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TECHNISCHE
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DISSERTATION

Synthese neuer Peptidomimetica unter Verwendung von *N*-Carboxycarbonylaminosäurehydrazid (= NXO) Bausteinen.

Ausgeführt am Institut für Angewandte Synthesechemie der Technischen Universität Wien zum Zwecke der Erlangung des akademischen Grades eines Doktors der technischen Wissenschaften (Ph.D.)

Ao. Univ. Prof. Dipl.-Ing. Dr. techn. Ulrich Jordis
Institutsnummer: E163
Institut für Angewandte Synthesechemie

von

Farhan Ahmed Khan
0728391
Josef Baumann Gasse 8a
Wien, A-1220.

Wien im Februar 2012

TECHNISCHE
UNIVERSITÄT
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Vienna University of Technology

Ph.D.Thesis

Synthesis of Novel Peptidomimetics Based on *N*-Carboxycarbonyl Amino Acids Hydrazide (=NXO) Building Blocks.

Conducted at the Institure of Applied Synthetic Chemistry, Technical University Vienna, for the purpose of receiving the academic title “Doktors der technischen Wissenschaften”(Ph.D.).

Ao. Univ. Prof. Dipl.-Ing. Dr. techn. Ulrich Jordis

Institute number: E 163
Institute of Applied Synthetic Chemistry

By

Farhan Ahmed Khan
0728391
Josef Baumann Gasse 8a
Vienna, A-1220.

Vienna February 2012

Acknowledgments

"Seek Knowledge from the cradle to the grave" [Prophet Muhammad (PBUH)]; According to this saying after getting my M.Sc. degree I put my efforts on a path which can bring me to higher studies. It was never so easy to get an admission here in Austria and also to complete the task assigned. With the prayers of my parents (especially Mukhtar Ahsan (grand-mother)) I started my journey.

There is a quote of a noble man:

God grant that not only the love of liberty but a thorough knowledge of the rights of man may pervade all the nations of the earth, so that a philosopher may set his foot anywhere on its surface and say: "**This is my country**". [Benjamin Franklin]

It will be unfair if I don't acknowledge Austria and Austrians. Austria is a very nice country with very nice people; one of them is my Ph.D. supervisor, Ao. Univ. Prof. Dipl.-Ing. Dr. techn. Ulrich Jordis. I want to express my heartiest gratitude to him for his kind, loving, supporting attitude throughout my stay. He supported me many times in making decisions which were quiet difficult for me to decide. He listened to my problems like a sincere friend when I don't have any friend around.
I am thankful to Prof. Dr. Norbert Haider for accepting request to be my second examiner.

Aristotle said: "Man is a social animal". I always enjoyed the company of my colleagues. I would like to thank Dr. Jaywant Phopase who helped me alot in the beginning of my project. I would also like to thank Kausar Hussain Shah, Dr.Laszlo Czollner, Sagar Beldar, Harshad Bidwai, Asmita Patil, Mag. Dorothea Peter and all those who I could not mention here for their support during this journey. I would also like to thank Dr. Mehri Magazehi and Dr. Constantin Rabong; one is like mother to me and other helped me like brother.

I would like to thanks Prof. Dr. Robert Konrat from Vienna Bio Center. for measuring NMR samples of complex molecules.

During this journey the greaest loss I suffered was the death of my Grand Mother. She eagerly wants to meet me and even on last day she was asking my brothers to open the door as I am waiting outside the door. I could not hold her hand in last moments and this gap cannot be filled.

I also pay my regards to all my family: Arshad Mehmood Khan (grand-father); Saeed Zafar Ahmed Khan (father) and Attia Tabassam (mother) they always pray for my success and prosperous life. How I can forget the love of my sisters and brothers without which I cannot climb up on this mountain.

In the last I would like to thank Govt. of Pakistan for financial support and Higher Education Commission of Pakistan who awarded this scholarship to me. In the last I would also like to thank ÖAD-Österreichischer Austauschdienst for processing my scholarship.

It is not the end of the journey:

Farhan Ahmed Khan
Vienna, January 2012.

for my Grand mother

Mrs. MUKHTAR AHSAN

Abstract

Modified peptides are of great importance in peptidomimetics. Peptide backbone consist of $(-NH-CHR-CO)_n$, most of the modifications have been done in these three repeating units of peptide backbone (i.e., NH, CHR and CO).

NXO building blocks are also an extension of the efforts to create and incorporate modified amino acids into modified peptides. In this project amino acids (both D/L) were modified by reversing functionalities on ends, incorporating oxalic acid functionalities at amine end and hydrazine functionalities at carboxylic acid end.

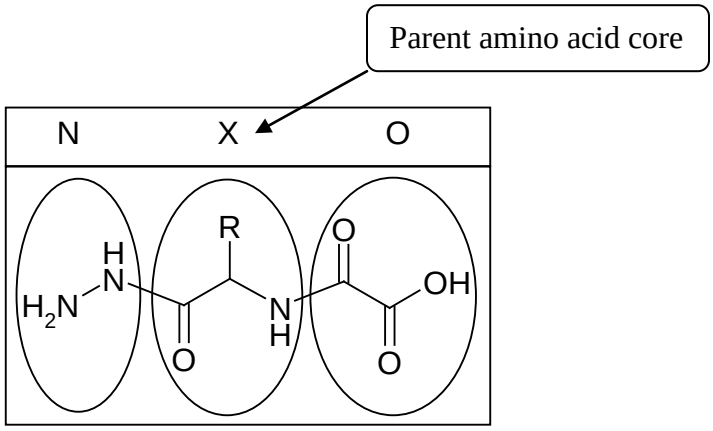


Figure: NXO building block

Some new NXO building blocks were prepared and identified. A modification on any nitrogen of hydrazine part is also reported important in peptide interaction with receptors, so some N-substituted hydrazines were synthesized and were used to create N-modified NXO building blocks. Secondary structure of proteins like beta-sheet and beta-turns were also modeled and synthesized, incorporating NXO building blocks in them; their conformational behavior was studied using high resolution NMR.

Zusammenfassung

Modifizierte Peptide nehmen eine hervorragende Stellung unter den Peptidomimetika ein. Das Peptidrückgrat besteht hierbei aus wiederholten Einheiten von (-NH-CHR-CO) n, wobei die häufigsten bekannten Modifikationen auf Veränderungen an den Einheiten NH, CHR- und CO abzielen.

Das NXO-Konzept versucht, modifizierte Aminosäuren in modifizierte Peptide einzubauen. Im Rahmen dieser Vorgehenseweise wurden beide, D- und L- Aminosäuren dahingehend verändert, dass die endständigen Funktionalitäten umgekehrt wurden – insofern enthält nun der N-Terminus eine Oxalsäurefunktion und der C-Terminus ein Säurehydrazid.

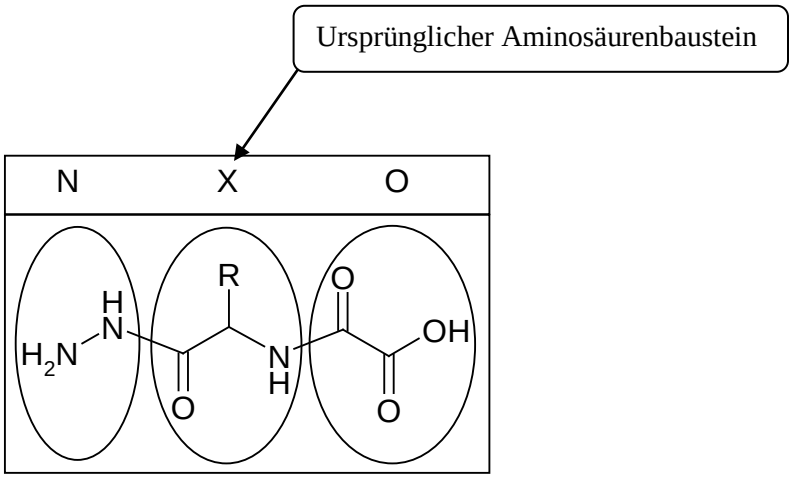


Figure: NXO-Einheit

Einige neue NXO-Synthesebausteine wurden dargestellt und charakterisiert. Modifikationen an Stickstoffen des Säurehydrazids besitzen im Rahmen der Rezeptor-Liganden Interaktion an Proteinen Bedeutung – daher wurden auch entsprechende N-modifizierte NXO-Synthesebausteine synthetisiert. NXO-modifizierte Mimetika von Sekundärstrukturen in Proteinen, wie beta-sheets und beta-turns, wurden zuerst als Modelle entworfen und dann dargestellt; ihr Konformationsverhalten wurde mit hochauflösender Kernresonanzspektroskopie untersucht.

Table of Contents

Result and Discussion Part

ABBREVIATIONS

Ala	alanine
Arg	arginine
Bn	benzyl
Boc	*tert*-butoxycarbonyl
Bz	benzoyl
Cbz/Z	benzyloxycarbonyl
CD	circular dichroism
Cys	cysteine
DCC	*N*-dicyclohexylcarbodiimide
DCE	dichloroethane
DCHU	dicyclohexyl urea
DCM	dichloromethane
DIC	diisopropylcarbodiimide
DIEA	diisopropylethylamine
DMAP	dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
Fmoc	9-fluorenylmethyloxycarbonyl
DOPA	3-(3,4-Dihydroxyphenyl-L-alanin)
Glu	glutamic acid
Gly	glycine
His	histidine
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	1-hydroxybenzotriazole
Ile	isoleucine
Leu	leucine
Lys	lysine
Met	methionine
NOE	nuclear overhauser enhancement
NOESY	nuclear overhauser effect spectroscopy
PG	protecting group
Phe	phenylalanine
Phg	phenylgylcine
Pro	proline

Sar	L-Sarcosin
Ser	serine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
Thr	threonine
Trp	tryptophan
Tyr	tyrosine
Val	valine

If not mentioned, amino acids have the L-conformation.

Peptides and Historical Developments in Peptidomimetics

Peptides are omnipresent in living organisms and are involved at some level in all physiological processes. They serve as agonist, antagonists [2011CBDD319], digestive intermediates, inhibitors [1996CPD225], transporters [2011BIOC77] and protectors. In nature, peptides are composed of more than twenty amino acids which are building blocks of proteins. D and L-amino acids are abundantly present in biologically active peptides.

During 1899 to 1908 Hermann Emil Fischer made his great contribution to knowledge of the proteins. He also proposed the term “Peptide” [1902CZ939]. In 1901 he discovered in collaboration with Fourneau, the synthesis of the first unprotected synthetic dipeptide glycyl-glycine. [1901BER2868].

The history of peptides comprises of an evolutionary phase of about hundred years. This science was born, when the peptide chemistry was only the gastric-juice-derived digests of protein and scientists were trying to re-synthesize these molecules from amino acids building blocks. Next problem was the development of protecting groups and also their selective removal. In next couple of years better peptide bond formation methods were developed using mixed anhydrides [1950JLAC117; 1950JLAC122], active esters [1955HCA69; 1955HCA83] and carbodiimides [1955JACS1067; 1956JACS1367]; they accelerated the pace of progress in this field. Improvement of synthetic methods like solid phase synthesis and purification methods like high performance liquid chromatography (HPLC) were also important developments in this field. Modifications in protein analogues with unnatural residues were always of great interest to scientists studying protein folding and factors influencing protein stability that can lead to novel biomaterials. Synthetic peptide chemistry still offers new horizons with many possibilities to protein engineering that was much more than the biology and limitations

of genetic code [Methods of Organic Chemistry (Houben-Weyl), Vol. E 22a (Synthesis of Peptides and Peptidomimetics), page 1-14].

Peptidomimetics as Drugs

Peptides are involved in most of the biological processes of living systems creating interest for scientists to manipulate their structure and observe the results. These new moieties which are known as *peptidomimetics* came into being as a result of these manipulations. A peptidomimetic is a molecule, which is expected to exert the same biological activity as the natural one with some improved advantages i.e. oral absorption, metabolic stability [2007DISS1on p2; 1989ARMC243]. In peptide-based drug design, there are several major considerations that limit clinical applications such as: (1) rapid degradation by many specific or non-specific peptidases under physiological conditions; (2) conformational flexibility which allows a peptide to bind to more than one receptor or receptor sub-type leading to undesirable side effects; (3) poor absorption and transportation because of their high molecular mass or the lack of specific delivery system, especially for some peptides which require the passage through the blood-brain-barrier (BBB) to act in the central nervous system (CNS). To counteract these problems, peptidomimetic drug design has emerged as an important tool and emerging science for both peptide chemists and medicinal chemists. [1996PAC1303]; these problems have brought organic chemistry, biochemistry and pharmacology together to dialogue and find solutions.

Complex biochemical pathways consist of a large number of potential target proteins, nucleic acids, or lipidic structures and they can potentially offer themselves for agonist or antagonist development. In many cases a protein fragment can be identified as either part of the unprocessed protein or a cleaved piece of protein that serves as a ligand or the target. Naturally occurring peptide drugs are just the beginning of a new world. With the advancement in the computer power and molecular interaction databases, ligands can be designed for those protein fragments where models show validated structural features. Due to their high specificity and low toxicity profile, peptide-based drugs can be a good choice as contemporary therapeutics.

An example of a recent peptide drug is T-20 (Enfuvirtide), which blocks HIV entry into cellular CD4 [2005DDT1085]. Another HIV drug candidate the CCR5 trans-membrane receptor ligand, RANTES (68 residues), is an example of the current trends in peptide modification [1998JEMED1215; 2004PNA16460].

