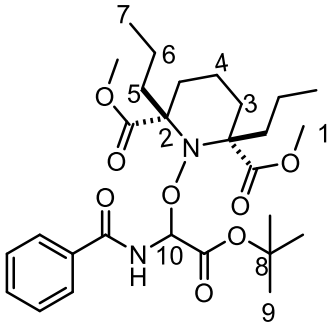
R_f = 0.3 (20% EtOAc/PE). **m.p.** 73-75 °C. **LRMS** (ESI+) *m/z,* (%): 242 (100, [M+H–CO_2Me]$^+$), 264 (25, [M+Na–CO_2Me]$^+$), 301 (5, [M+H]$^+$), 323 (70, [M+Na]$^+$). **HRMS** (ESI+) *m/z*: ([M+Na]$^+$): calculated for $C_{15}H_{26}O_5NNa$: 323.1703; found: 323.1705. **Anal. Calcd** for $C_{15}H_{26}NO_5$: C, 59.98; H, 8.73; N, 4.66. Found: C, 59.77; H, 8.27; N, 4.94.

***cis*-2,6-bis(methoxycarbonyl)-1-hydroxy-2,6-dipropylpiperidine (36)**



R_f = 0.5 (20% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.30 (s, 1H, OH), 3.69 (s, 6H, CH_3-1), 2.28-2.11 (m, 2H, CH_2-5), 2.03 (ddd, *J* = 13.4, 12.3, 4.5 Hz, 2H, CH_2-3), 1.89-1.67 (m, 2H, CH_2-3), 1.64-1.38 (m, 8H, CH_2-5, CH_2-6), 1.20-1.08 (m, 2H, CH_2-4), 0.91 (t, *J* = 7.3 Hz, 6H, CH_3-7). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 174.8 (C=O), 68.3 (C2), 51.0 (C1), 41.0 (C3), 32.4 (C5), 18.2 (C6), 16.4 (C4), 14.1 (C7). **LRMS** (ESI+) *m/z,* (%): 242 (40, [M-CO_2Me]$^+$), 324 (100, [M+Na]$^+$). **HRMS** (ESI+) *m/z*: ([M+Na]$^+$): calculated for $C_{15}H_{27}O_5NNa$: 324.1781; found: 324.1782.

***tert*-Butyl *N*-benzoyl-(2*R* and 2*S*)-(*cis*-(2,6-bis(methoxycarbonyl)-2,6-dipropylpiperidine-1-yl)oxy)glycinate (37)**

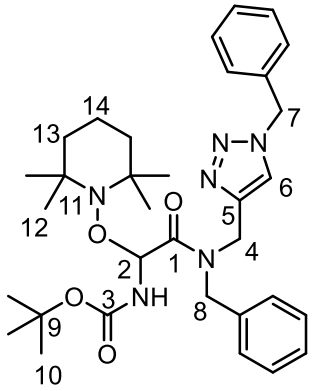


A freshly prepared LHMDS solution (*n*-BuLi (0.6 mL, 0.93 mmol, 1.6 M in hexane) and HMDS (0.2 mL, 0.93 mmol) in THF (1 mL) at −78 °C) was added dropwise to a solution of *tert*-butyl glycinate (**4q**) (100 mg, 0.42 mmol) in THF (1.5 mL) at −78 °C and the mixture was stirred for 30 min. Nitroxide **NO3** (281 mg, 0.94 mmol) was added and the mixture was stirred for 1 h on warming to 0 °C. The reaction mixture was quenched by a few drops of sat. NH_4Cl solution, filtered through a short pad of Celite® and the solvent was evaporated at reduced pressure. The crude product was purified by column chromatography (10%

EtOAc/cyclohexane) to give oxygenated product **37** (132 mg, 58%) as 1:1 diastereomeric mixture.

R_f = 0.5 (20% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.98-7.75 (m, 4H, CH_{Ar}, CH_{Ar}*), 7.62-7.52 (m, 2H, CH_{Ar}, CH_{Ar}*), 7.51-7.41 (m, 4H, CH_{Ar}, CH_{Ar}*), 7.31 (d, *J* = 8.8 Hz, 1H, NH), 6.66 (br s, 1H, NH*), 6.14 (d, *J* = 9.2 Hz, 1H, CH-10), 5.83 (br s, 1H, CH-10*), 3.66 (s, 6H, CH_3-1*), 3.62 (s, 6H, CH_3-1), 2.53-2.47 (m, 4H, CH_2-3, CH_2-3*), 2.24-2.00 (m, 12H, CH_2-5, CH_2-5*, CH_2-6, CH_2-6*, CH_2-3, CH_2-3*), 1.52-1.14 (m, 12H, CH_2-5, CH_2-5*, CH_2-6, CH_2-6*, CH_2-4, CH_2-4*), 1.55 (s, 9H, CH_3-9), 1.53 (s, 9H, CH_3-9*), 0.99 (t, *J* = 6.9 Hz, 6H, CH_3-7), 0.58 (t, *J* = 7.2 Hz, 6H, CH_3-7*). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 173.0 (C=O, C=O*), 169.3 (C=O, C=O*), 166.3 (C=O, C=O*), 133.4 (C_{Ar}, C_{Ar}*), 132.2 (CH_{Ar}, CH_{Ar}*), 128.65 (CH_{Ar}), 128.61 (CH_{Ar}*), 127.31 (CH_{Ar}), 127.26 (CH_{Ar}*), 83.2 (C10, C10*), 83.1 (C8, C8*), 71.2 (C2), 69.4 (C2*), 51.06 (C1), 50.95 (C1*), 42.9 (C5), 42.5 (C5*), 40.4 (C3, C3*), 33.6 (C6), 33.5 (C6*), 28.1 (C9*), 27.9 (C9), 17.9 (C4*), 17.1 (C4), 14.5 (C7), 14.4 (C7*). **IR**: ν [cm^{-1}] 3346, 2959, 2931, 2875, 2852, 1736, 1672, 1524, 1487, 1457, 1368, 1341, 1260, 1225, 1153, 1017, 1001, 987, 972, 711. **LRMS** (ESI+) *m/z,* (%): 535 (10, $[M+H]^+$), 557 (100, $[M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{28}H_{42}O_8N_2Na$: 557.2833; found: 557.2835.

***N*-Benzyl-*N'*-(*tert*-butyloxycarbonyl)-*N*-[(1-benzyltriazol-4-yl)methyl]-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)glycinamide (39)**

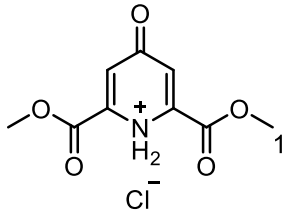


Aqueous sodium ascorbate solution (10 µL, 0.002 mmol, 0.2 M) and copper(II) sulfate pentahydrate solution (50 µL, 0.022 mmol, 0.4 M) were added to a mixture of *N*-benzyl-*N'*-(*tert*-butyloxycarbonyl)-*N*-propargyl-2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)glycinamide (**5d**) (100 mg, 0.219 mmol) and benzyl azide (0.5 M in DCM, 0.44 mL, 0.219 mmol) in a H_2O/^{t}BuOH mixture (2 mL, 1:1) and the heterogeneous mixture was stirred vigorously at rt overnight. The reaction mixture was diluted with EtOAc (10 mL), washed with sat. $NaHCO_3$ solution (3 mL), dried over $MgSO_4$ and the solvent was removed in vacuum. The residue was

purified by column chromatography (20% EtOAc/cyclohexane) to obtain **39** (75 mg, 58%) as 2:1 rotameric mixture as colorless oil.

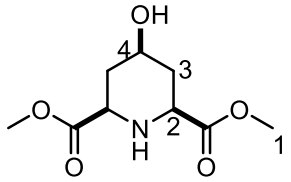
R_f = 0.45 (20% EtOAc/PE). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.51 (s, 1H, CH-6, A), 7.45 (s, 1H, CH-6, B), 7.41-7.24 (m, 20H, CH_{Ar}, A+B), 6.26 (d, *J* = 10.7 Hz, 1H, NH, B), 6.09 (d, *J* = 10.7 Hz, 1H, CH-2, B), 6.01 (d, *J* = 10.6 Hz, 1H, CH-2, A), 5.76 (d, *J* = 10.6 Hz, 1H, NH, A), 5.56-5.43 (m, 4H, CH_2-7, A+B), 4.98 (d, *J* = 16.3 Hz, 1H, CH_2-8, A), 4.81 (d, *J* = 16.5 Hz, 1H, CH_2-4, B), 4.72 (d, *J* = 16.5 Hz, 1H, CH_2-4, B), 4.63-4.60 (m, 4H, CH_2-8, A+B, CH_2-4, A), 4.42 (d, *J* = 14.7 Hz, 1H, CH_2-4, A), 1.50-1.28 (m, 12H, CH_2-13, CH_2-14, A+B), 1.45 (s, 18H, CH_3-10, A+B), 1.21 (s, 3H, CH_3-12, B), 1.17 (s, 3H, CH_3-12, A), 1.13 (s, 3H, CH_3-12, B), 1.05 (s, 3H, CH_3-12, B), 1.03 (s, 6H, CH_3-12, A+B), 0.94 (s, 3H, CH_3-12, A), 0.89 (s, 3H, CH_3-12, A). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 168.4 (C=O, A), 167.9 (C=O, B), 154.7 (C=O, B), 154.2 (C=O, A), 144.0 (C5, B), 143.8 (C5, A), 136.3 (C_{Ar}, B), 135.7 (C_{Ar}, A), 134.5 (C_{Ar}, A+B), 129.2 (CH_{Ar}, B), 129.1 (CH_{Ar}, A), 128.79 (CH_{Ar}, A), 128.75 (CH_{Ar}, B), 128.6 (CH_{Ar}, A+B), 128.2 (CH_{Ar}, A), 128.1 (CH_{Ar}, B), 127.8 (CH_{Ar}, A+B), 127.5 (CH_{Ar}, A+B), 123.4 (C6, A), 122.3 (C6, B), 81.7 (C2, B), 80.4 (C2, A), 79.94 (C9, A), 79.88 (C9, B), 60.6 (C11, B), 60.4 (C11, A), 59.67 (C11, B), 59.65 (C11, A), 54.3 (C7, B), 54.2 (C7, A), 50.4 (C8, A), 48.0 (C8, B), 41.4 (C4, B), 40.2 (C4, A), 40.1 (C13, A+B), 33.4 (C12, B), 33.3 (C12, A), 33.0 (C12, B), 32.6 (C12, A), 28.3 (C10, B) , 28.2 (C10, A), 20.53 (C12, B), 20.49 (C12, B), 20.4 (C12, A), 17.1 (C14, A+B). **IR**: v [cm^{-1}] 3294, 2964, 2927, 1715, 1649, 1496, 1450, 1365, 1259, 1241, 1161, 1133, 1046, 1013, 985, 953, 921, 798, 723, 697, 665. **LRMS** (ESI+) *m/z*, (%): 591 (30, $[M+H]^+$) , 613 (100, $[M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{33}H_{46}N_6O_4Na$: 613.3473, found: 613.3474.