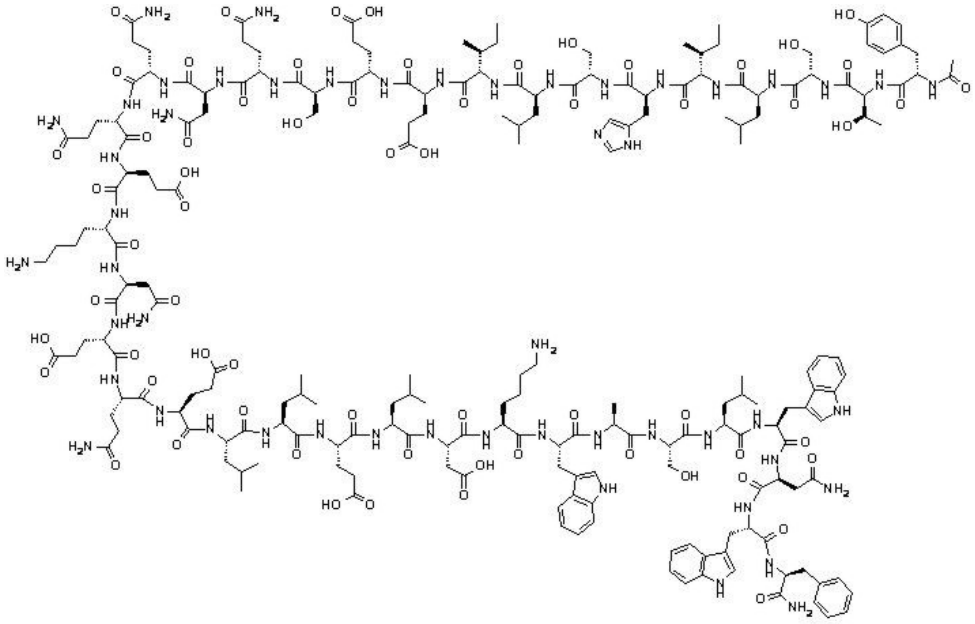


Figure: Enfuvirtide a peptide based drug for HIV infection.

Although peptide receptor agonists / antagonists or enzyme inhibitors can play very important role as modulators of disease processes, they suffer from several limitations that restrict their development into drugs. These limitations are (1) rapid metabolism by proteolysis, (2) poor absorption from gastrointestinal tract, (3) rapid excretion and (4) lack of receptor specificity [2001DISS2].

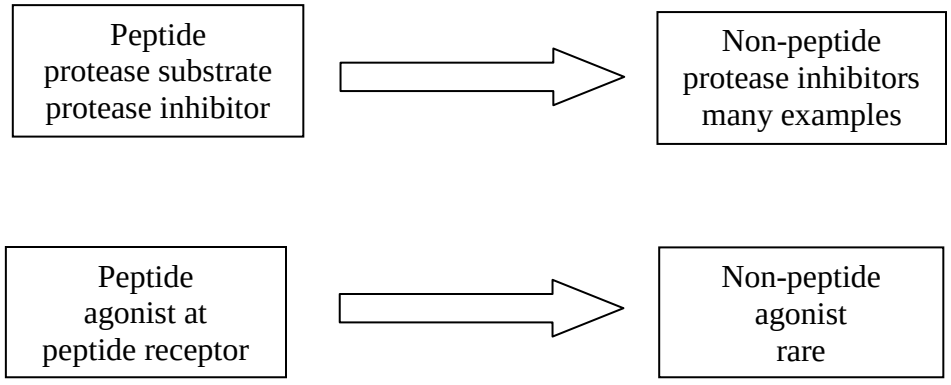


Figure: Transformation of peptides to non-peptides. [2001DISS2]

Peptidomimetic Discovery Strategy

Despite their enormous diversity in biological function and structure; peptides and proteins are endowed with properties that have induced and stimulated the development of peptidomimetics. Clearly, peptides can be considered as the “stem” of a phylogenetic molecular development tree from which branches of oligomeric peptidomimetics such as peptoids, peptidosulfonamides, urea peptidomimetics, as well as β-peptides have sprouted. It is still a challenge to efficiently synthesize these oligomeric species and also study their structural and biological properties. Combining peptides and peptidomimetics led to the emergence of peptide–peptidomimetic hybrids in which one or more (proteinogenic) amino acid residues have been replaced with these mimetic residues, the influence of these replacements on biological activity can then be studied by scanning, to evaluate to what extent a peptide can be transformed into a peptidomimetic structure while maintaining, or even improving, its biological properties [2011CHBC1626]. Following is the most commonly used route for peptidomimetic design and discovery.

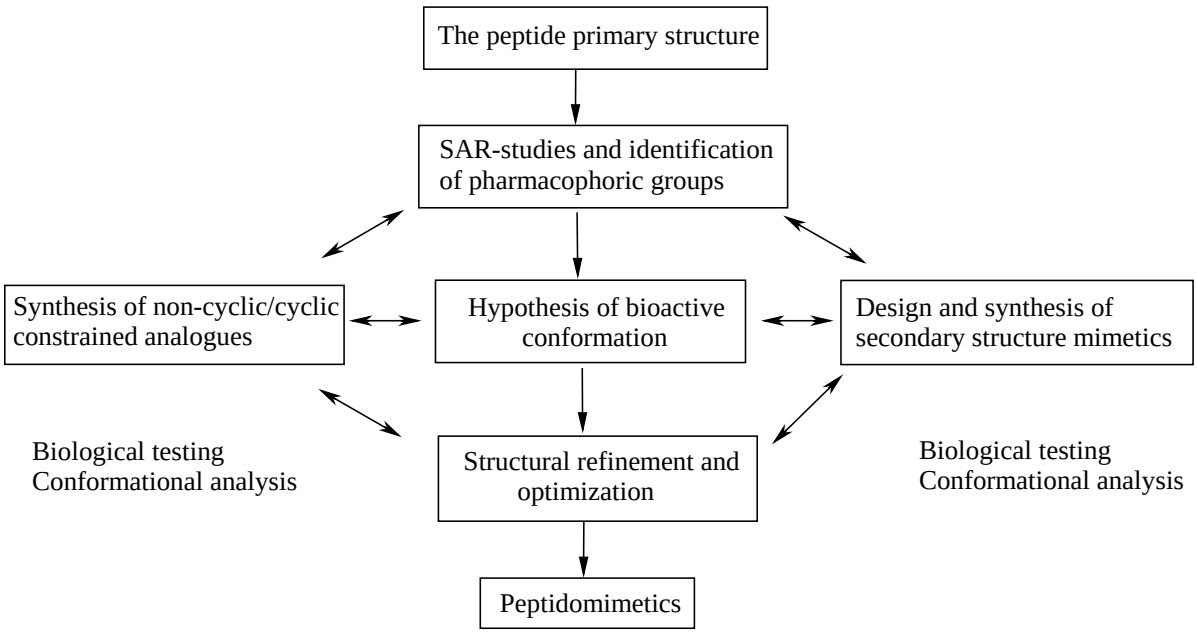


Figure: Peptidomimetic discover strategy. [2001DISS2]

Following is the *de novo* approach of drug design with all the steps involved like SAR and biophysical studies, in the development of a peptidomimetic candidate to use as a drug.

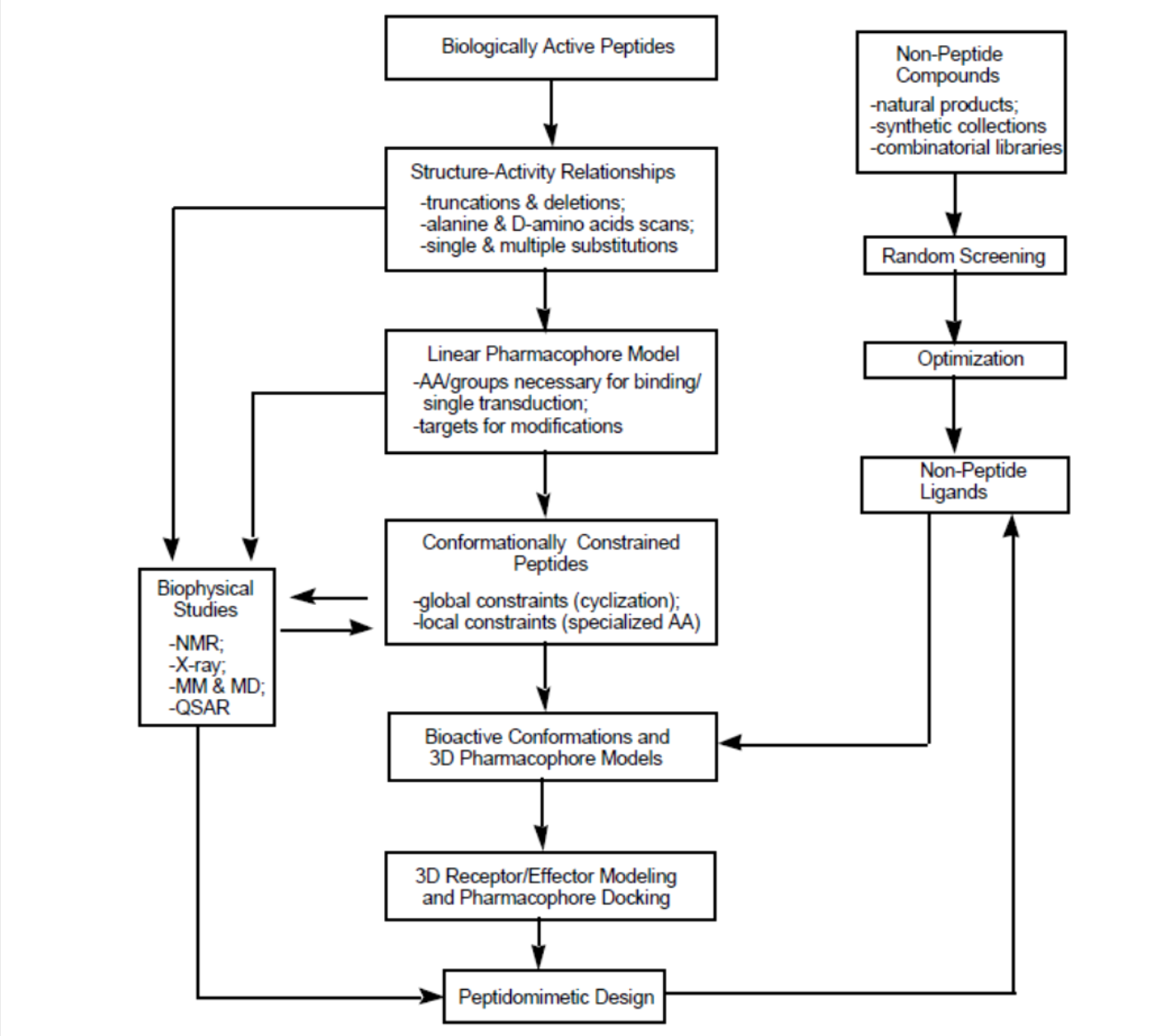


Figure: A *de novo* approach for peptidomimetic design. [2000CMCH945]

The design of peptidomimetics with a specific structure, conformation and topographical properties is a major issue in medicinal chemistry [2011IJMOS2853]. Topographical considerations are an important approach for exploring the stereo-chemical requirements for receptor recognition and for signal transduction [1997BPS219; 2011IJMOS2853]. The initial steps are to determine a smallest active sequence and pharmacophoric groups.

Structure-activity relationships (SAR) of the peptide should also be extensively studied. Bioactive conformation(s) are defined by the introduction of non-cyclic and / or cyclic constrains at various positions in the peptide to reduce the conformational freedom. The essential amino acid side-chains are positioned onto the scaffolds, preferably small, poly-functional rings of defined stereochemistry, using structural information gathered in previous steps. Finally designed peptidomimetics should retain the pharmacophoric groups in a defined, spatial relationship for docking onto the receptor protein.

Cyclic and Non-cyclic Constraints

Constrained analogues sometimes enhance the proteolytic stability, because proteolytic enzymes e.g. peptidases prefer conformationally flexible substrates in extended conformations. Secondly selectivity can also be enhanced by reducing conformers that produce undesirable bioactivity. [1985TN392; 1998CMCH29; 2007DISS1 on p 6].

Non-cyclic and cyclic constraints play important roles in proteins; their incorporation into the peptides is discussed in more detail in coming pages:

Non-cyclic Constraints

Amino acids and peptides naturally exist with a conformational bias which starts at the backbone level. This spatial orientation of the backbone peptide bonds and side chains are defined by torsional (or dihedral) angles commonly represented as Φ, Ψ, ω and χ as illustrated in following figure. [2011IJMOS2853]

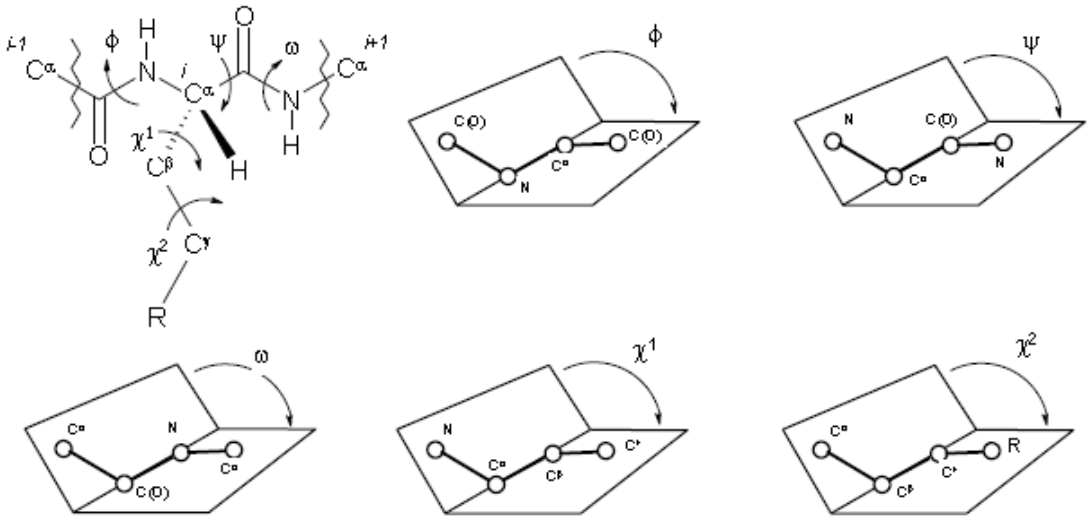


Figure: Conformations of peptides: definitions of the Φ, Ψ, ω, χ^1 and χ^2 torsional angles.