Chelidamic acid dimethyl ester hydrochloride (41)



To a suspension of Chelidamic acid hydrate (5 g, 25.94 mmol) in dry MeOH (60 mL), dimethoxypropane (30 mL) was added, followed by addition of conc. HCl (11.6 M, 3.4 mL, 38.91 mmol). The mixture was refluxed under a $CaCl_2$ drying tube for 4 h, cooled to rt and stirred overnight. The solvent was removed in vacuum, the residue was washed with diethyl ether, the insoluble HCl salt was obtained as pale yellow solid. (6.15 g, 95%).
The analytical data according to the literature.[144]

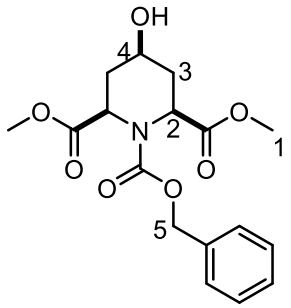
***cis*-2,6-Bis(methoxycarbonyl)-4-hydroxypiperidine (42)**



A suspension of Rh/Al_2O_3 (150 mg) in MeOH (4 mL) was pre-activated under H_2 (15 bar) for 1h in an autoclave. A solution of chelidamic ester hydrochloride **41** (1000 mg, 4.04 mmol) in MeOH (8 mL) was added to the suspension of pre-activated catalyst and stirred for 24 h. The reaction mixture was filtered through a pad of Celite® and the filtrate was evaporated. The residue was dissolved in a minimum amount of water (10 mL), Na_2CO_3 (642 mg, 6.06 mmol) was added at 0 °C, EtOAc (25 mL) was added and stirred for 10 min. Layers were separated and the aqueous layer was extracted with EtOAc (3x25 mL), organic layers were combined, dried over $MgSO_4$, the solvent was removed in vacuum and the residue was purified by column chromatography to obtain **27** (40% EtOAc/cyclohexane) (160 mg, 20%) and **42** (60% EtOAc/cyclohexane) (520 mg, 60%) as colorless solid.
R_f = 0.45 (100% EtOAc). **m.p**.135 °C. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 3.85-3.77 (m, 1H, CH-4), 3.78 (s, 6H, CH_3-1), 3.44 (d, *J* = 11.8 Hz, 2H, CH-2), 2.48-2.29 (m, 2H, CH_2-3), 1.79 (br s, 1H, NH), 1.65 (br s, 1H, OH), 1.49-1.26 (m, 2H, CH_2-3). **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 172.1 (C=O), 68.5 (C4), 56.4 (C2), 52.3 (C1), 37.8 (C3). **IR**: v [cm^{-1}] 3321, 3137, 2961, 2922, 2860, 1735, 1427, 1349, 1328, 1302, 1252, 1198, 1161, 1116, 1065, 1006, 949, 881, 840, 789, 761, 727. **LRMS** (ESI+) *m/z*, (%): 218 (60, $[M+H]^+$), 240 (100, $[M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_9H_{15}NO_5Na$: 240.0842, found: 240.0845.

The analytical data according to the literature.[144]

***cis*-*N*-(Benzyloxycarbonyl)-2,6-bis(methoxycarbonyl)-4-hydroxypiperidine (44)**

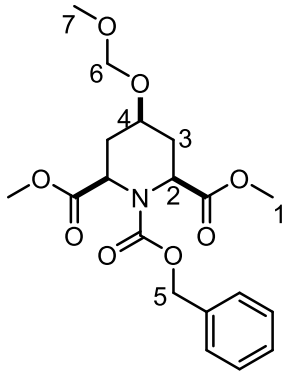


Benzyl chloroformate (2.2 mL, 15.19 mmol) was added to a mixture of piperidine **42** (2.2 g, 10.13 mmol) and $NaHCO_3$ (1.70 g, 20.06 mmol) in CH_2Cl_2 (48 mL)/ H_2O (12 mL) at rt, and stirring was continued overnight. After the completion of the reaction, brine (20 mL) was added and the mixture was extracted with CH_2Cl_2 (4x30 mL). The combined organic layers were dried over $MgSO_4$, the solvent was removed and the residue was purified by column chromatography (25% EtOAc/cyclohexane) to obtain **44** (2.90 g, 82%) as colorless oil.

R_f = 0.5 (50% EtOAc). **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 7.49-7.31 (m, 5H, CH_{Ar}), 5.30-5.17 (m, 2H, CH_2-5), 5.11-4.92 (m, 2H, CH_2-2), 4.19-4.14 (m, 1H, CH-4), 3.77 (s, 6H, CH_3-1), 2.56-2.41 (m, 2H, CH_2-3), 1.91-1.80 (m, 2H, CH_2-3), 1.68 (br s, 1H, OH). **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 173.7 (C=O), 155.8 (C=O), 136.2 (C_{Ar}), 128.5 (CH_{Ar}), 128.2 (CH_{Ar}), 128.0 (CH_{Ar}), 68.1 (C5), 61.5 (C4), 52.6 (C1), 50.4 (C2), 31.8 (C3). **IR**: v [cm^{-1}] 3475, 2952, 1747, 1701, 1438, 1410, 1335, 1290, 1209, 1087, 1061, 769, 700. **LRMS** (ESI+) *m/z*, (%): 352 (10, $[M+H]^+$), 374 (100, $[M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{17}H_{21}NO_7Na$: 374.1210, found: 374.1209.

The analytical data according to the literature.[144]

***cis*-*N*-(Benzyloxycarbonyl)-2,6-bis(methoxycarbonyl)-4-[(methoxy)methoxy]piperidine (45)**

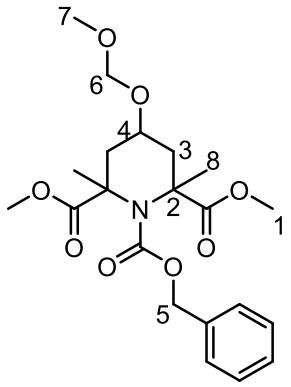


Bromomethyl methyl ether (1.9 mL, 22.77 mmol) was added dropwise to a solution of alcohol **44** (2.0 g, 5.69 mmol) and DIPEA (5.9 mL, 34.15 mmol) in CH_2Cl_2 (40 mL) at 0 °C. The reaction mixture was warmed to rt after 30 min and stirred overnight. After completion, brine (30 mL) was added to the reaction mixture at 0 °C, the layers were separated, and the aqueous was extracted with CH_2Cl_2 (3x40 mL). The combined organic layers were dried over $MgSO_4$, the solvent was removed in vacuum and the residue was purified by column chromatography (20% EtOAc/cyclohexane) to obtain **45** (2.1 g, 93%) as pale yellow oil.

R_f = 0.4 (50% EtOAc).**^{1}H NMR** (400 MHz, $CDCl_3$): δ = 7.43-7.29 (m, 5H, CH_{Ar}), 5.21 (s, 2H, CH_2-5), 4.80 (br s, 2H, CH-2), 4.58 (s, 2H, CH_2-6), 4.02-3.91 (m, 1H, CH-4), 3.70 (s, 6H, CH_3-1), 3.34 (s, 3H, CH_3-7), 2.52-2.34 (m, 2H, CH_2-3), 2.01 (ddd, *J* = 13.3, 7.4, 3.7 Hz, 2H, CH_2-3). **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 171.7 (C=O), 156.0 (C=O), 136.2 (C_{Ar}), 128.5 (CH_{Ar}), 128.1 (CH_{Ar}), 128.0 (CH_{Ar}), 94.1 (C6), 67.9 (C5), 66.0 (C4), 55.3 (C7), 52.1 (C1), 51.6 (C2), 29.8 (C3). **IR**: v [cm^{-1}] 2951, 2895, 1736, 1701, 1437, 1407, 1326, 1292, 1198, 1175, 1149, 1100, 1073, 1031, 989, 916, 769, 735, 698. **LRMS** (ESI+) *m/z*, (%): 396 (5, $[M+H]^+$), 418 (100, $[M+Na]^+$), 813 (10, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{19}H_{25}NO_8Na$: 418.1472, found: 418.1475.

The analytical data according to the literature.[144]

***N*-(Benzyloxycarbonyl)-2,6-bis(methoxycarbonyl)-4-[(methoxy)methoxy]-2,6-dimethyl piperidine (46)**

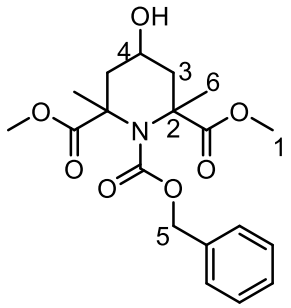


NaHMDS (1 M in THF, 22 mL, 22.00 mmol) was added dropwise to a solution of piperidine **45** (2.9 g, 7.33 mmol) in THF (45 mL) at −60 °C and the mixture was stirred for 60 min. MeI (4.6 mL, 73.34 mmol) was added dropwise at −78 °C. The reaction mixture was stirred at −78 °C for 90 min, warmed to rt and stirred overnight. After completion, sat. NH_4Cl solution was added, the layers were separated, and the aqueous was extracted with EtOAc (2x40 mL). The combined organic layers were dried over $MgSO_4$, the solvent was removed in vacuum and the residue was purified by column chromatography (20% EtOAc/cyclohexane) to obtain **46** (2.9 g, 93%) as pale yellow oil as inseparable 1:1:3 diastereomeric mixture.

R_f = 0.4 (50% EtOAc).**^{1}H NMR** (400 MHz, $CDCl_3$): δ = 7.40-7.30 (m, 15H, CH_{Ar}, CH_{Ar}*, CH_{Ar}**), 5.22-4.97 (m, 6H, CH_2-5, CH_2-5*, CH_2-5**), 4.72 (s, 2H, CH_2-6**), 4.68 (s, 2H, CH_2-6), 4.64 (s, 2H, CH_2-6*), 4.16-4.10 (m, 1H, CH-4**), 4.07-4.02 (m, 1H, CH-4*), 3.96 (tt, *J* = 11.6, 3.8 Hz, 1H, CH-4), 3.77 (s, 9H, CH_3-1*, CH_3-1**), 3.55 (s, 3H, CH_3-1*), 3.36 (s, 15H, CH_3-1, CH_3-7, CH_3-7*, CH_3-7**), 2.22-2.01 (m, 6H, CH_2-3, CH_2-3*, CH_2-3**), 1.98-1.83 (m, 6H, CH_2-3, CH_2-3*, CH_2-3**), 1.72 (s, 3H, CH_3-8**), 1.69 (s, 3H, CH_3-8**), 1.63 (s, 6H, CH_3-8, CH_3-8*), 1.60 (s, 6H, CH_3-8, CH_3-8*). **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 175.1 (C=O*), 174.3 (C=O, C=O**), 154.7 (C=O, C=O*, C=O**), 135.6 (C_{Ar}, C_{Ar}*, C_{Ar}**), 128.50 ($CH_{Ar,}$ CH_{Ar}**), 128.47 (CH_{Ar}*), 128.43 (CH_{Ar}, CH_{Ar}**), 128.39 (CH_{Ar}*), 128.3 (CH_{Ar}), 128.21 (CH_{Ar}*), 128.18 (CH_{Ar}**), 95.2 (C6*), 95.2 (C6), 94.9 (C6**), 67.9 (C5), 67.7 (C5*), 67.6 (C5**), 67.1 (C4**), 66.9 (C4), 66.4 (C4*), 63.2 (C2), 62.4 (C2), 61.7 (C2*, C2**), 61.0 (C2*, C2**), 55.5 (C7, C7*, C7**), 52.6 (C1**), 52.3 (C1, C1*), 41.8 (C3, C3*, C3**), 41.4 (C3, C3*, C3**), 23.6 (C8, C8*, C8**), 22.2 (C8, C8*, C8**). **IR**: ν [cm^{-1}] 2995, 2952, 1737, 1696, 1455, 1435, 1394, 1333, 1290, 1256, 1213, 1191, 1146, 1129, 1087, 1077, 1029, 1001, 775, 752, 735, 699. **LRMS** (ESI+) *m/z*, (%): 424 (25, $[M+H]^+$), 446 (100, $[M+Na]^+$), 869 (40,

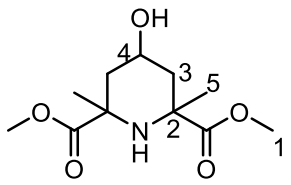
[2M+Na]$^+$). **HRMS** (ESI+) *m/z*: ([M+Na]$^+$): calculated for $C_{21}H_{29}NO_8Na$: 446.1785, found: 446.1780.