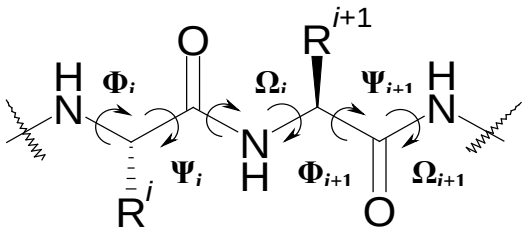


Figure: Definition of torsional angles. [2007DISS1]

Non-cyclic constraints can be manipulated using (1) amino acid manipulations, (2) peptide backbone modifications, (3) peptoids and (4) extension of the backbone peptide-chain.

Amino Acid Manipulations: D-amino acids, α,α- or β,β-disubstituted amino acids, or conformationally restricted amino acids are the tools which can be used to induce non-cyclic constrains and allows to probe the local conformational requirements for recognition and deduce the probable position of e.g. turns. For example *N*-alkyl amino acids restrict the torsional space due to the increased steric bulk of the alkyl group and prevent the participation of the amide hydrogen in hydrogen bonding interactions. [1993T3547]

Peptide backbone modifications: Isosteric exchange of units in the peptide-chain and the introduction of additional fragments, like analogues containing amide bond (*CONH*) isosteres (CH=CH, CH=CF, CH_2CH_2, CH_2NH, NHCO, CSNH, CH_2O, CH_2S, $COCH_2$, $CH(OH)CH_2$, etc) have been extensively utilize to address aspects of peptide structure and function, including rotational freedom in the backbone, modifications of local and total polarity, oral bioavailability, intra- and intermolecular hydrogen-bond patterns and hydrophobicity. [2000CMCH945; 1993AG(E)1244; Spatola, A. F. In *Chemisty and Biochemistry of Amino Acids, Peptides and Proteins*; Weinstein, B., Ed.; Marcel Dekker: New York, 1983; Vol. 7, p 267-357].

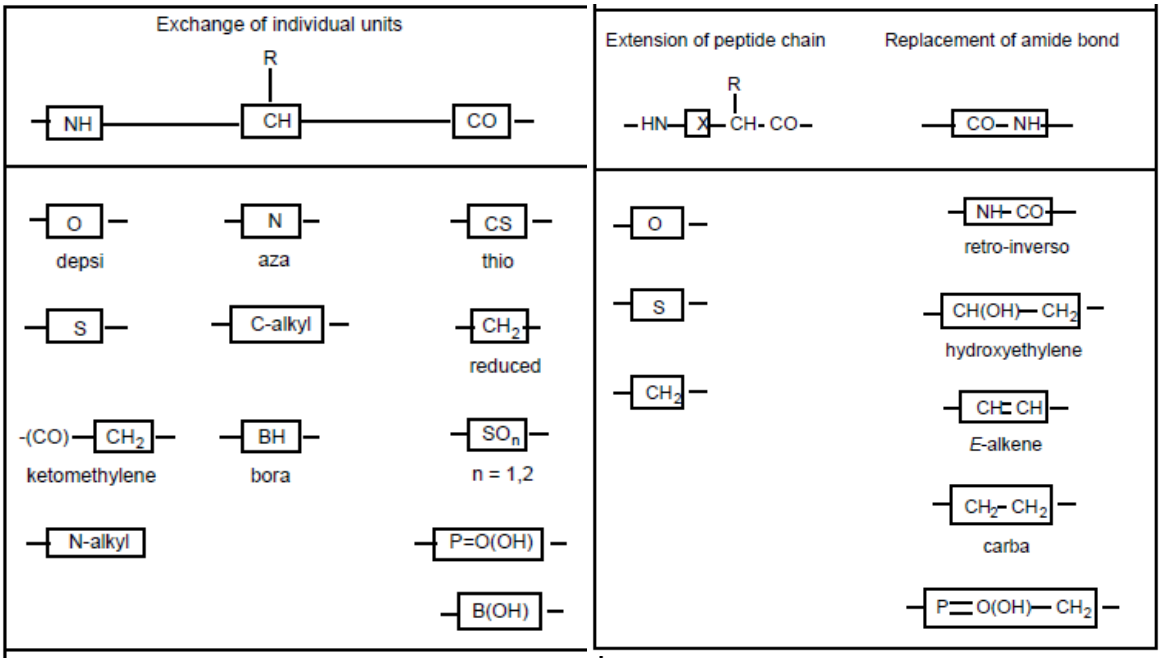


Figure: The most frequent modifications to the peptide backbone. [2000CMCH945]

Peptoids *N*-Alkylated glycines are joined together in a retro-inverse manner to maintain the relative orientation of the carbonyl groups to the R-groups. The α-CHR groups have been replaced by NR units and the NH groups by CH_2 units as shown in figure. Peptoids are not chiral, since the side-chains corresponding to the normal amino acids are attached to the nitrogen [1993AG(E)543; 1992PNA9367]. They are non sensitive to proteolysis [1995DDR20].

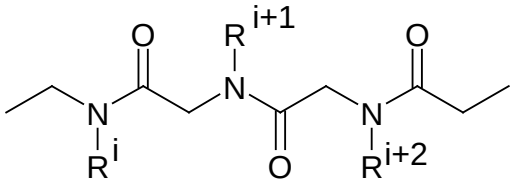
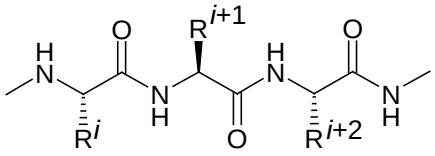
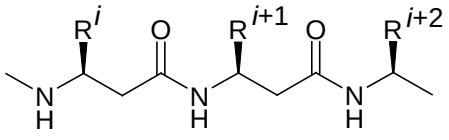


Fig: Peptoid.

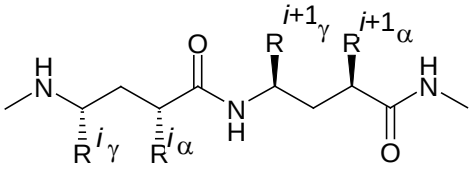
Extension of the backbone peptide-chain can also make it resistant to peptidases activity. Some examples can be observed in the synthesis of oligomers of *β*-amino acids, i.e. *β*-peptides. These *β*-peptides [1997CC2015; 2000JOC4766] can adopt several well-defined secondary structures, preferentially helical [1998ACR173], hairpin [1994NSB584] and *β*-sheet conformations. In a similar manner γ-peptides are synthesized from γ-amino acids [1999TL4925; 2001DISS2].



α-peptide



β-peptide



γ-peptide

Figure: Comparison of an α-peptide, a β-peptide and a γ-peptide. [2001DISS2]

Cyclic Constraints

Many naturally occurring peptides occur as cyclic structures. In general, cyclization imposes conformational constraints on linear peptides [Molecular Biology and Biotechnology Edited by Robert A. Meyers (**1995**) p 660]. These effects are most pronounced when the cycles are di-, tri-, or tetra- peptides, but can also be observed in larger cyclic peptides where additional conformational constraints are incorporated [1995CMCH654; 1998CMCH29].

Short range cyclizations: These types of cyclizations can be observed within a single amino acid residue or between two neighbouring amino acids to a *dipeptide mimetic* [1990IJPPR287]. Following are the few examples:

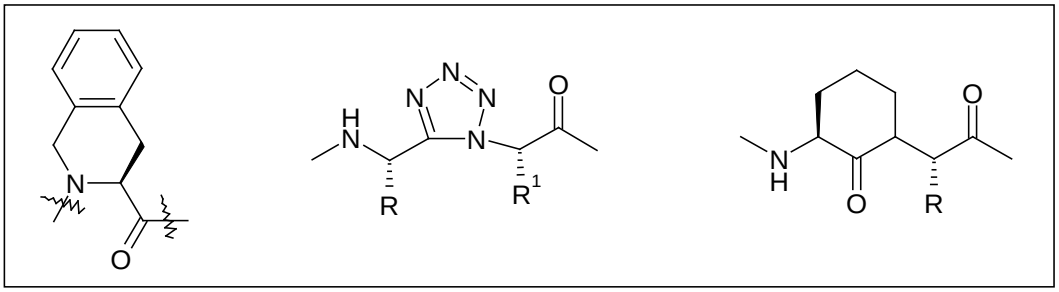


Figure: Different examples of short range cyclization. [1994AG(E)1699]

Long-range cyclizations: These kinds of cyclizations can be divided into cyclizations involving C and N termini, backbone to backbone cyclizations [1991BPS745], side-chain to side-chain cyclizations and a combination of side-chain to backbone cyclizations [1990BJ249].

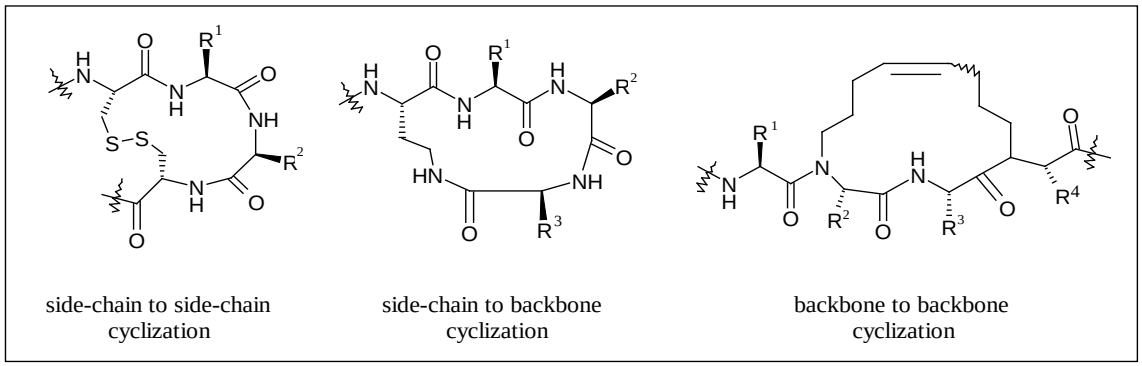


Figure: Examples of a disulfide-cyclization, an amide-cyclization and a ring-closing metathesis cyclization. [1990BJ249]

1: NXO Amino Acid Building Blocks

Peptides are rarely used as therapeutics especially due to their stability issues. Naturally occurring amino acids are therefore modified to increase the stability, reduce the bio-degradation and enhance the reactivity properties.

Generally peptide backbone (—NH —CHR— CO)$_n$ is modified at any of the repeating element (NH, CHR, and CO) or a combined (CONH) unit [2010JOC2492]. A graphical representation of modifications in amino acids with reverse functionality is given in the figure:

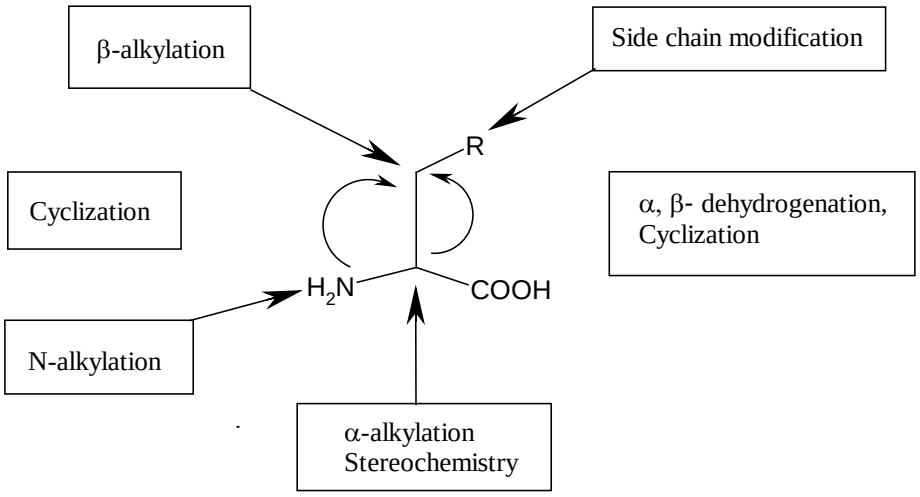


Figure: General strategy for amino acid modification.

Our group introduced the NXO-concept as a novel kind of amino acid modification and a number of such building blocks were prepared [2010JOC2492] and used.

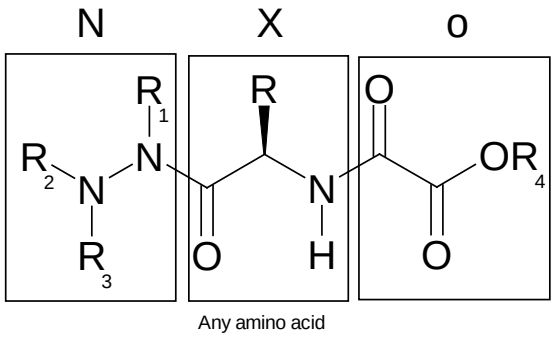


Figure: X- any amino acid, R_1-R_4- H, alkyl, aryl, protecting groups. [2010JOC2492]

These modified amino acids with end group modifications can duplicate the acceptor-donor pattern of normal tripeptides and can also be seen as a combination of a oxalo-retro-modification with an azapeptide modification.

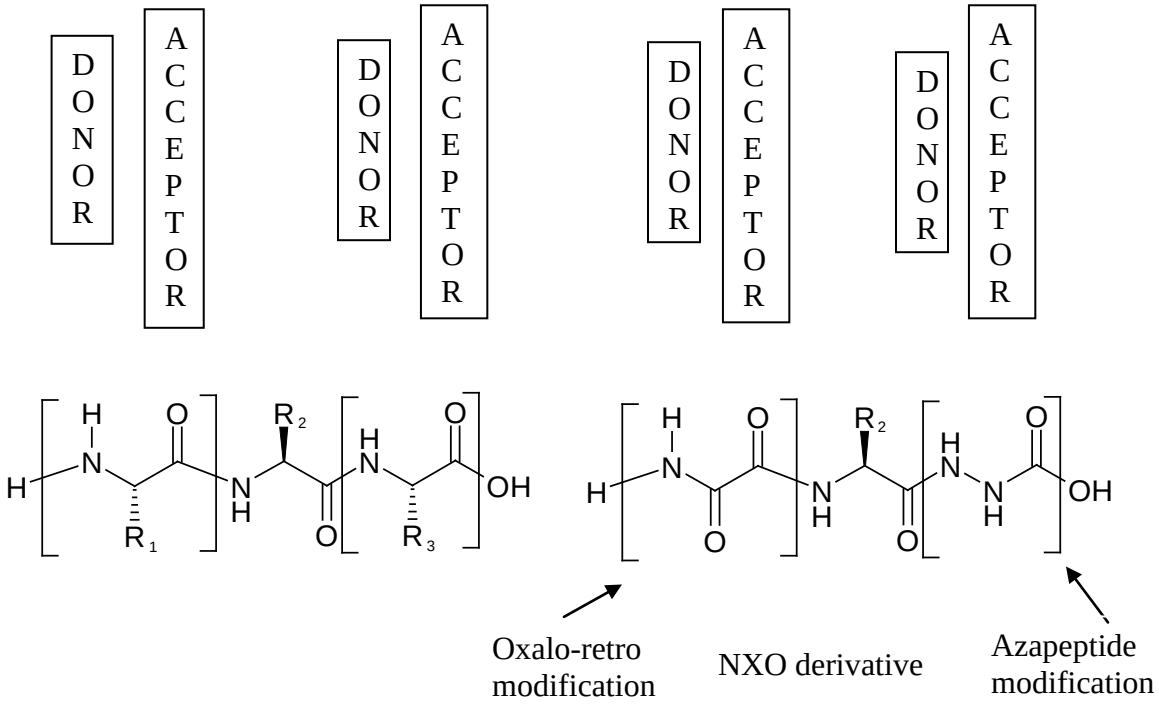


Figure: Representation of acceptor-donor pattern and modification at Cα-carbons.

Nomenclature

A three letter abbreviation NXO was introduced for such building blocks; where “N” is for hydrazide part and “O” represents the oxamide part, while “X” is a replacement of three letter abbreviation of the parent amino acid (which can be any amino acid, natural, unnatural, alpha, beta, gamma etc.).

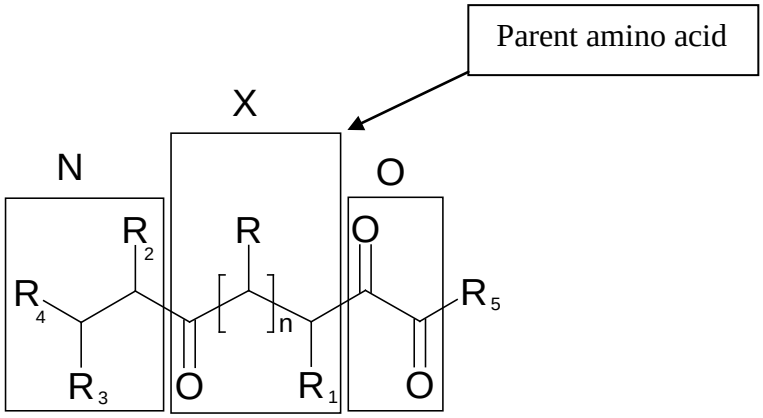


Figure: NXO nomenclature.

Sr. No.	R_1	R_2	R_3,R_4	R_5	X	NXO-name
FK-29	H	H	H, Fmoc	OBu^t	Met	Fmoc-NMetO-OBu^t
FK-32	H	H	H, Cbz	OBu^t	Met	Cbz-NMetO-OBu^t
FK-76	H	H	H, Boc	OMe	Met	Boc-NMetO-OMe
FK-78	H	H	H, Fmoc	OBu^t	Thr	Fmoc-NThrO-OBu^t
FK-81	H	H	H, Fmoc	OBu^t	Phg	Fmoc-NphgO-OBu^t

Intermediates for NXO building blocks

For the preparation of NXO-building blocks a series of key intermediates were prepared on larger scale with improved yields and higher purity compared to the reported literature [2008T6788; 2000CJC942; 1972JOC3404].

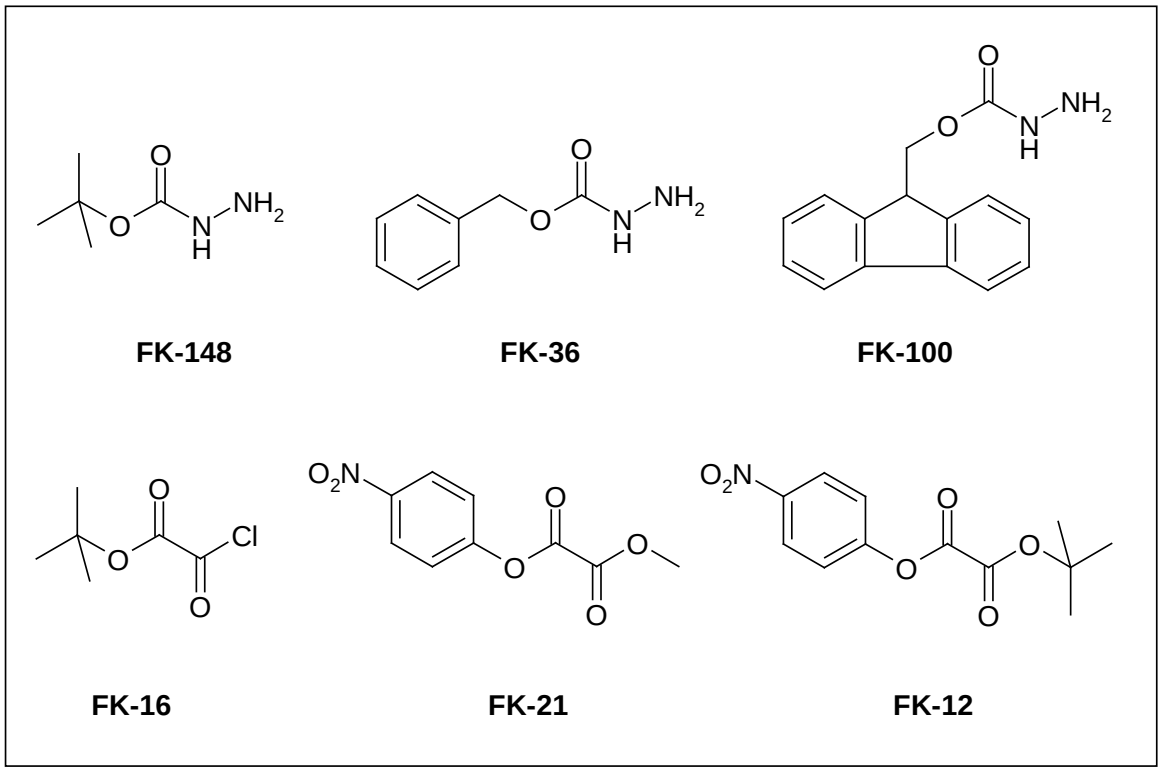
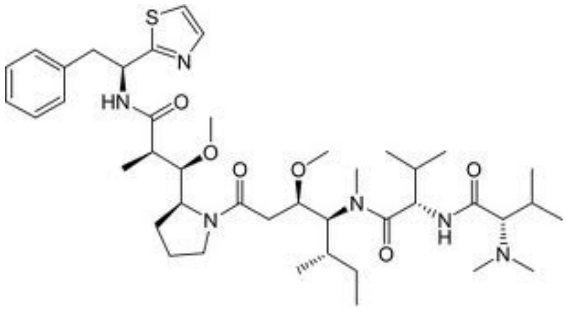


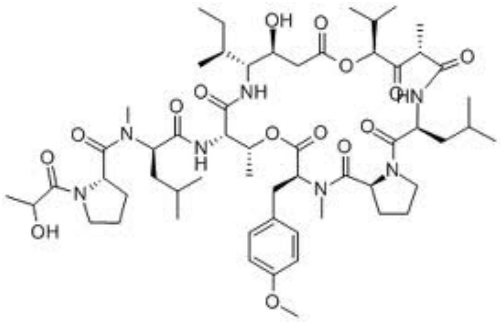
Figure: Intermediates for NXO building blocks.

2: *N*-substituted NXO Building Blocks

N-substituted amino acids are present in many naturally occurring peptides and depsipeptides such as cyclosporine, dolastatin and didemnin [1999JPS56] especially *N*-methyl amino acids has been observed in wide variety of naturally occurring peptides and contribute to a wide range of biological effects including anticancer, antidiuretic [1993T9151; 1983JMC555], antibiotic [WO2009013262], antitumor [2003BMCL455], antiviral and immunosuppressive activity [1997TL5085]. *N*-Methylation in amino acids has been proved to improve certain properties in therapeutics like membrane permeability, conformational rigidity and proteolytic stability. [2002OL3767].



Dolastatin



Didemnin B

Hydrazine derivatives are precursors in organic synthesis of peptidomimetics and are commonly used in pharmaceutical, agrochemical and polymer industry. Hydrazine based peptidomimetics (aza-peptide derivatives) are active against diseases like hepatitis, SARS and HIV [2008T6788].

Hydrazine moiety is one of the components of NXO-building blocks; a change in substituent on any of the nitrogen can be potentionally valuable. A number of *N*-substituted mono protected hydrazines were prepared according to literature reported methods and incorporated them in *N*-substituted NXO-building blocks.

3: Secondary structures of proteins

Foldamers are discrete artificial oligomers with size about 500-5000 amu. They fold into definite secondary structures (i.e., helices, turns, sheets) and can mimic bio-macromolecules [2011EJOC3648]. Synthetic foldamers (peptidomimetics) have attracted a lot of attention recently. Discovering predictable and well-defined secondary structures and harnessing bioactivities in enzyme mimics as well as drugs is one example of this development [2010T9733]. A major challenge in the field of peptidomimetics is the design and synthesis of conformationally constrained analogues that mimic essential secondary structural elements [2010T4474]. Secondary structures like β-turns are often located on the surface of proteins where they can undergo post-translational modification and serve as important recognition elements in receptor-ligand interactions of peptides and proteins and are considered as initiation sites for protein folding [2008BPS380; 2004JOC3500]. A general approach to the synthesis of peptidomimetic compounds involves the use of non-peptide building blocks, which enforce or stabilize a particular type of β-turn, when inserted into a peptide chain.

Helices and sheet structures are uni-directional; therefore, loops are required to reverse the direction of polypeptide chain

Rigidity is also a two-edged sword in designing and testing of peptidomimetics for binding target protein receptors. Compounds that are too flexible may pay such high entropic penalties on binding that the process becomes energetically unfavorable. Severe conformational constrains can also prevent molecules from adapting to the binding site at all [2004JOC701; 1992JACS10690; 1993BMCL803; 1998AG (E) 2755].

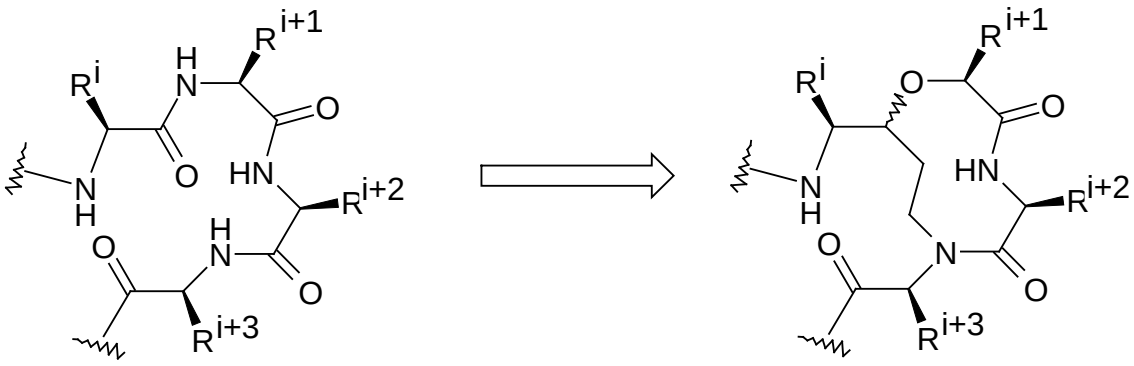


Figure: Transformation of a *β*-turn into conformationally restricted mimetic.

Freidinger was a pioneer in the field of peptidomimetics and developed dipeptide γ-, δ-, ε -lactams as constrained scaffolds to stabilize turn conformations [2010T4474; 2003JMC5553; 1982JOC104].

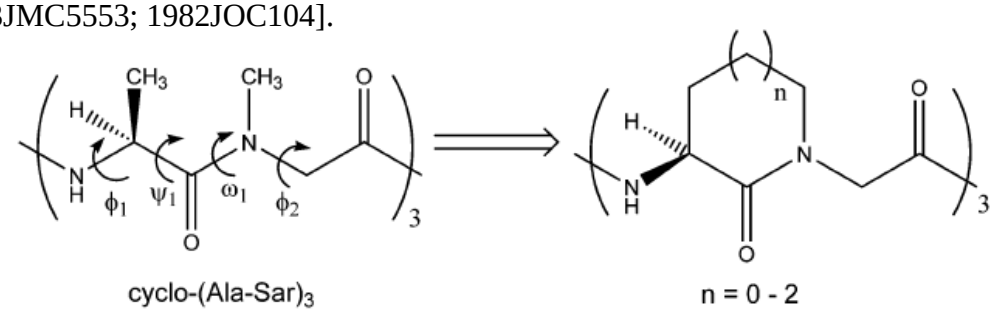


Figure: Initial application of dipeptide lactam. [2003JMC5553]

In recent years several methodologies have been followed for the development of such molecules including liquid phase and solid phase synthesis [2004JOC701; 2000T9809]. Generally turns are grouped by their hydrogen bonding and by their backbone dihedral angles.