_N_-(Benzyloxycarbonyl)-2,6-bis(methoxycarbonyl)-4-hydroxy-2,6-dimethylpiperidine (47)



Bromotrimethylsilane (1 mL, 7.67 mmol) was added dropwise to a solution of diastereomeric mixture of methoxymethyl ether **46** (1300 mg, 3.07 mmol) in CH_2Cl_2 (25 mL) at 0 °C and the solution was stirred for 2 h. After completion, saturated $NaHCO_3$ solution (10 mL) was added dropwise (10 mL) and the mixture was extracted with CH_2Cl_2 (3x20 mL). The combined organic layers were washed with brine (20 mL) and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (20% EtOAc/cyclohexane) to obtain **47** (870 mg, 75%) as pale yellow oil as inseparable 1:1:3 diastereomeric mixture.
R_f = 0.4 (50% EtOAc).**^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.42-7.30 (m, 15H, CH_{Ar}, CH_{Ar}*, CH_{Ar}**), 5.21-5.01 (m, 6H, CH_2-5, CH_2-5*, CH_2-5**), 4.40-4.27 (m, 1H, CH-4*), 4.20-4.05 (m, 2H, CH-4, CH-4**), 3.79-3.28 (m, 18H, CH_3-1, CH_3-1*, CH_3-1**), 2.62-2.54 (m, 2H, CH_2-3*), 2.47 (d, *J* = 12.9 Hz, 2H, CH_2-3*), 2.28 (ddd, *J* = 12.9, 6.1, 2.4 Hz, 2H, CH_2-3**), 2.23-2.16 (m, 2H, CH_2-3**), 2.08-2.00 (m, 2H, CH_2-3), 1.92-1.84 (m, 5H, CH_2-3, OH, OH*, OH**), 1.81 (s, 6H, CH_3-6*), 1.73 (s, 6H, CH_3-6**), 1.61 (s, 6H, CH_3-6). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 174.4 (C=O, C=O*, C=O*), 154.8 (C=O, C=O*, C=O*), 135.5 (C_{Ar}, C_{Ar}*, C_{Ar}**), 128.5 (CH_{Ar}, CH_{Ar}**), 128.44 (CH_{Ar}), 128.42 (CH_{Ar}*), 128.35 (CH_{Ar}*), 128.33 (CH_{Ar}), 128.25 (CH_{Ar}**), 128.22 (CH_{Ar}*), 128.1 (CH_{Ar}**), 68.0 (C5), 67.8 (C5*), 67.7 (C5**), 62.2 (C4*), 61.6 (C4, C4**), 61.5 (C2, C2*, C2**), 61.1 (C2, C2*, C2**), 52.6 (C1*), 52.5 (C1), 52.3 (C1**), 43.8 (C3), 43.0 (C3*), 40.9 (C3**), 26.0 (C6*), 22.4 (C6, C6**). **IR**: ν [cm^{-1}] 3488, 2996, 2952, 1739, 1697, 1455, 1435, 1395, 1330, 1291, 1257, 1214, 1145, 1132, 1087, 1029, 1003, 776, 752, 700. **LRMS** (ESI+) *m/z*, (%): 380 (30, [M+H]$^+$), 402 (100, [M+Na]$^+$), 781 (40, [2M+Na]$^+$). **HRMS** (ESI+) *m/z*: ([M+Na]$^+$): calculated for $C_{19}H_{25}NO_7Na$: 402.1523, found: 402.1525.

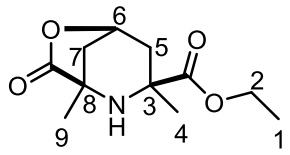
2,6-Bis(methoxycarbonyl)-4-hydroxy-2,6-dimethylpiperidine (48)



Pd/C was added to a solution of benzyloxycarbonyl protected piperidine **47** (750 mg, 1.98 mmol) in methanol (10 mL) and the suspension was stirred under a H_2 atmosphere (15 bar) at rt overnight. After completion the reaction mixture was filtered through a Celite® pad and the filtrate was evaporated in vacuum to obtain the piperidine **48** (400 mg, 82%) as colorless oil as inseparable 1:1:3 diastereomeric mixture, which was directly used in the next step without further purification.

R_f = 0.6 (100% EtOAc).**^{1}H NMR** (400 MHz, $CDCl_3$) δ = 4.30-4.20 (m, 2H, CH-4*, CH-4**), 4.13-4.05 (m, 1H, CH-4), 3.76-3.71 (m, 18H, CH_3-1, CH_3-1*, CH_3-1**), 3.04 (dd, *J* = 14.3, 4.9 Hz, 1H, CH_2-3*), 2.57-2.36 (m, 12H, CH_2-3, CH_2-3*, CH_2-3**, OH, OH*, OH**, NH, NH*, NH**), 2.17-2.07 (m, 1H, CH_2-3**), 1.86 (d, *J* = 11.5 Hz, 1H, CH_2-3**), 1.68-1.60 (m, 1H, CH_2-3*), 1.48 (dd, *J* = 13.8, 2.9 Hz, 2H, CH_2-3), 1.36 (s, 6H, CH_3-5*), 1.33 (s, 12H, CH_3-5, CH_3-5**). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 178.8 (C=O, C=O*, C=O**), 63.9 (C4*), 63.6 (C4, C4**), 58.3 (C2*), 55.8 (C2, C2**), 52.7 (C1*), 52.3 (C1), 51.8 (C1**), 42.5 (C3*), 39.5 (C3), 37.0 (C3**), 30.6 (C5), 30.2 (C5*), 29.8 (C5**). **IR**: v [cm^{-1}] 3449, 2953, 1729, 1707, 1452, 1435, 1294, 1266, 1209, 1138, 1106, 1044, 986, 951. **LRMS** (ESI+) *m/z*, (%): 246 (100, $[M+H]^+$), 268 (70, $[M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{11}H_{19}NO_5Na$: 268.1155, found: 268.1154.

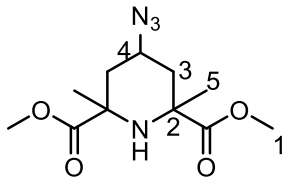
***cis*-Ethyl 1,3-dimethyl-7-oxo-6-oxa-2-azabicyclo[3.2.1]octane-3-carboxylate (48)**



A solution of alcohol **48** (100 mg, 0.41 mmol) in DMF (2 mL) was added dropwise to a suspension of NaH (21 mg, 0.53 mmol) in DMF (2 mL) at 0 °C and the reaction mixture was stirred for 30 minutes before the addition of the solution of 1-azido-2-(2-(2-iodoethoxy)ethoxy)ethane (139 mg, 0.49 mmol) in DMF (1 mL). The reaction mixture was warmed to rt and stirring was continued overnight. A few drops of NH4Cl solution was added to the reaction mixture, filtered through Celite® and solvents were removed under reduced pressure. The residue was purified by column chromatography (20% EtOAc/cyclohexane) to obtain **48** (40 mg, 44%) as colorless solids and recrystallized from EtOAc/pentane.

R_f = 0.6 (100% EtOAc).**^{1}H NMR** (400 MHz, $CDCl_3$): δ = 4.76-4.69 (m, 1H, CH-6), 4.30-4.08 (m, 2H, CH_2-2), 3.04 (ddd, *J* = 14.3, 4.9, 2.0 Hz, 1H, CH_2-7), 2.74 (s, 1H, NH), 2.11 (ddd, *J* = 11.5, 6.1, 2.0 Hz, 1H, CH_2-5), 1.85 (d, *J* = 11.5 Hz, 1H, CH_2-5), 1.65 (d, *J* = 14.3 Hz, 1H, CH_2-7), 1.35 (s, 6H, CH_3-4, CH_3-9), 1.29 (t, *J* = 7.1 Hz, 3H, CH_3-1). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 177.3 (C=O), 175.9 (C=O), 74.3 (C6), 61.9 (C2), 58.0 (C8), 56.1 (C3), 42.5 (C5), 36.9 (C7), 30.2 (C9), 20.1 (C4), 14.0 (C1).

4-Azido-2,6-bis(methoxycarbonyl)-2,6-dimethylpiperidine (50)

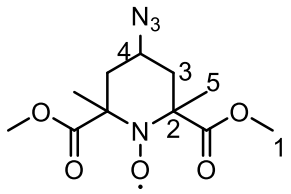


Methanesulfonyl chloride (0.16 mL, 2.10 mmol) was added dropwise to a solution of alcohol **48** (430 mg, 1.75 mmol) and DIPEA (0.67 mL, 3.86 mmol) in CH_2Cl_2 (13 mL) at −10 °C. The reaction mixture was warmed to rt and stirred for 2 h. After completion, the reaction mixture was treated with sat. $NaHCO_3$ (5 mL), and the mixture was extracted with CH_2Cl_2 (2x15 mL). The organic layers were combined, dried over $MgSO_4$, the solvent was removed under reduced pressure to obtain mesylate (600 mg) and the crude mixture was used in the next step without further purification.

Sodium azide (120 mg, 1.85 mmol) was added to a solution of the crude mixture of mesylated alcohol (600 mg, 1.85 mmol) in DMF (12 mL) and the mixture was heated to 80 °C overnight. After completion, the reaction mixture was diluted with EtOAc (10 mL), washed with brine (5 mL), the solvent was evaporated in vacuum and the residue was purified by column chromatography (20% EtOAc/cyclohexane) to obtain **50** (210 mg, 45%) as colorless oil as single diastereomer.

R_f = 0.4 (50% EtOAc).**^{1}H NMR** (400 MHz, $CDCl_3$): δ = 4.06 (tt, *J* = 12.1, 4.2 Hz, 1H, CH-4), 3.68 (s, 6H, CH_3-1), 2.54 (s, 1H, NH), 2.46-2.37 (m, 2H, CH_2-3), 1.34 (s, 6H, CH_3-5), 1.31-1.18 (m, 2H, CH_2-3). **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 175.7 (C=O), 58.0 (C2), 53.8 (C4), 52.1 (C1), 38.8 (C3), 29.6 (C5). **IR**: v [cm^{-1}] 3344, 2957, 2091, 1722, 1444, 1425, 1266, 1207, 1164, 1139, 1127, 1089, 1072, 1034, 977, 924, 782, 760, 727, 694. **LRMS** (ESI+) *m/z*, (%): 271 (10, $[M+H]^+$), 293 (100, $[M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{11}H_{18}N_4O_4Na$: 293.1220, found: 293.1218.

4-Azido-2,6-bis(methoxycarbonyl)-2,6-dimethyl-piperidine-N-oxyl (51)



*m*CPBA (150 mg, 0.61 mmol, 70%) was added portionwise to a solution of piperidine **50** (150 mg, 0.55 mmol) in CH_2Cl_2 (5 mL) at 0 °C and the reaction mixture was stirred at rt for 5 h. The orange colored reaction mixture was diluted with CH_2Cl_2 (10 mL), washed with 5% $NaHCO_3$ (2x5 mL), the combined organic layers were dried over $MgSO_4$, the solvent was evaporated under reduced pressure and the residue was purified by column chromatography (5% EtOAc/cyclohexane) to obtain **51** (82 mg, 52%) as orange solid.

R_f = 0.4 (20% EtOAc). **m.p.** 71-73 °C. **LRMS** (ESI+) *m/z*, (%): 308 (100, $[M+Na]^+$), 593 (20, $[2M+Na]^+$). **HRMS** (ESI+) *m/z*: ($[M+Na]^+$): calculated for $C_{11}H_{17}N_4O_5Na$: 308.1091, found: 308.1089.

6.4 X-Ray Crystallographic Data

Crystallographic data were collected on either Bruker D8 VENTURE Kappa Duo PHOTONIII by IμS micro-focus sealed tube with MoKα (0.71073) or Nonius-KappaCCD equipped with ApexII detector also with MoKα (0.71073) radiation at low temperature preserved by Cryostream Cooler. The structures were solved by direct methods (XT)[149] and refined by full matrix least squares based on F^2 (SHELXL2019).[150] The hydrogen atoms on carbon were fixed into idealized positions (riding model) and assigned temperature factors either $H_{iso}(H) = 1.2\ U_{eq}$(pivot atom) or $H_{iso}(H) = 1.5\ U_{eq}$(pivot atom) for methyl moiety. One compound (**49**) crystallized in non-centrosymmetric space group $P2_1$, however determination of absolute configuration[151] is not reliable due to lack of atoms with significant anomalous dispersion.
The X-ray crystallographic data were deposited at the Cambridge Crystallographic Data Centre (CCDC) and can be obtained free of charge from the Centre via its website (www.ccdc.cam.ac.uk/getstructures). The crystallographic data are summarized in following tables (Table 5-9).