At the level of hydrogen bonds, the nomenclature is similar to that of helices.

- An **α-turn** is characterized by (a) hydrogen bond(s) in which the donor and acceptor residues are separated by *four* residues ($i \rightarrow i \pm 4$).
- A **β-turn** (the most common form) is characterized by (a) hydrogen bond(s) in which the donor and acceptor residues are separated by *three* residues ($i \rightarrow i \pm 3$).
- A **γ-turn** is characterized by (a) hydrogen bond(s) in which the donor and acceptor residues are separated by *two* residues ($i \rightarrow i \pm 2$).
- A **π-turn** is characterized by (a) hydrogen bond(s) in which the donor and acceptor residues are separated by *five* residues ($i \rightarrow i \pm 5$).
- Finally, an **ω-loop** is a catch-all term for a longer loop with no internal hydrogen bonding

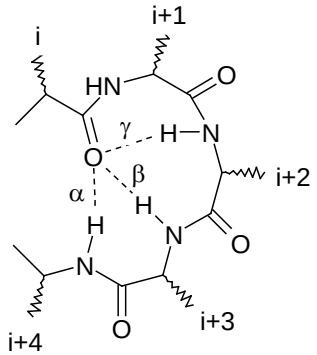


Figure: Distinction between general α-, *β*-, and *γ*-turns. [2005CRV793]

β-turns consist of a tetrapeptide sequence in which the α_{Ci}-α_{Ci+3} distance is shorter than 7.1A° [1993T3467; 1977JMB135]. Such turns are often stabilized by an intra-molecular hydrogen bond between the carboxyl oxygen of the *i* residue and the amide proton of the *i* + 3 residue, which leads to the formation of a 10-membered ring-type structure. β-turns are often located on the protein surface and hence play important roles in the molecular recognition events of biological systems [2004OL3183].

At least eight forms of the β-turns have been identified; mainly varying in whether a *cis* isomer of a peptide bond is involved and on the dihedral angles of the central two residues. The classical and inverse β-turns are usually distinguished with a prime, *e.g.*, type I and type I' β-turns.

Ideal angles for different β-turn type. Types VIa1, VIa2 and VIb turns are subject to the additional condition that residue $(i + 2)$(*) must be a *cis*-proline

Type	φ_{i+1}	ψ_{i+1}	φ_{i+2}	ψ_{i+2}
I	-60	-30	-90	0
II	-60	120	80	0
VIII	-60	-30	-120	120
I'	60	30	90	0
II'	60	-120	-80	0
VIa1	-60	120	-90	0*
VIa2	-120	120	-60	0*
VIb	-135	135	-75	160*
IV	turns excluded from all the above categories			

A reverse β-turn is a motif made up of four amino acids fragment and can invert the chain direction. It is stabilized by intra-molecular hydrogen bonding between the carbonyl oxygen of the first residue (*i*) and the amide proton of the fourth one (*i* +3) [2011JOC833; 2008BPS380; 1993T3467].

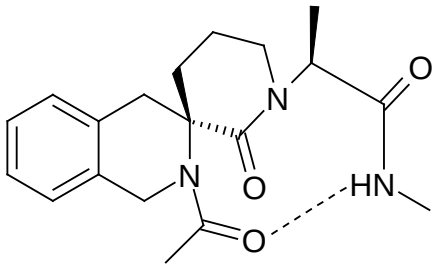


Figure: Structure of a reverse beta-turn. [2011JOC833]

Lactams have been shown to be a useful new type of conformational constraint in peptides. Bioactive conformation and biological potency of peptide may be increased by incorporation of a lactam [1982JOC104]. Ring size of the lactam has been shown to have an important effect on conformation and also on biological potency of an analogue [1980IJPPR464]. Several molecules like spirolactams have been reported so far which mimic the beta turn structures [1993JOC860] some examples are given below:

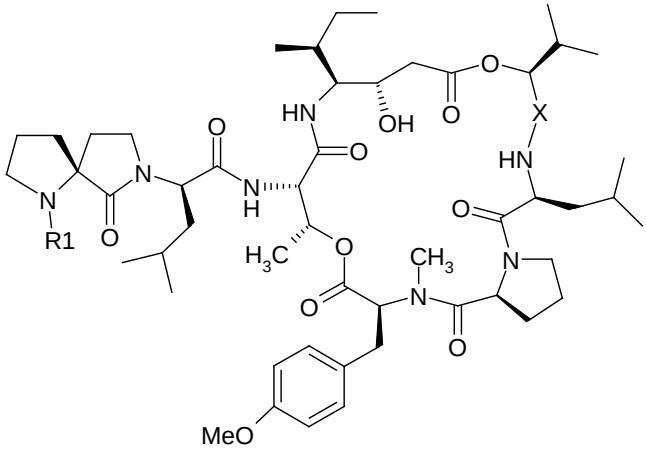


Figure: Spirolactam analogue of Aplidine. [2004JMC5700]

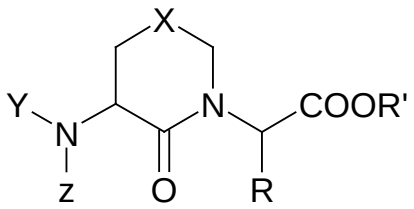


Figure: lactam-bridged peptide (where Y and Z are protecting group or H; X= $(CH_2)_{0-2}$ or S). [1982JOC104]

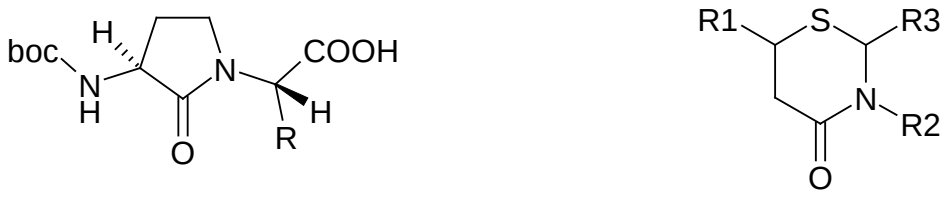


Figure: 5 and 6 membered ring structures of lactam-bridged dipeptides. [1982JOC104]

If a β-strand is folded back on itself to form an antiparallel association with the succeeding region of polypeptide, there must necessarily be residues forming a chain reversal. This chain reversal with two corner residues and an i to i+3 hydrogen bond leads to a conformational feature known as a β-turn. Some β-turn conformations are shown in the following figure [2006BPS13].

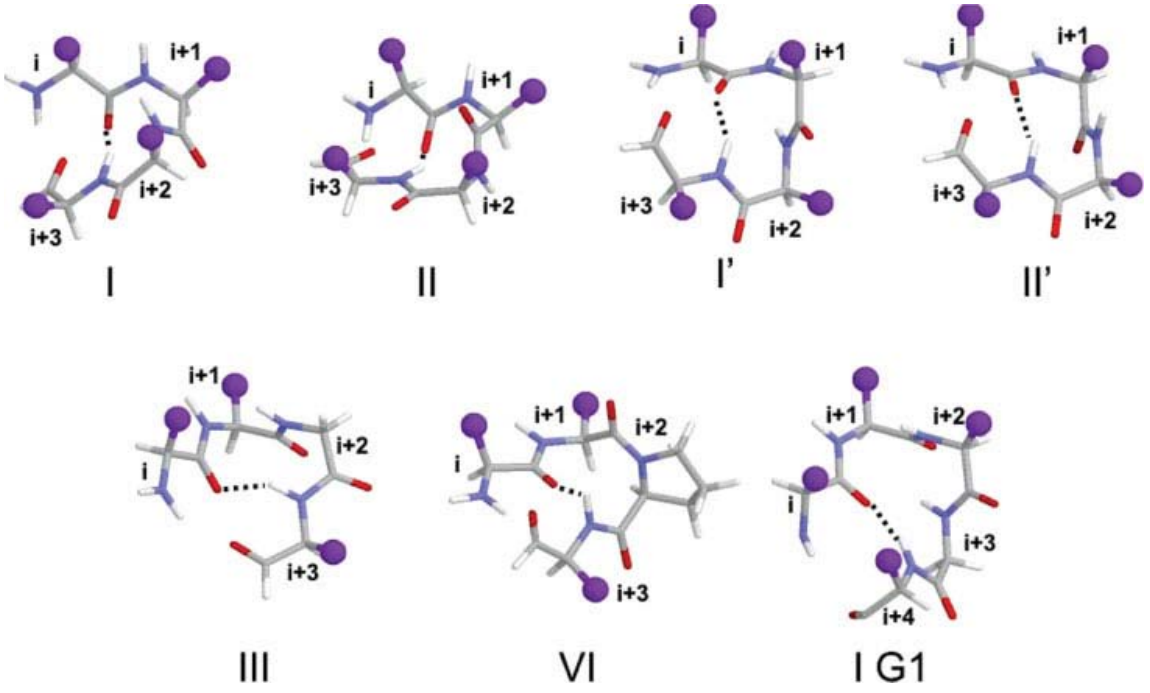
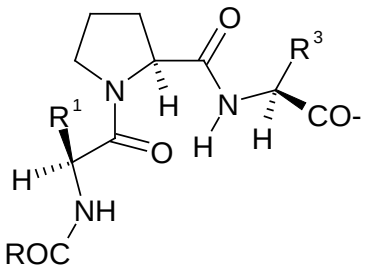
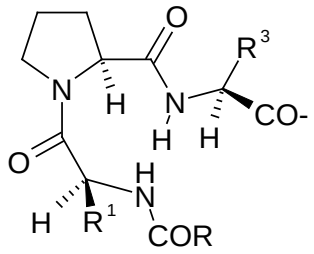


Figure: Typical stereochemically allowed β-turn conformations that enable the polypeptide chain to reverse its direction. All side chains are shown in the L-configuration. Types I (and its mirror image I') and II (and its mirror image II') are typically found in proteins. The type III turn has i+1ϕ and i+2ψ and angles near -60, -30, the value for a 3_{10} helix. In a type I turn with a G1 bulge, the i+3 residues occupy α_1.conformational space. Glycine, with its steric ability to visit this conformational space, is frequently found in such turns, although the small polar residues Asp and Asn can also occupy the i+3 position of type I G1 bulge turns. The type I, I', II, II', and III turns are canonical, while the I G1 turn is turn 4 from CRABP I(PDB file 1cbi). [2006BPS13]

Proline is unique in behavior amongst other amino acids; it is the only amino acid whose C^{α}-N bond is the part of pyrrolidine ring. Proline is quite often observed at (i +1) position of β-turn structure [1977JMB135]. Prolines role to control peptide structure is important and it also serves to expose recognition sites for the protein-protein interactions on the surface of proteins [1995BBR1115]. The peptide bond in X-Pro was observed to exist both in cis and trans- forms in nature while interconversions of *cis* and *trans*-forms of small peptides are quite slow in water [1993APC1], with ribonuclease and bradykinin being an example [1979JACS2455; 2007DISS1].



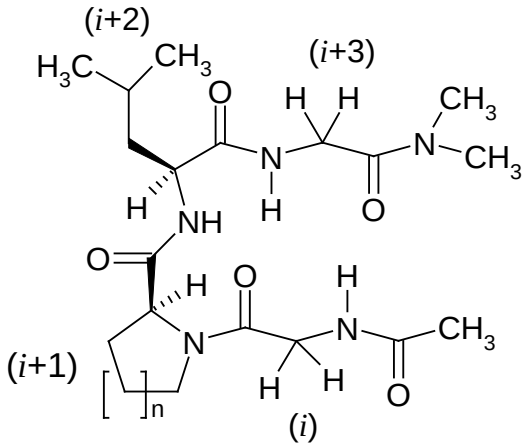
trans - X - Pro - Y



cis - X - Pro - Y

3.1 Conformational studies of β-turn structure in tetra-peptides containing Proline.

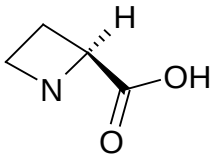
Takashi Hayashi and co-workers prepared and studied three tetrapeptides (**a,b,c**) having L-2-azetidine-carboxylic acid (4-membered ring **d**), L-proline (5-membered ring **e**), and L-2-piperidinecarboxylic acid (6-membered ring **f**) respectively at the (i+1) position of tetrapeptide sequence [1997TL3039].



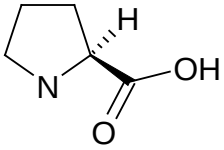
n= 0 : Ac-Gly-Aze-Leu-Gly-NMe_2 **(a)**

n= 1 : Ac-Gly-Pro-Leu-Gly-NMe_2 **(b)**

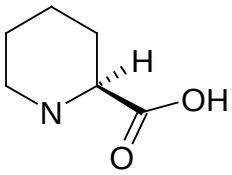
n= 2 : Ac-Gly-Pip-Leu-Gly-NMe_2 **(c)**



L-2-azetidinecarboxylic acid **(d)**



L-Proline **(e)**



L-2-piperidinecarboxylic acid **(f)**

NOE cross peaks which support β-turn structure were observed in all the three peptides **(a,b,c)**, the NOE cross peak between both terminals of peptide was observed only in the NOESY spectra of **(b).** that indicates that 5-membered ring side chain in proline **(e)** plays a very important role in the formation of β-hairpin structure. [2007DISS1on p145]

3.2 Design of β-turn mimic

Proline containing NXO-peptides were designed by replacing the carbonyl group at (*i*) position, proline (D or L) at (*i*+1), NH group at (*i*+2) and glycine at (*i*+3) in analogy to Takashi [1997TL3039]. The methyl amino and dimethyl amino groups were used as end group protection. The substitution at R^3 at the (*i*+2) position is important [1993T3467] as the orientation of the side chain is dependent on it. Nowick, et al. have reported that a cyano-ethyl group at R^3 can impose strong molecular constraints to control the orientation of the side chain to stabilize the intermolecular hydrogen-bonded confirmation [2006OBC3869]. We planned to place a methyl group at this position due to its medical significance [1997TL5085; 2001B8237].

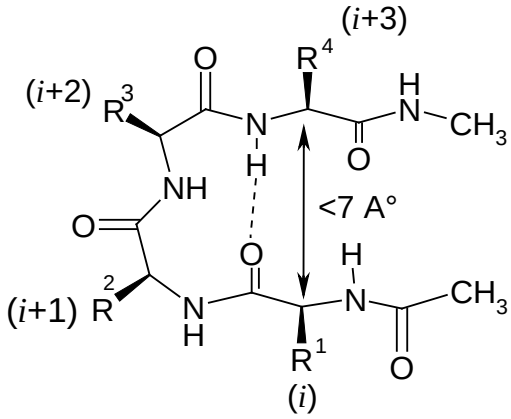


Figure: β-Turn

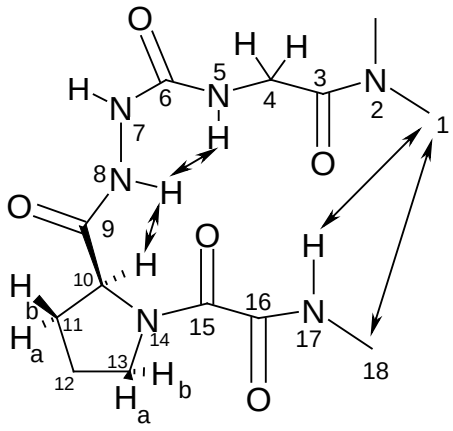


Figure: β-Turn analogue (FK-166)

Beta turn mimic using L-proline was reported by Jaywant in his dissertation [2007DISS1]. Molecular modeling suggested that an analogue with D-Proline will be more stable and can show better hydrogen bonding pattern. This analogue was prepared along-with L-analogue with some changes in the synthetic route to get better yield and high purity. The molecular modeling results were further confirmed by NMR results.

The detailed synthetic route for the synthesis of β-turn mimic (FK-166) is described in (Scheme 1).

4: NXO β-sheet mimic & NXO β-turn mimic

Using Molecular Operating Environment, Version 2007.09 (MOE, © 1997-2007 Chemical Computing Group Inc.). a beta-sheet was designed and its geometry was optimized.

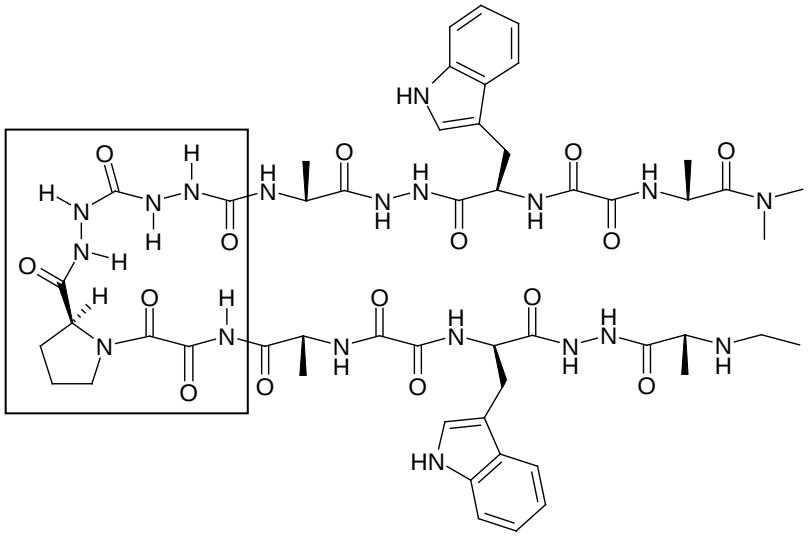


Figure: β-sheet containing NXO β-turn mimic with carbohydrazide substructure.

The first step of this project was the preparation of a NXO β-turn with carbohydrazide substructure as shown in figure. The detailed synthetic route for the preparation of NXO β-turn mimic with carbohydrazide substructure is shown in the (Scheme 2).

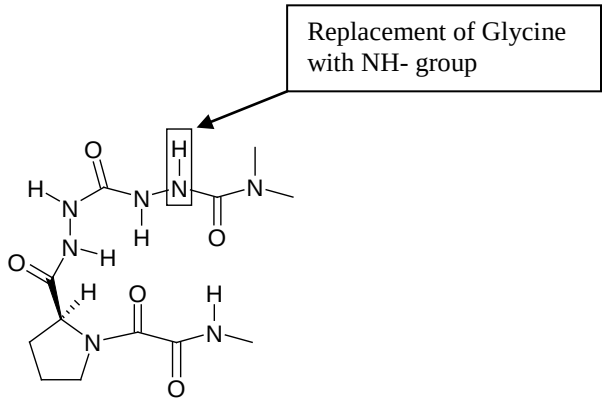
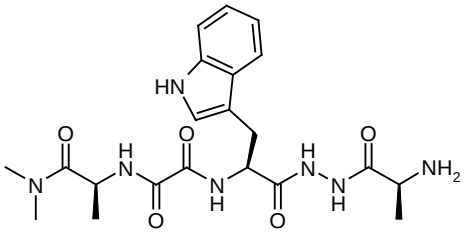


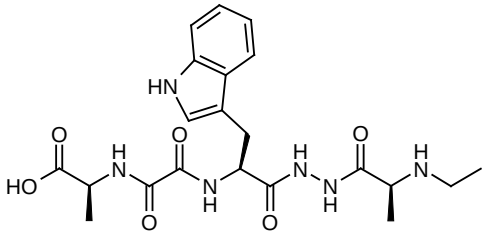
Figure: NXO β-turn mimic with carbohydrazide substructure.

5. Modified tripeptides A and B

According to the calculated structure of β-sheet, two modified peptides were designed; the detailed synthetic route for the preparation of modified tripeptide (A) and modified tripeptide (B) is given in (Scheme 6 and 7).



Modified tripeptide (A)



Modified tripeptide (B)

6. Rearranged Product

In a methyl ester hydrolysis reaction using mild base like lithium hydroxide in methanol, an unexpected product is repeatedly observed and its structure was confirmed by NMR and an alternate synthetic route (Scheme 9). The new structure was initially named as "Rearranged Product" and was reported in [2009ECSOC1]

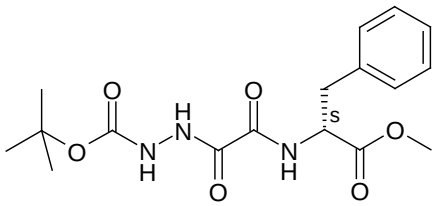


Figure: Structure of "Rearranged Product".

RESULTS AND DISCUSSION

1. NXO building blocks

A series of novel NXO-compounds were prepared according to the reported procedure [2010JOC2492] and using a number of natural and unnatural amino acids with different combinations of protecting groups on the oxalyl and hydrazide ends.

In a particular example of L-methionine a little difference in yields was observed. Maximum yield was obtained with a combination of *tert*-butyl and Fmoc (FK-29) on ends resulting in 78% of product, a combination of *tert*-butyl and Cbz (FK-32) resulted in 66% yield and a Boc and methyl ester protection (FK-76) gave 60% yield.

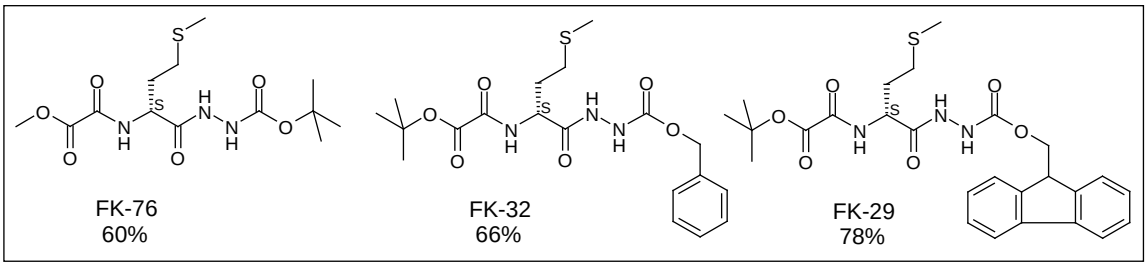


Figure: NXO-building blocks with L-methionine.

Two similar NXO building blocks were prepared FK-78 and FK-92. L-threonine was used in FK-78 with *tert*-butyl and Fmoc protection on ends resulted in 45% yield while the same NXO building block with D-threonine gave only 63% yield in my hands.

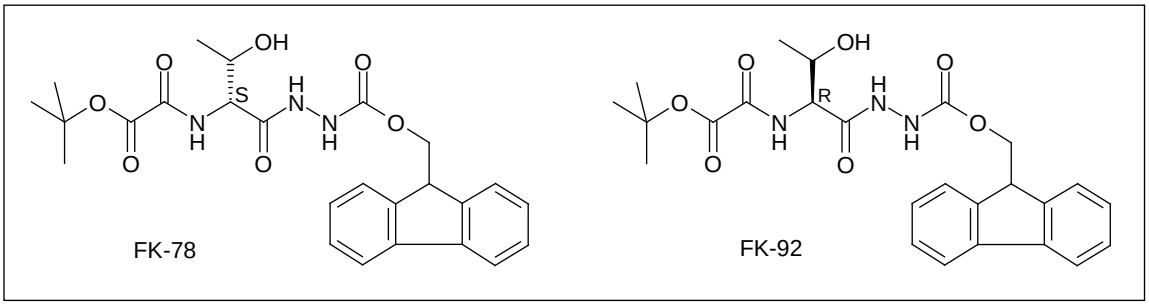


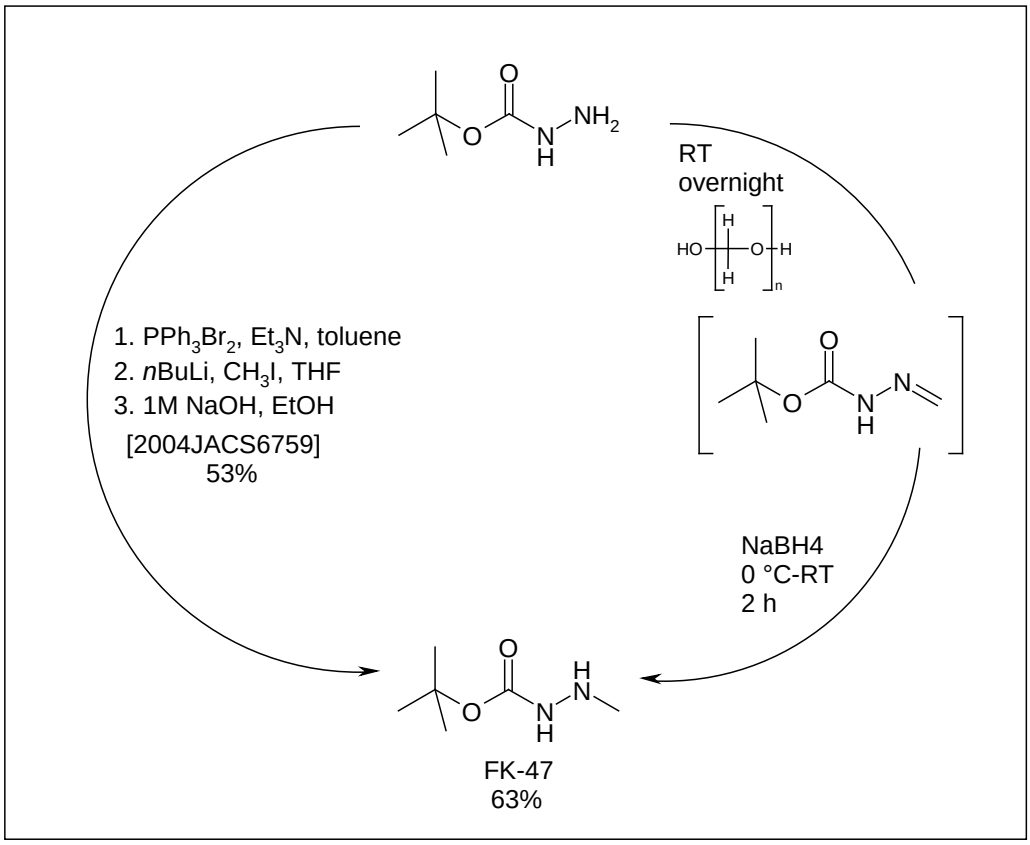
Figure: NXO-building blocks with L/D-threonine.

It was observed that NXO-compounds with a combination of methyl ester and Boc group at ends show low yields as compare to other combinations of protecting groups. NXO building blocks were synthesized in a range of 45-78% yield.

2. Synthesis of *N*-substituted hydrazines and their application in NXO building blocks.

2.1 Synthesis of *tert*-butyl *N*-(methylamino) carbamate (FK-47)

To synthesize FK-47 a reported procedure in [2004JACS6759] was found lengthy and was not cost effective. A reductive amination was carried out by reacting *tert*-butyl carbazate and paraformaldehyde and without separating the intermediate it was reduced by sodium borohydride to get the target compound (FK-47). It was observed that this compound partially decomposed when kept at room temperature so it is suggested to store it in refrigerator.



Scheme: Comparison of synthetic routes for FK-47.

FK-50, FK-57 & FK-58 were prepared according to the reported procedures [2008T6788; 2004JACS6759; 2007JMC4789] without any further modifications. FK-45 was prepared according to the reported procedure [1976JOC3805] but the reduction of double bond to get FK-46 did not work in my hands; some other reducing procedures like (1) hydrogenation using 10% w/w Pd/C in MeOH, (2) $NaBH_4$ in MeOH, (3) $NaBH(OAc)_3$ in MeOH were applied but unfortunately these reactions were not successful in my hands.