Table 5: Crystal data, data collection, and refinement parameters for 5h-*minor*

Compound	**5h**-*minor*
CCDC	2047587
Formula	$C_{25}H_{47}N_3O_6$
M.W.	485.65
Crystal system	Monoclinic
Space group	*C*2
a [Å]	24.0449 (13)
b [Å]	9.7434 (5)
c [Å]	12.3578 (7)
α [°]	90
β [°]	96.880 (2)
γ [°]	90
Z	4
V[Å^3]	2874.3 (3)
D_x [g cm^{-3}]	1.122
Crystal size [mm]	0.40 × 0.19 × 0.14
Crystal color, shape	Colorless, bar
μ [mm^{-1}]	0.64
T_{min},T_{max}	0.82, 0.91
Measured reflections	24056
Independent diffractions $(R_{int})^a$	5407 (0.023)
Observed diffract. [I>2σ(I)]	5359
No. of parameters	319
R^b	0.030
$wR(F^2)$	0.079
GOF^c	1.04
Residual electron density [e/Å^3]	0.17, -0.17
Flack parameter	0.01 (10)

$^aR_{int} = \Sigma \left| F_o^2 - F_{o,mean}^2 \right| / \Sigma F_o^2$; $^bR(F) = \Sigma \left| \left| F_o \right| - \left| F_c \right| \right| / \Sigma \left| F_o \right|$; $wR(F^2) = [\Sigma(w(F_o^2 - F_c^2)^2)/(\Sigma w(F_o^2)^2)]^{½}$;
$^cGOF = [\Sigma(w(F_o^2 - F_c^2)^2)/(N_{diffrs} - N_{params})]^{½}$

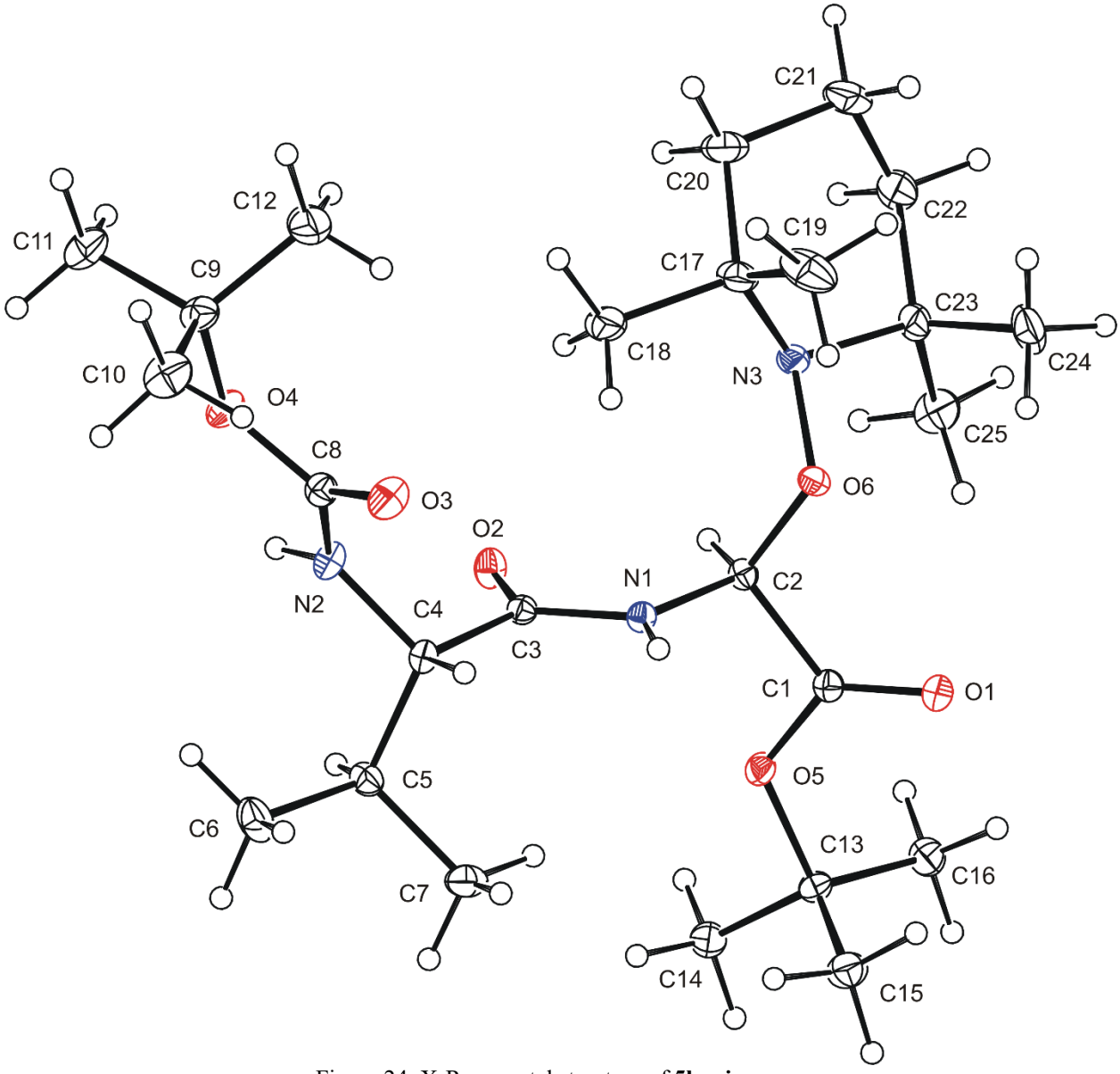


Figure 24: X-Ray crystal structure of **5h minor**

Table 6: Crystal data, data collection, and refinement parameters for 32

Compound	**32**
CCDC	2353509
Formula	$C_{24}H_{34}N_2O_8$
M.w.	478.53
Crystal system Space group	Triclinic *P* -1 (No.2)
a [Å]	10.7585 (4)
b [Å]	10.8373 (3)
c [Å]	11.0398 (3)
α [°]	74.326 (1)°
β [°]	86.395 (1)°
γ [°]	79.690 (1)°
Z	2
V[Å^3]	1219.15 (7)
Temperature	120
D_x [g cm^{-3}]	1.304
Wavelength, Å	0.71073
Crystal size [mm]	0.39 × 0.29 × 0.18
Crystal color, shape	Plate, colorless
μ [mm^{-1}]	0.10
T_{min},T_{max}	0.94, 0.98
Measured reflections	58918
Independent diffractions *(R_{int}[a])*	6027, (0.024)
Observed diffract. [I>2σ(I)]	5800
No. of parameters	314
R[b]	0.034
wR(F^2) for all data	0.088
GOF[c]	1.07
Residual electron density [e/Å^3]	0.42, −0.19
Absolute structure parameter	0.5

[a] $R_{int} = \Sigma \left| F_o^2 - F_{o,mean}^2 \right| / \Sigma F_o^2$; [b] $R(F) = \Sigma \left| \left| F_o \right| - \left| F_c \right| \right| / \Sigma \left| F_o \right|$; $wR(F^2) = [\Sigma(w(F_o^2 - F_c^2)^2)/(\Sigma w(F_o^2)^2)]^{½}$;
[c] $GOF = [\Sigma(w(F_o^2 - F_c^2)^2)/(N_{diffrs} - N_{params})]^{½}$

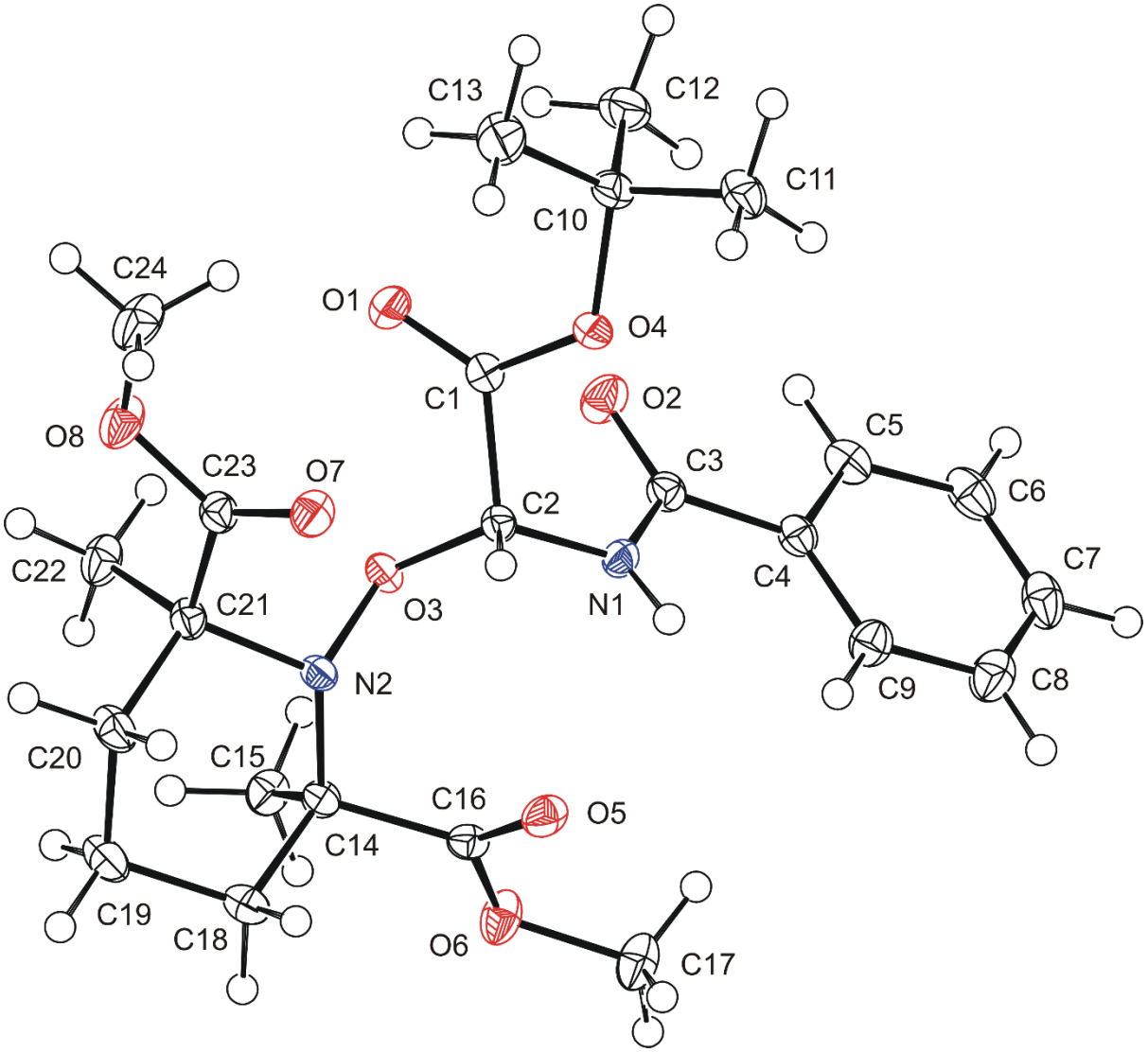


Figure 25: X-Ray crystal structure of **32**

Table 7: Crystal data, data collection, and refinement parameters for NO3

Compound	**NO3**
CCDC	2353511
Formula	$C_{15}H_{26}NO_5$
M.w.	300.37
Crystal system Space group	Orthorhombic *Pnma* (No. 62)
a [Å]	8.4879 (9)
b [Å]	19.706 (2)
c [Å]	9.6947 (11)
α [°]	
β [°]	
γ [°]	
Z	4
V[Å³]	1621.6 (3)
Temperature	120
D_x [g cm^{-3}]	1.230
Wavelength, Å	0.71073
Crystal size [mm]	0.70 × 0.67 × 0.15
Crystal color, shape	prism, red-orange
μ [mm^{-1}]	0.09
T_{min},T_{max}	0.84, 0.99
Measured reflections	7639
Independent diffractions *(R_{int}[a])*	1914, (0.029)
Observed diffract. [I>2σ(I)]	1516
No. of parameters	102
R[b]	0.044
wR(F^2) for all data	0.113
GOF[c]	1.06
Residual electron density [e/Å^3]	0.28, −0.20
Absolute structure parameter	0.5

[a] $R_{int} = \Sigma \left| F_o^2 - F_{o,mean}^2 \right| / \Sigma F_o^2$; [b] $R(F) = \Sigma \left| \left| F_o \right| - \left| F_c \right| \right| / \Sigma \left| F_o \right|$; $wR(F^2) = [\Sigma(w(F_o^2 - F_c^2)^2)/(\Sigma w(F_o^2)^2)]^{1/2}$;
[c] $GOF = [\Sigma(w(F_o^2 - F_c^2)^2)/(N_{diffrs} - N_{params})]^{1/2}$