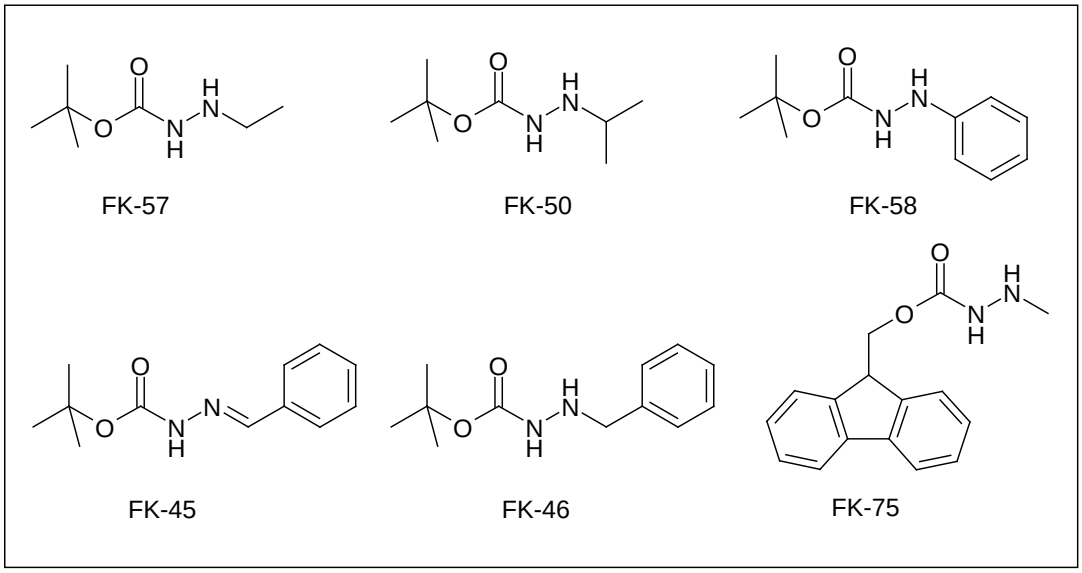


Figure: *N*-substituted hydrazines synthesized to use in NXO building blocks.

2.2 Fmoc-2-methylhydrazine (FK-75)

It was prepared with an improved yield of 75%, using the reported method [1999JOC7388; 64%].

N-substituted mono-protected hydrazines were incorporated in NXO building blocks, using reported DCC coupling method [2010JOC2492].

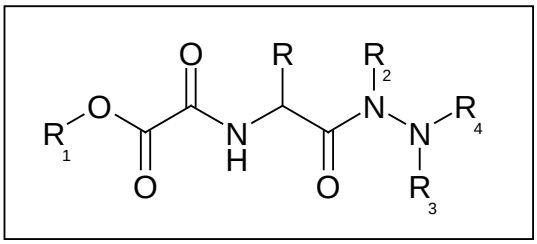


Figure: General formula for *N*-substituted NXO building blocks.

Novel N-substituted NXO building blocks were prepared and isolated as shown in the following table:

Sr.No.	R1	R2	R3	R4	R= amino acid	NXO-name	Lab Journal number	Isolated yield(%)
1	Me	CH_3	H	Boc	Phe	Boc-N(Me)PheO-OMe	FK-62	37
2	Me	CH_3CH_2	H	Boc	Phe	Boc-N(Et)PheO-OMe	FK-63	37
3	Me	$(CH_3)_2CH$	H	Boc	Phe	Boc-N(iPr)PheO-OMe	FK-52	35
4	Me	CH_3CH_2	H	Boc	Trp	Boc-N(Et)TrpO-OMe	FK-154	24
5	Bu^t	CH_3	H	Fmoc	Met	Fmoc-N(Me)MetO-OBu^t	FK-79	46

3. NXO beta-turn mimic

Structural modeling and Geometry optimization

Calculations were performed using Molecular Operating Environment, Version 2007.09 (MOE, © 1997-2007 Chemical Computing Group Inc.). input geometries were obtained from stochastic conformational searches employing the OPLS-AA forcefield potential parameters. Conformational space was explored with an energy cutoff of 20kcal using 0.0001Å Cartesian perturbation before 0.001Å RMS-gradient minimization with full dihedral minimization and bond rotation in 30° steps for a minimum of >100000 random geometries. The lowest energy conformers thus obtained were freed of restraints, partial charges on titratable groups adjusted according to standardized pK_A values and then subjected to Molecular Dynamics simulations in an NVT ensemble. Dynamics were run at 290K with a 0.001ps timestep, using the Nose-Poincaré-Anderson algorithm for solving the equations of motion. Pressure and temperature responses were set to 0.5 and 0.1ps relaxation time, respectively. Trajectory coordinates were stored at 0.5ps intervals for data analysis. The occurrence of a hydrogen bond was registered if the donor-acceptor distance was <3.5Å with a donor-hydrogen-acceptor angle of 90°<angle<180°.

β-turn having D-proline was calculated to be more suitable and stable by our molecular modeling calculation. The synthesis of NXO β-turn mimic was done according to (Scheme 1).

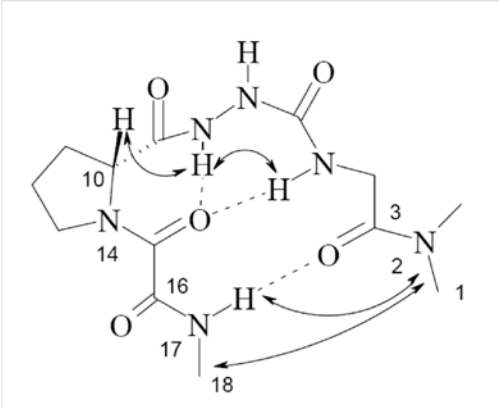
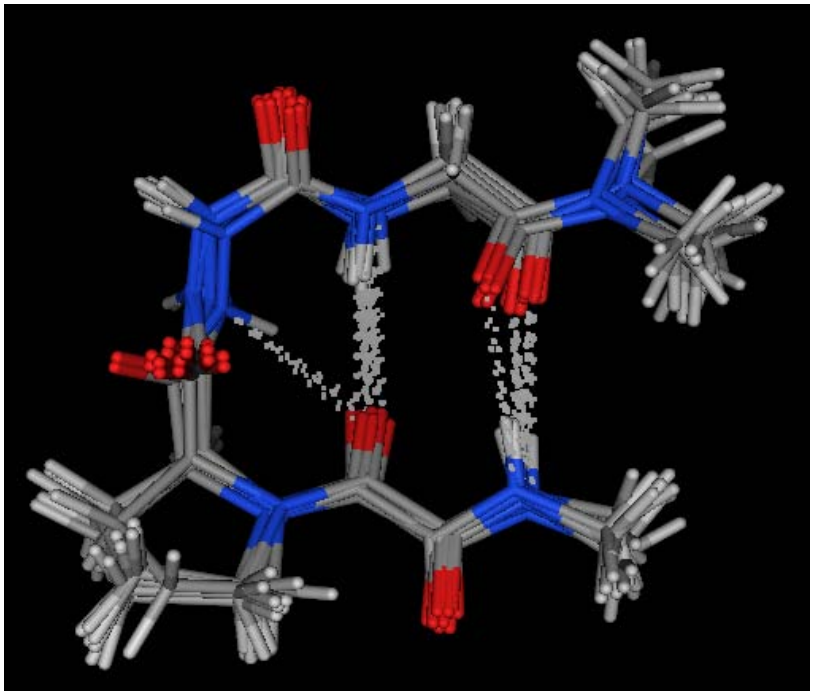
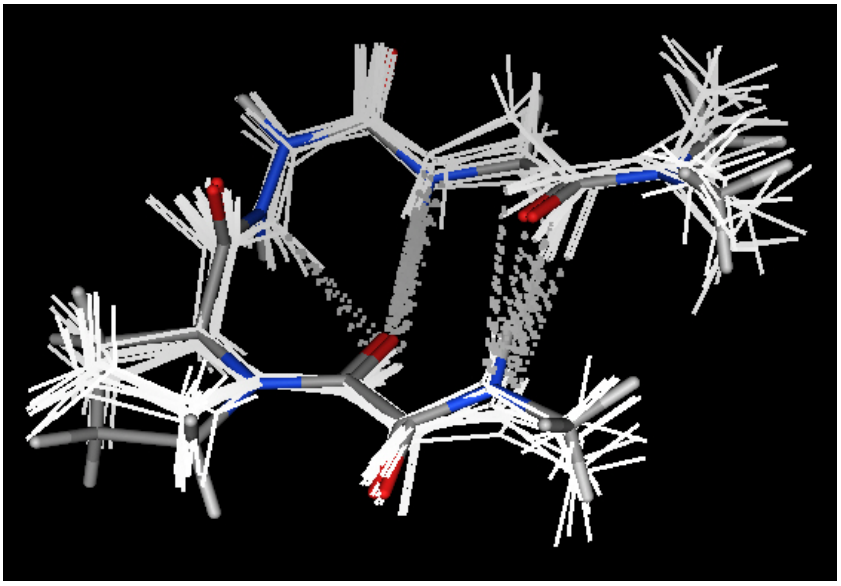


Figure: NXO β-turn (FK-166) indicating observed NOEs.

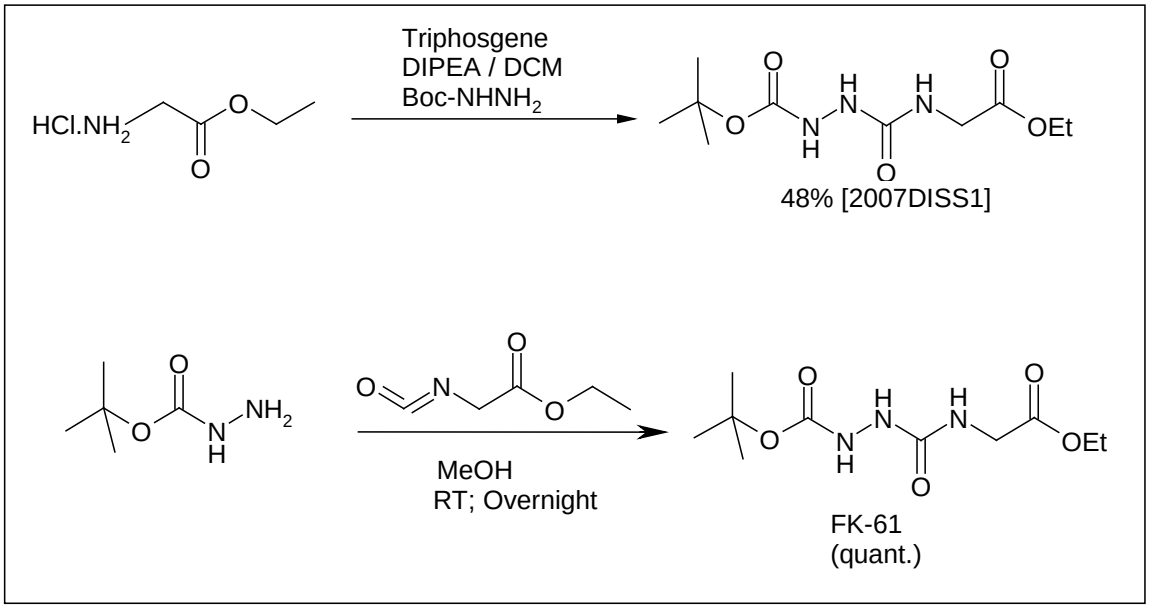


Picture: The ten energy-lowest geometries from a ROESY-restrained stochastic search within the OPLS-AA forcefield are shown superposed. Dotted lines indicate hydrogen bonding.

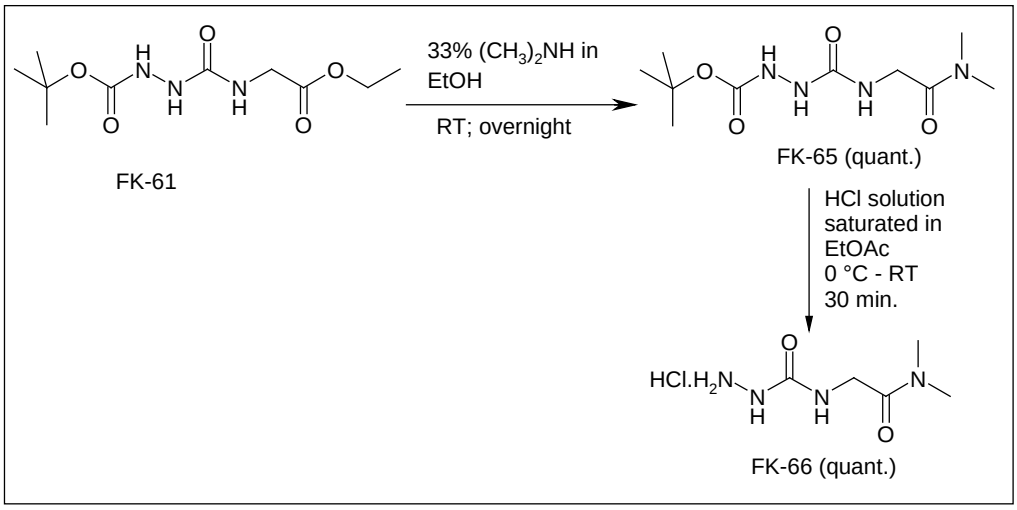


Picture: RMSD overlap of OPLS-AA in H2O mimimized (colored model) and the core RMSD region sampled in the 100 ns MD run at 290K at RMSD 1.02 -1.13. Dotted lines indicate hydrogen bonding.

FK-61 was prepared with an improved method using isocyanatoacetate and product was obtained in quantitative yield.