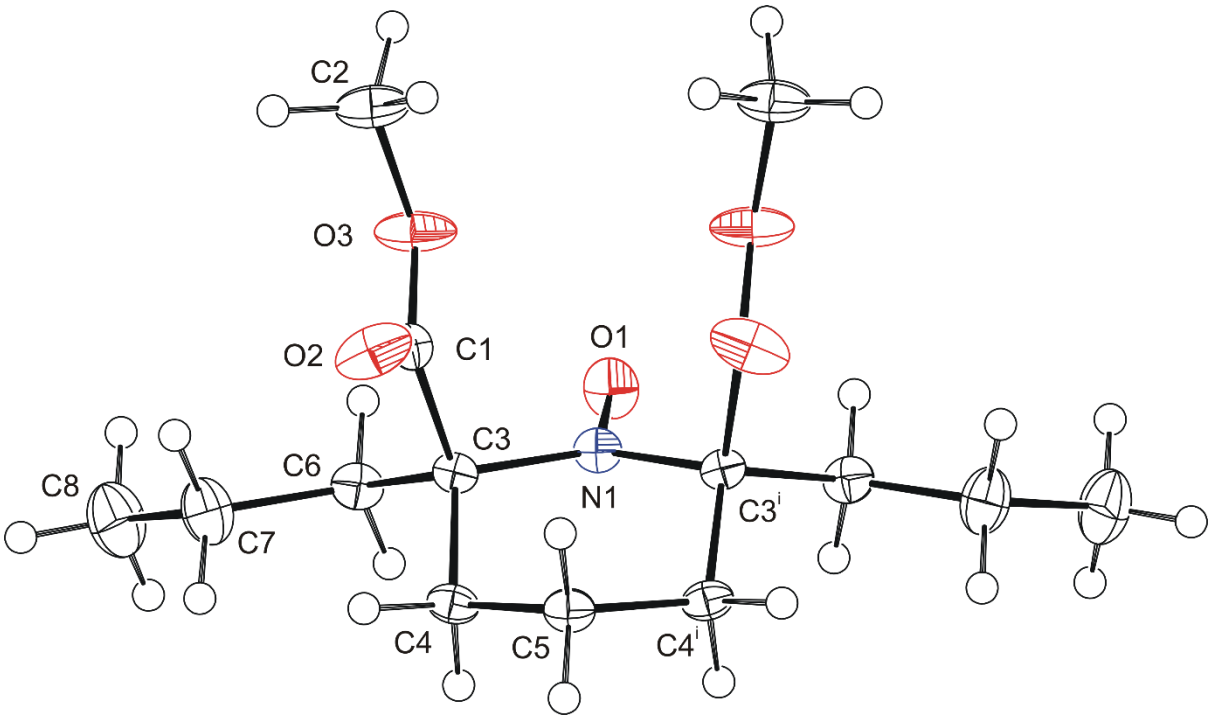


Figure 26: X-Ray crystal structure of **NO3**

Table 8: Crystal data, data collection, and refinement parameters for 37

Compound	**37**
CCDC	2353510
Formula	$C_{28}H_{44}N_2O_9$
M.w.	552.65
Crystal system	Triclinic
Space group	*P*-1 (No. 2)
a [Å]	10.3156 (2)
b [Å]	12.4051 (3)
c [Å]	12.8902 (3)
α [°]	72.696 (1)
β [°]	71.487 (1)
γ [°]	89.100 (1)
Z	2
V[Å^3]	1487.70 (6)
Temperature	120
D_x [g cm^{-3}]	1.234
Wavelength, Å	0.71073
Crystal size [mm]	0.49 × 0.37 × 0.25
Crystal color, shape	Prism, colourless
μ [mm^{-1}]	0.09
T_{min},T_{max}	0.93, 0.98
Measured reflections	28249
Independent diffractions *(R_{int}[a])*	7334, (0.018)
Observed diffract. [I>2σ(I)]	6861
No. of parameters	359
R[b]	0.035
wR(F^2) for all data	0.093
GOF[c]	1.03
Residual electron density [e/Å^3]	0.37, −0.21
Absolute structure parameter	0.5

[a] $R_{int} = \Sigma \left| F_o^2 - F_{o,mean}^2 \right| / \Sigma F_o^2$; [b] $R(F) = \Sigma \left| \left| F_o \right| - \left| F_c \right| \right| / \Sigma \left| F_o \right|$; $wR(F^2) = [\Sigma(w(F_o^2 - F_c^2)^2)/(\Sigma w(F_o^2)^2)]^{½}$;
[c] $GOF = [\Sigma(w(F_o^2 - F_c^2)^2)/(N_{diffrs} - N_{params})]^{½}$

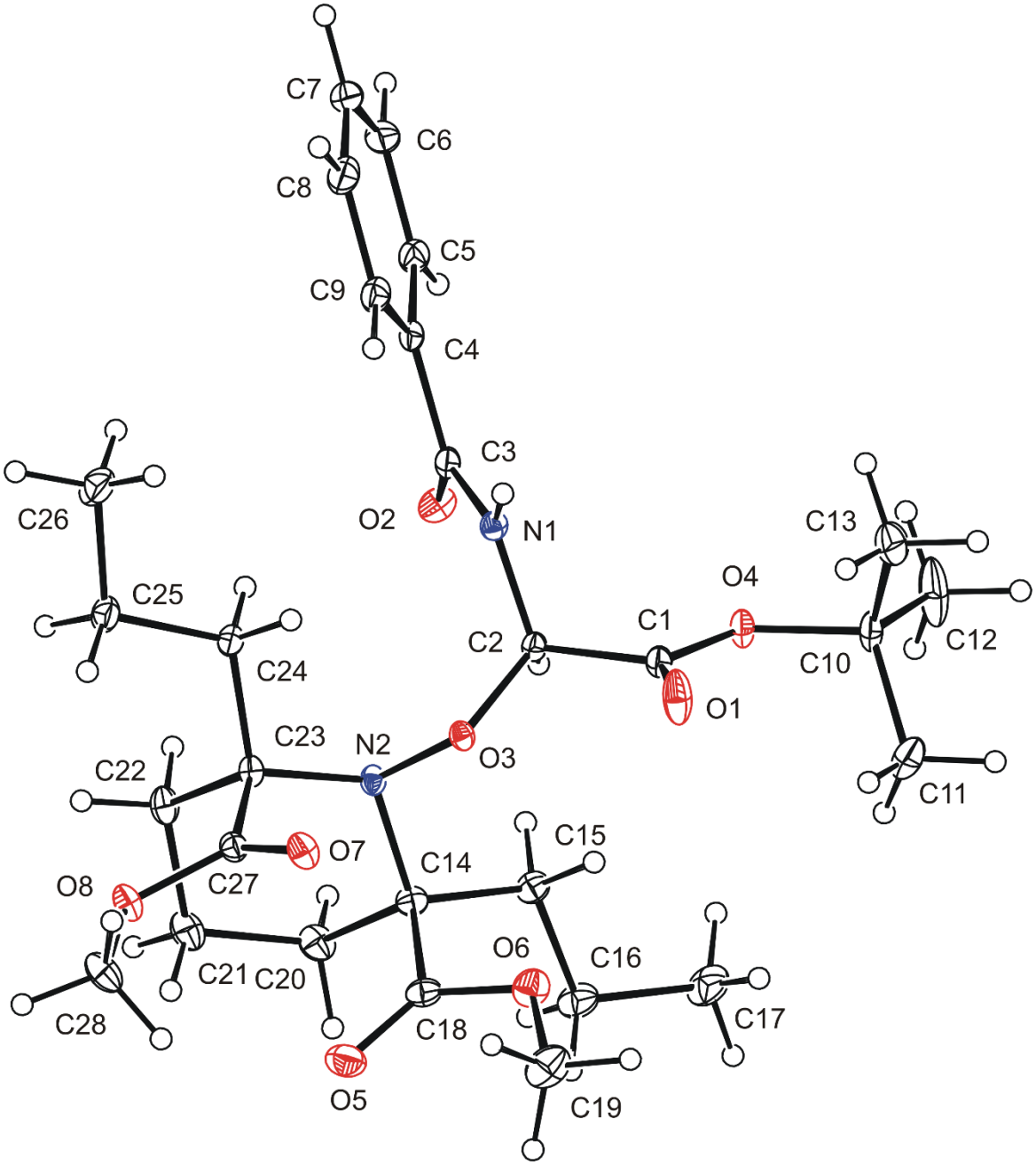


Figure 27: X-Ray crystal structure of **37**

Table 9: Crystal data, data collection, and refinement parameters for 49

Compound	**49**
CCDC	2353512
Formula	$C_{11}H_{17}NO_4$
M.w.	227.25
Crystal system Space group	Monoclinic $P\,2_1$ (No.4)
a [Å]	6.9930 (3)
b [Å]	16.1963 (7)
c [Å]	10.4238 (4)
α [°]	
β [°]	102.845 (1)°
γ [°]	
Z	4
$V[Å^3]$	1151.06 (8)
Temperature	120
D_x [g cm^{-3}]	1.311
Wavelength, Å	0.71073
Crystal size [mm]	0.25 × 0.20 × 0.13
Crystal color, shape	Plate, colourless
μ [mm^{-1}]	0.10
T_{min},T_{max}	0.91, 0.99
Measured reflections	21344
Independent diffractions $(R_{int}{}^a)$	5179, (0.021)
Observed diffract. [I>2σ(I)]	5008
No. of parameters	295
R^b	0.032
$wR(F^2)$ for all data	0.085
GOF^c	1.03
Residual electron density [e/Å^3]	0.41, −0.19
Absolute structure parameter	0.2 (2)

$^a R_{int} = \Sigma \left| F_o^2 - F_{o,mean}^2 \right| / \Sigma F_o^2$; $^b R(F) = \Sigma \left| \left| F_o \right| - \left| F_c \right| \right| / \Sigma \left| F_o \right|$; $wR(F^2) = [\Sigma(w(F_o^2 - F_c^2)^2)/(\Sigma w(F_o^2)^2)]^{½}$;
^{c}GOF = $[\Sigma(w(F_o^2 - F_c^2)^2)/(N_{diffrs} - N_{params})]^{½}$

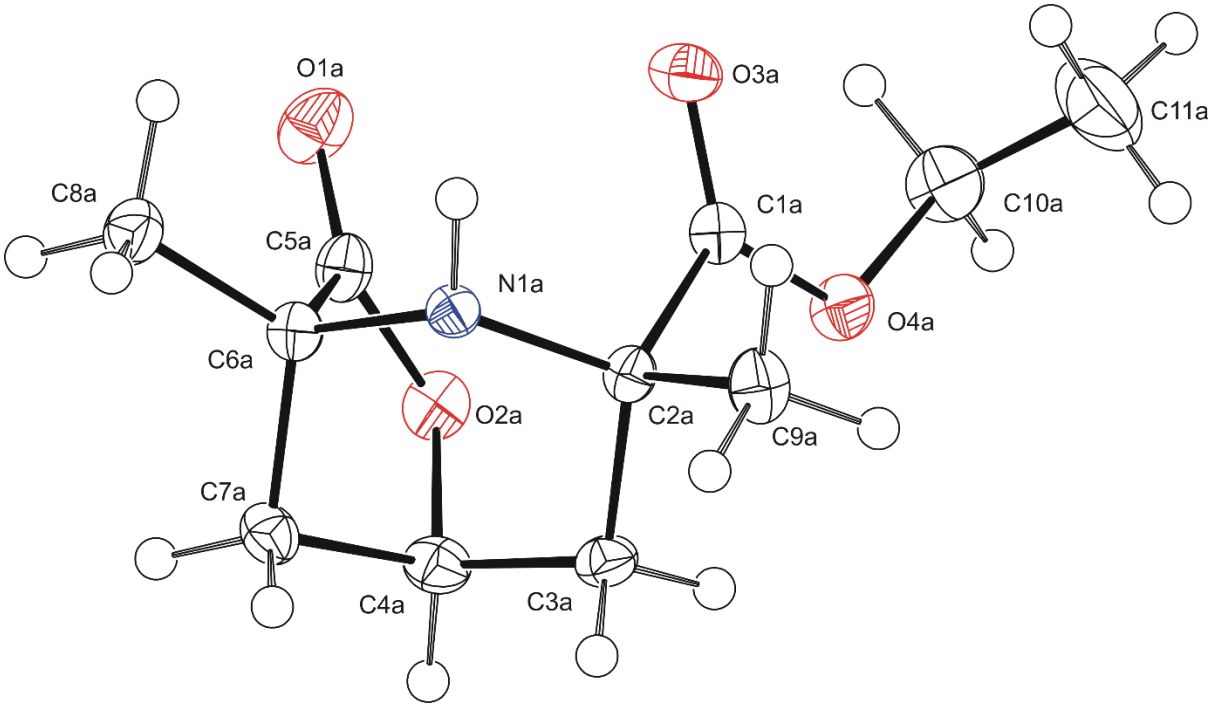
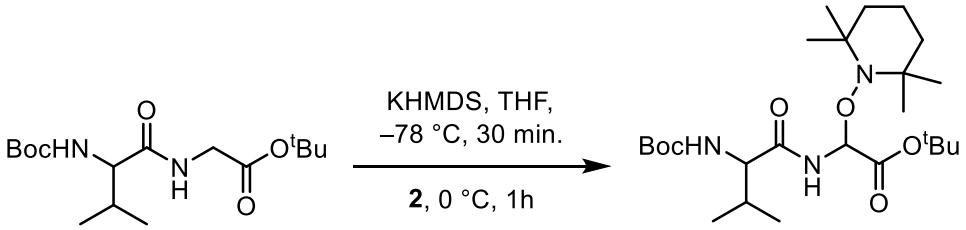


Figure 28: X-Ray crystal structure of **49**

6.5 Chiral HPLC Data

Chiral HPLC analyses were performed to confirm that epimerization is not taking place during oxygenation of dipeptides. Boc-D,L-Val-Gly-O^{t}Bu (*rac*-**4h**) was synthesized according to general procedure D using *N*-Boc-D,L-valine and *tert*-butyl glycinate•HCl. Oxygenation of *rac*-**4h** gave rise to TEMPO adduct *rac*-**5h** as 2:1 diastereomeric mixture by ^{1}H NMR. HPLC analysis was performed on an Agilent 1260 Infinity analytical HPLC system with CHIRALART Amylose-SA column (column No. 0425068363) using hexane/isopropanol in a 90:10 ratio with a flow rate of 0.5 mL/min. 10 µL of the sample solution in hexane (1 mg/1 mL) was injected and the peaks were detected on a Diode Array detector (wavelength 220 nm, labelled as DAD1 C) and external light scattering detector (Polymer Laboratories PL-ELS 2100, labelled as UIB1 A).

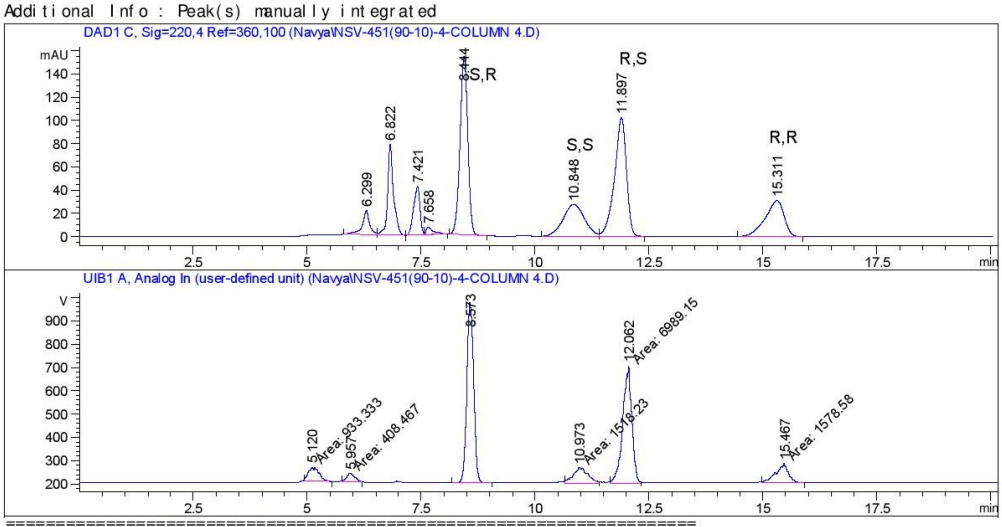


rac-**4h** *rac*-**5h** 81%, d.r. = 2:1

HPLC chromatograms of *rac*-**5h** show 4 peaks corresponding to two enantiomeric pairs of diastereomers in a 2:1 ratio. The DAD detected peaks at 6.3, 6.8, 7.4 and 7.6 min do not belong to the product *rac*-**5h** (Figure 29). The sample of **5e** displays only 2 peaks corresponding to two diastereomers. The DAD detected peaks at 5.9, 6.3, 6.8, 7.3 and 9.0 min do not belong to the product **5h** (Figure 30).

Sample Info : Hexane:iPrOH, 90:10, 10 microL inj., conc. 1 mg/mL, flow 0.5 mL/min, Amylose SA

Additional Info : Peak(s) manually integrated



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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 25.0000
Sample Amount: : 1.00000 [ng/ul] (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=220,4 Ref=360,100

Peak #	Ret Time [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.299	BV	0.1490	238.64360	20.94011	3.4735
2	6.822	VB	0.1271	700.82910	78.15158	10.2006
3	7.421	BV R	0.1366	405.17657	41.60771	5.8974
4	7.658	VB E	0.1454	71.69949	6.17852	1.0436
5	8.444	BB	0.1820	1822.32947	153.50259	26.5242
6	10.848	BV	0.3826	891.19781	27.32055	12.9715
7	11.897	VB	0.2633	1856.00208	101.99798	27.0143
8	15.311	BB	0.3397	884.56787	31.01978	12.8750
Totals :				6870.44600	460.71883	

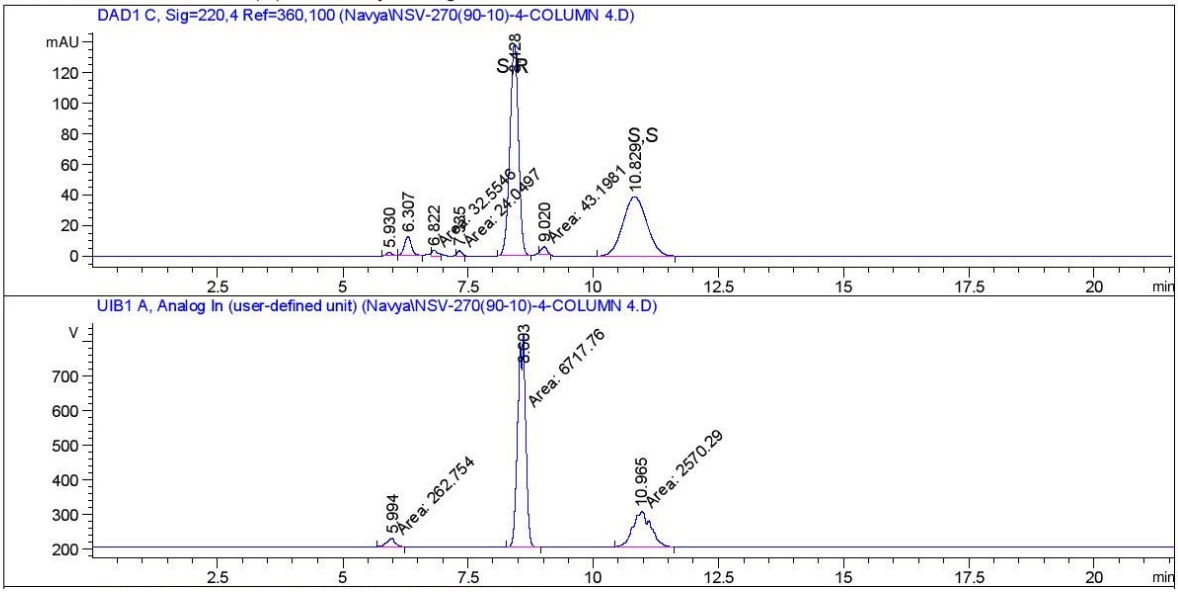
Signal 2: UIB1 A, Analog In (user-defined unit)

Peak #	Ret Time [min]	Type	Width [min]	Area [V*s]	Height [V]	Area %
1	5.120	MM	0.2629	933.33325	59.16908	4.7840
2	5.957	MM	0.2006	408.46667	33.93346	2.0937
3	8.573	VV R	0.1314	8081.81201	774.05139	41.4249
4	10.973	MM	0.3776	1518.22864	67.01394	7.7820
5	12.062	MM	0.2308	6989.15039	504.65808	35.8242
6	15.467	MM	0.3190	1578.57813	82.47910	8.0913
Totals :				1.95096e4	1521.30504	

Figure 29: Chiral HPLC data of *rac*-**5h**.

Sample Info : Hexane:iPrOH, 90:10, 10 microL inj., conc. 1 mg/mL, flow 0.5 mL/min, Amylose SA

Additional Info : Peak(s) manually integrated



Signal 1: DAD1 C, Sig=220,4 Ref=360,100

Peak #	Ret Time [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.930	BV	0.0817	17.51731	2.54889	0.5516
2	6.307	VB	0.1265	121.34050	12.58765	3.8207
3	6.822	MM	0.1357	32.55458	3.99942	1.0251
4	7.335	MM	0.1115	24.04967	3.59642	0.7573
5	8.428	BB	0.1833	1655.10059	138.59001	52.1144
6	9.020	MM	0.1418	43.19812	5.07899	1.3602
7	10.829	BB	0.3891	1282.14026	38.69282	40.3709
Totals :				3175.90101	205.09420	

Signal 2: UIB1 A, Analog In (user-defined unit)

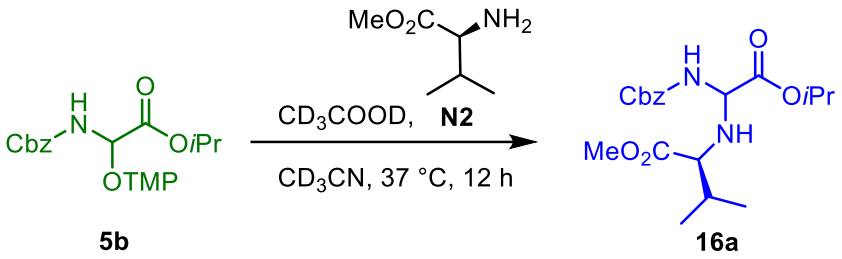
Peak #	Ret Time [min]	Type	Width [min]	Area [V*s]	Height [V]	Area %
1	5.994	MM	0.1841	262.75415	23.78591	2.7511
2	8.603	MM	0.1818	6717.76074	615.74359	70.3371
3	10.965	MM	0.4137	2570.29443	103.55491	26.9118
Totals :				9550.80933	743.08441	

Figure 30**:** Chiral HPLC data of **5h**.

6.6 Reaction Kinetics by ^{1}H NMR Spectroscopy

The reaction kinetics of the substitution reaction were studied by ^{1}H NMR spectroscopy. The glycine alkoxyamine **5b** in CD_3CN was reacted with nucleophiles L-valine methyl ester **N2** and tripeptide Boc-Ala-Cys-Ala-OMe **N3** catalyzed by acetic acid-d4 in NMR tube at 37 °C and the reaction was monitored over 12 h.

a) Reaction kinetics of formation of aminal **16a**



L-Valine methyl ester (**N2**) (3.2 mg, 0.025 mmol) was added to a solution of glycine alkoxyamine **5b** (10 mg, 0.025 mmol) in CD_3CN (0.5 mL) in an NMR tube. Prior to the commencement of the reaction monitoring at 37 °C, CD_3COOD (2 µL, 0.035 mmol) was added to the reaction mixture and ^{1}H NMR spectra were measured at regular intervals.

Concentration (c) = 0.05 M;

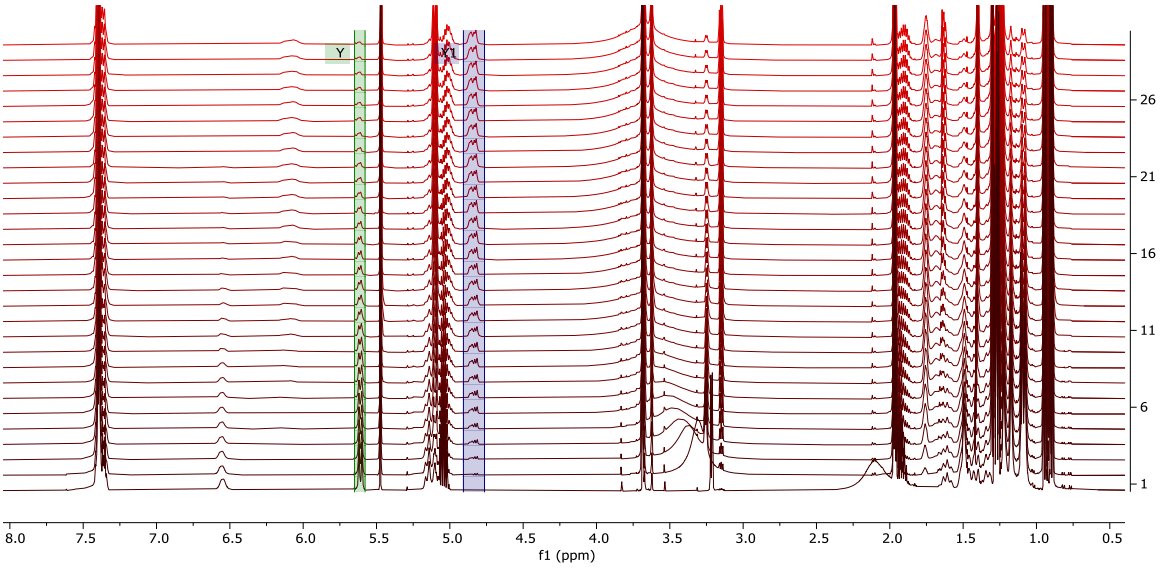
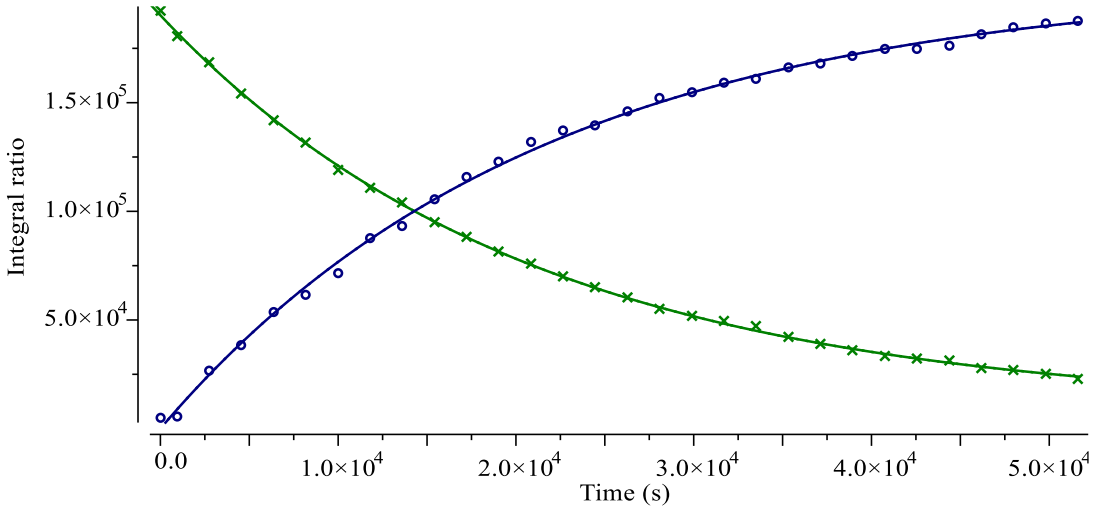


Figure 31: Stacked ^{1}H NMR spectra for analysis of reaction kinetics of formation of aminal product **16a**



Y' = B+F*exp(-Time *G)

B=8.8e+3; F=1.8e+5; G=0.000048;

Error: 4.1e-7

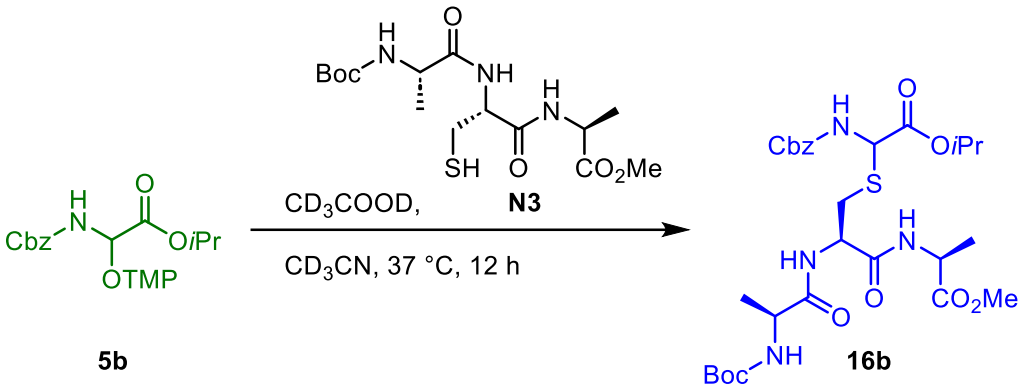
Y1' = B+F*exp(-Time *G)

B=2.1e+5; F=-2.1e+5; G=0.000047;

Error: 9.0e-7

Figure 32: Reaction kinetic plot of formation of aminal product **16a**

b) Reaction kinetics of formation of thiohemiaminal **16b**



Boc-Ala-Cys-Ala-OMe (**N3**) (12.9 mg, 0.025 mmol) was added to a solution of glycine alkoxyamine **5b** (10 mg, 0.025 mmol) in CD_3CN (0.5 mL) in an NMR tube. Prior to the commencement of the reaction monitoring at 37 °C, CD_3COOD (2 µL, 0.033 mmol) was added to the reaction mixture and ^1H NMR spectra were measured at regular intervals.

Concentration (c) = 0.05 M;

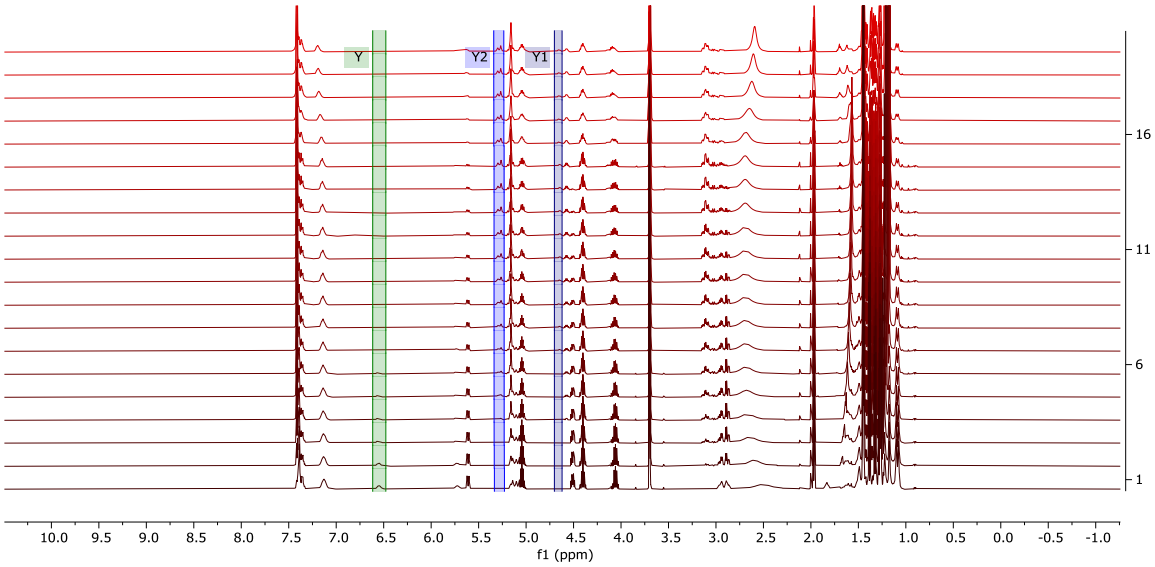
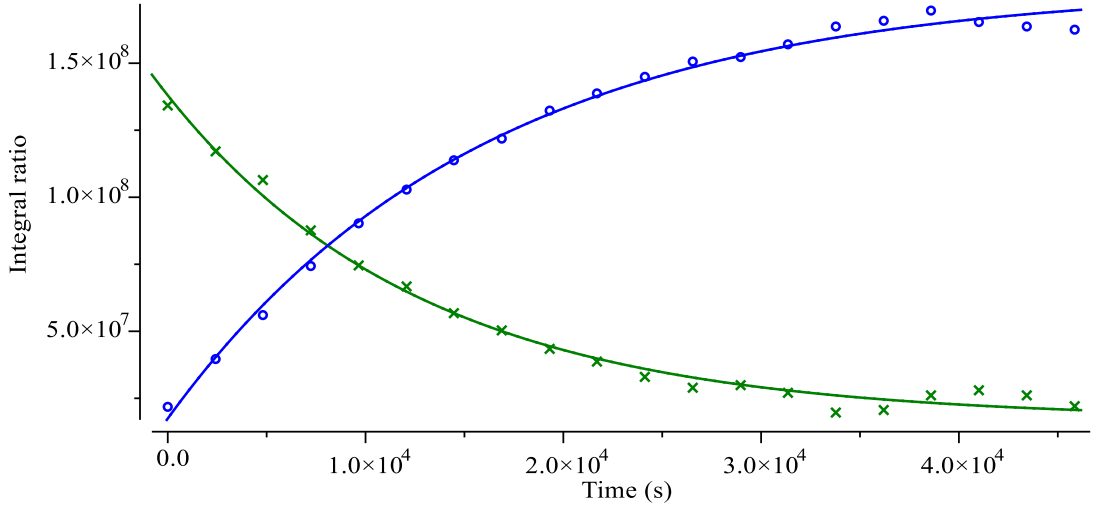


Figure 33: Stacked ^{1}H NMR spectra for analysis of reaction kinetics of formation of thiohemiaminal product **16b**



Y' = B+F*exp(-Time *G)

B=1.7e+7; F=1.2e+8; G=0.000077;

Error: 0.0000031

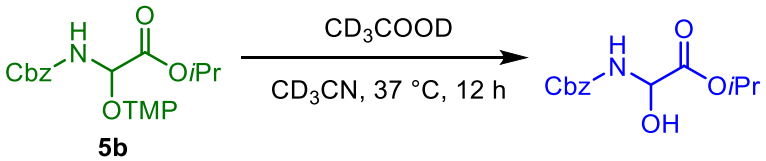
Y2' = B+F*exp(-Time *G)

B=1.8e+8; F=-1.6e+8; G=0.000063;

Error: 0.0

Figure 34: Reaction kinetic plot of formation of thiohemiaminal product **16b**

c) Reaction kinetics of alkoxyamine **5b** without nucleophile



5b

CD_3COOD (2 µL, 0.033 mmol) was added to the solution of glycine alkoxyamine **5b** (10 mg, 0.025 mmol) in CD_3CN (0.5 mL) in an NMR tube at 37 °C. ^{1}H NMR spectra were measured at regular intervals.

Concentration (c) = 0.05 M;

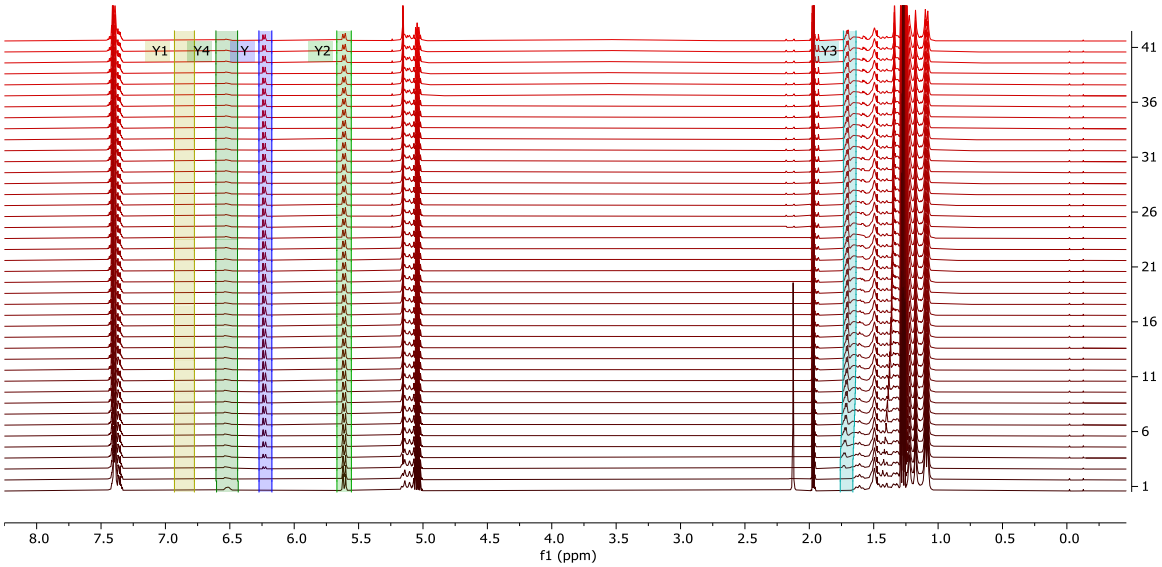
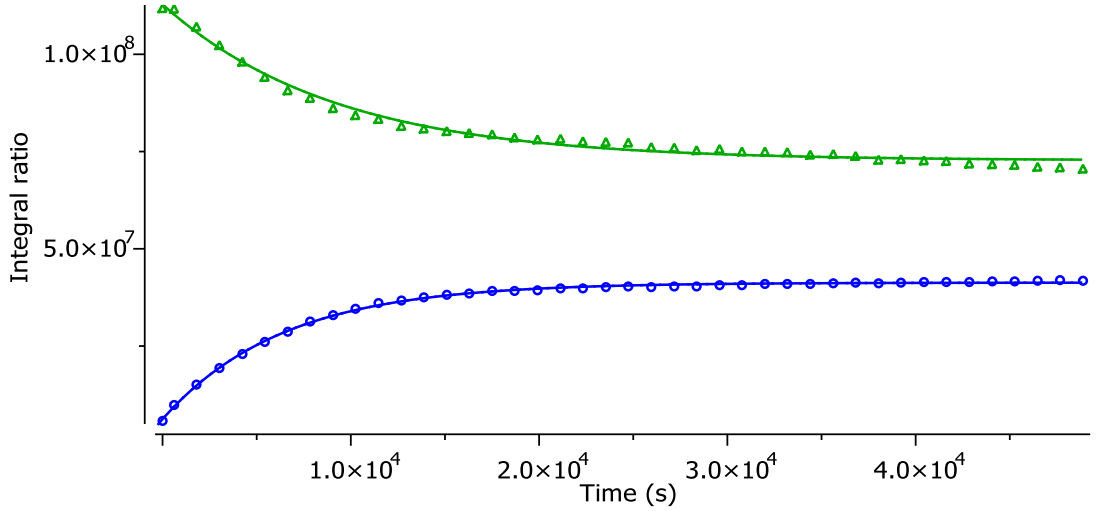


Figure 35: Stacked ^{1}H NMR spectra for analysis of reaction kinetics of alkoxyamine **5b** without nucleophile



Y' = B+F*exp(-Time *G)

B=4.1e+7; F=-3.5e+7; G=0.00016;

Error: 0.0

Y2' = B+F*exp(-Time *G)

B=7.3e+7; F=4.0e+7; G=0.00011;

Error: 0.0

Figure 36: Reaction kinetic plot of alkoxyamine **5b** without nucleophile

6.7 DMSO titration experiment by ^{1}H NMR Spectroscopy

1) DMSO titration of **5k** solution

The diastereomeric mixture of glycine alkoxyamine **17bD1** (2.5 mg, 0.005 mmol) was dissolved to a 10 mM solution in $CDCl_3$ (500 µL, 0.03% TMS). DMSO-d6 (5-50 µL) was injected and the NMR tube was shaken to ensure homogeneity. ^{1}H NMR spectra were recorded for each sample.

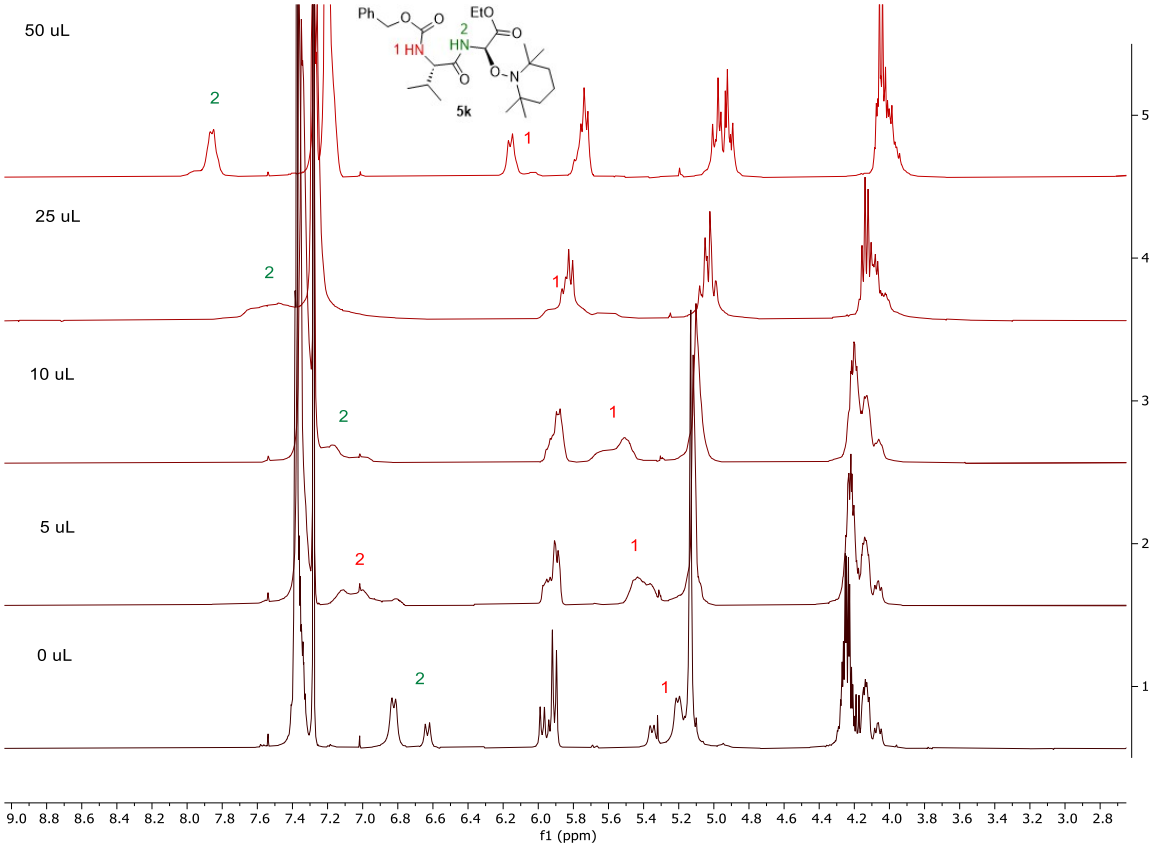


Figure 37: Stacked ^{1}H NMR spectra for analysis of DMSO titration (5-50 µL) of $CDCl_3$ solution of the 2:1 diastereomeric mixture of **5k**.

2) DMSO titration of **17bD1** solution

The major diastereomer of dipeptide glycine aminal **17bD1** (2.2 mg, 0.005 mmol) was dissolved to a 10 mM solution in $CDCl_3$ (500 µL, 0.03% TMS). DMSO-d6 (5-50 µL) was injected and the NMR tube was shaken to ensure homogeneity. ^{1}H NMR spectra were recorded for each sample.

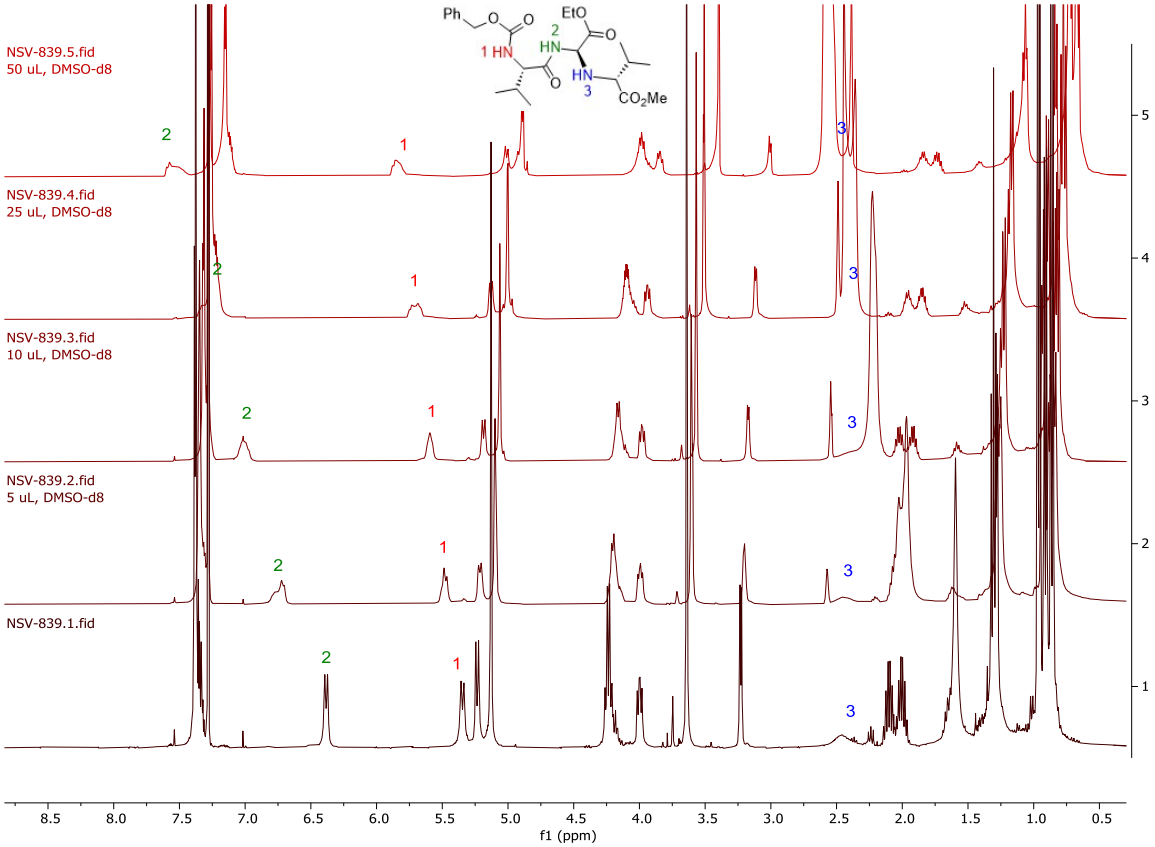


Figure 38: Stacked ^{1}H NMR spectra for analysis of DMSO titration (5-50 µL) of $CDCl_3$ solution of **17bD1**

6.8 Homolysing Temperature Screening of The Glycine Alkoxyamines

Glycine alkoxyamine **32** and **37** were analyzed by EPR spectroscopy on elevated temperature to monitor homolysis.

Experimental procedure:

A solution of glycine alkoxyamine **32** or **37** (1 mg, 0.002 mg) in toluene (2 mL) (c= 1 mM) was measured on an EPR spectrometer at various temperatures, starting from 305 K to 373 K. The EPR spectra were observed to match to the corresponding nitroxides. The samples that underwent heating to 373 K were subsequently analyzed after a period of 24 hours, and the EPR signals exhibited similar intensity.

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