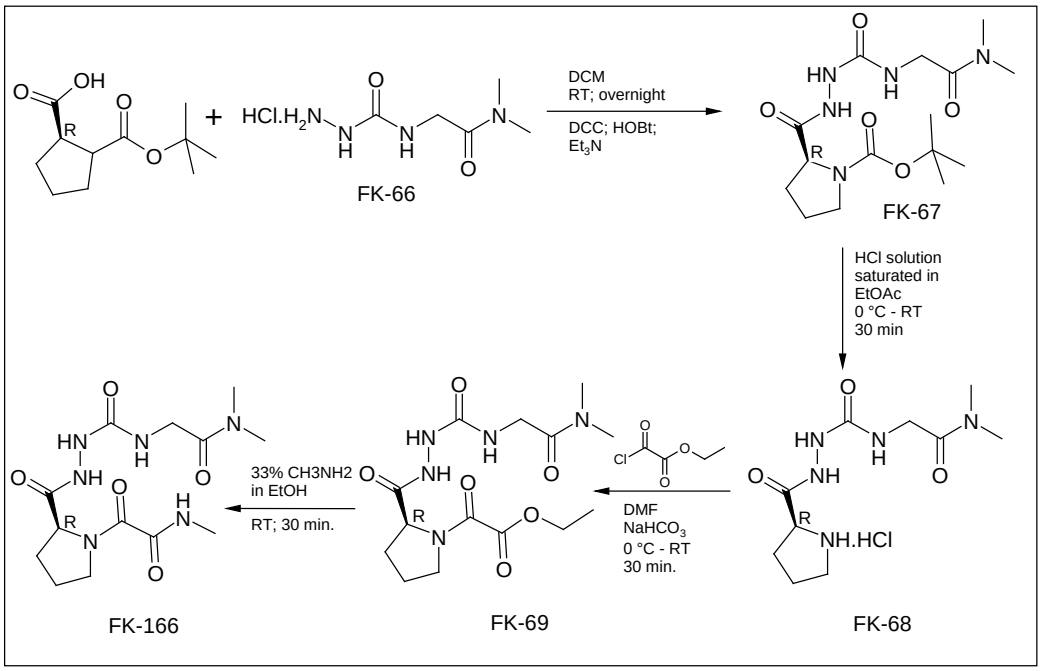


Subsequent treatment of FK-61 with 33% *N,N*-dimethylamine solution in ethanol followed by Boc-deprotection using hydrochloric acid solution saturated in EtOAc generated FK-66 in quantitative yield.



This hydrochloric salt of FK-66 was neutralized and coupled with Boc-Pro-OH to get FK-67 in 69% yield. Boc was removed once again with hydrochloric acid solution saturated in EtOAc to get FK-68 and this salt was neutralized with $NaHCO_3$ and reacted with ethyl oxalyl chloride but the resultant product FK-69 was reported to be unstable

[2007DISS1], so the reaction mixture (FK-69) was immediately reacted with 33% methyl amine solution in ethanol to get the target molecule FK-166.

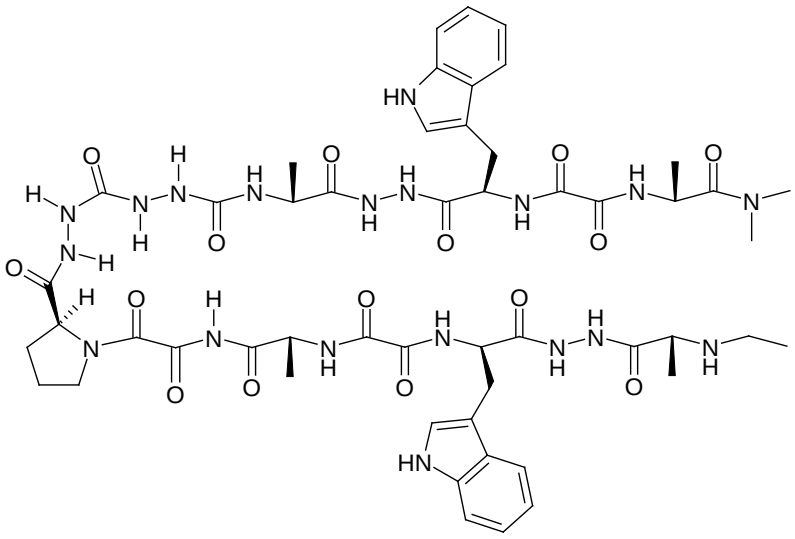


NMR Analysis of β-turn mimic FK-166

This beta turn mimic was analyzed using high resolution NMR to check the hydrogen bonding pattern. The results of NOESY experiments are summarized in table below. The ROESY and TOCSY spectra show cross peaks typical of β-turn especially cross peaks signals between 1 and 18. These spectra were recorded on 600 MHz NMR spectrometer at 295k in D2O.

^{1}H	Observed NOEs			
1	4		18	17
5	4		7	8
7	5		8	
8	10	13	7	5
17	18		1	
18	17		1	

4. NXO beta-sheet mimic



This project consisted of two parts:

(a) NXO β-turn mimic with carbohydrazide substructure

(b) Modified tripeptide (A) and (B)

4.1 NXO β-turn mimic with carbohydrazide substructure

A β-turn mimic with carbohydrazide substructure was aimed with two objectives:

(1) compare it with previously synthesized β-turn mimic FK-166

(2) incorporate it in NXO β-sheet mimic with some modifications

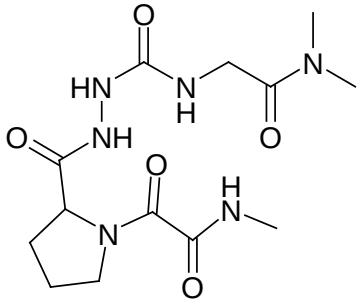


Figure: NXO β-turn mimic (FK-166)

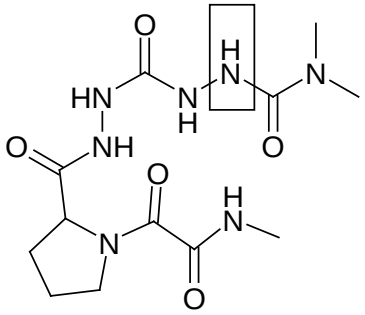


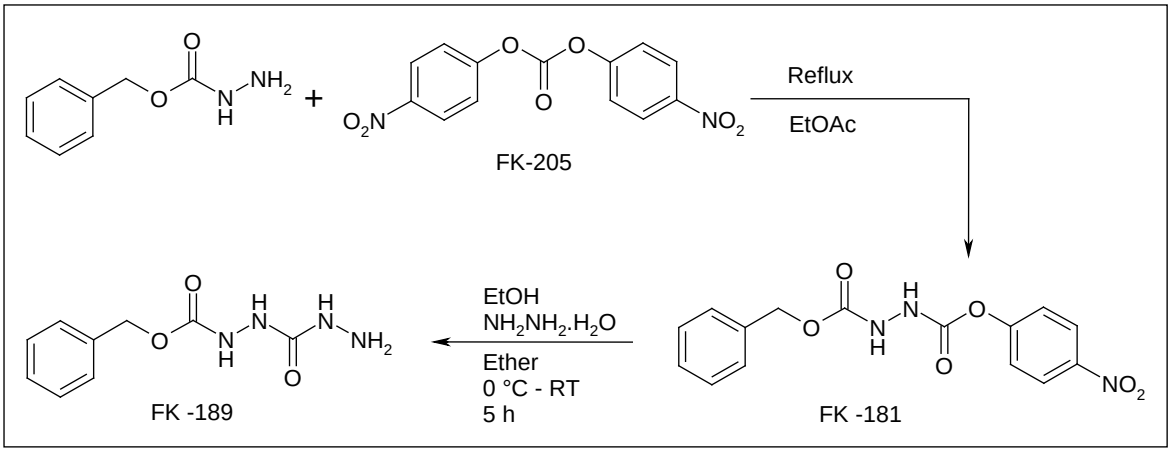
Figure: NXO β-turn mimic with carbohydrazide substructure

4.1.1 Synthesis of mono-protected carbohydrazide

In order to prepare an orthogonally protected carbohydrazide, a number of reagents were tried out which can incorporate a carbonyl group between two differently protected carbaztes. One reported method by Gante [1965CB3340; 71%] worked nicely for Cbz-carbohydrazide (FK-189) but with only 46% yield in my hands, this lower yield is might be a result of quality of hydrazine hydrate used (Gante used 100% hydrazine hydrate and it was approx. 60% in reaction FK-189), detailed synthetic route is shown in (Scheme 3).

4.1.1.1 Gante's route for synthesis of FK-189

A mono-protected carbohydrazide (FK-189) was prepared in a two step procedure reported by Gante [1965CB3340]. Bis-(*p*-nitrophenyl) carbonate was prepared as FK-205 according to reported procedure [2010AG(E)3049]. During the synthesis of FK-205, after filtering the target compound from the reaction mixture, an off-white solid was precipitated out and was insoluble in solvents like DCM, EtOAc and MeOH.



Scheme: Synthetic route for the preparation of mono-protected carbohydrazide.

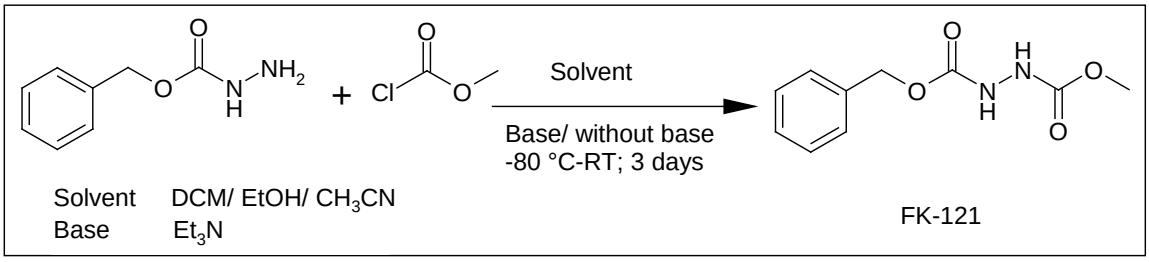
The compound FK-181 and FK-189 were synthesized and confirmed by 1H and 13C NMR and elemental analysis.

In order to increase the yield two reagents were tried out i.e. methyl chloroformate and *N,N*-carbonyldiimidazole.

Parallel screening reactions were carried out with temperature range of -80 °C to refluxing conditions with different solvents and also with and without base. Microwave conditions were also applied in some reactions. All the reactions were monitored on regular intervals by GCMS to check the presence of the target compound in reaction mixture.

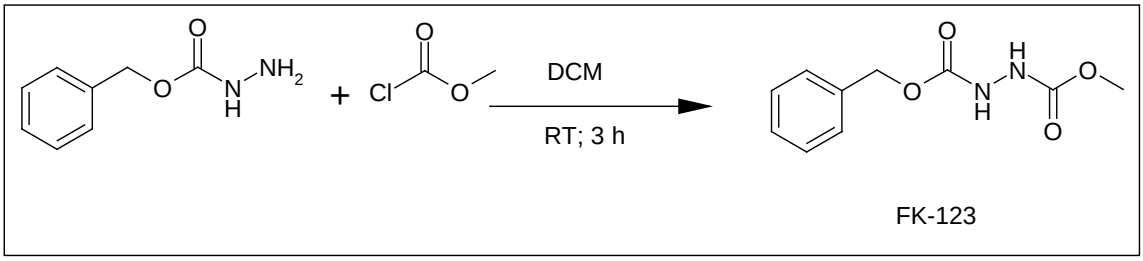
4.1.1.2 Methyl chloroformate reactions

Methyl chloroformate was used in a series of parallel reactions as shown below. Cbz-$NHNH_2$ was reacted with methyl chloroformate (1.0 equiv.) in solvents (DCM/CH_3CN/EtOH), with and without triethylamine (1.1 equiv.) as base and stirring at -80 °C to room temperature for 3 days with constant monitoring of samples with GCMS.



Sr.No.	Lab code	Reaction conditions	Remarks
1	FK-121-1	DCM & Et_3N	Mass peak of the target compound was observed in GCMS
2	FK-121-2	DCM	Mass peak of the target compound was observed in GCMS
3	FK-121-3	EtOH & Et_3N	A mixture was observed in GCMS
4	FK-121-4	EtOH	A mixture was observed in GCMS
5	FK-121-5	CH_3CN & Et_3N	Mass peak of the target compound was observed in GCMS
6	FK-121-6	CH_3CN	Mass peak of the target compound was observed in GCMS

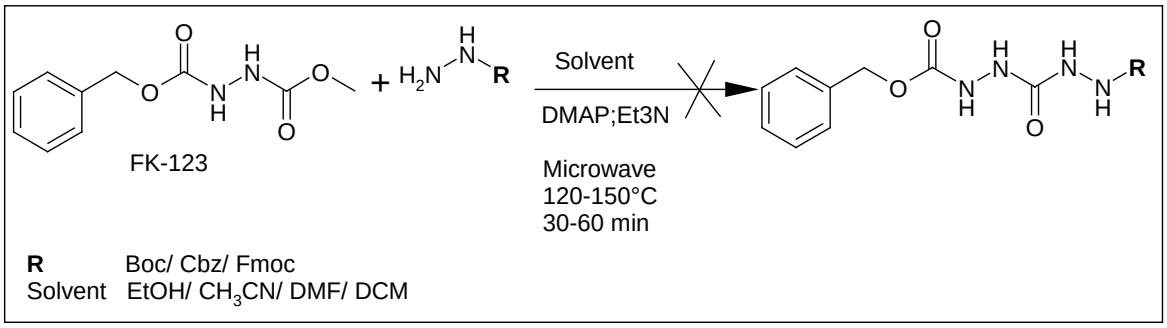
It was observed that the best conditions for this reaction were DCM as solvent, without triethylamine as a base and the reaction was completed in 3 hrs at room temperature.



The target compound (FK-123) was obtained in an amount of 3.2 g in 48% yield and was used for the second addition of protected hydrazide.

4.1.1.3 Addition of mono-protected carbazate to FK-123

A second series of parallel reactions was carried out to optimize reaction conditions for a reaction between FK-123 and mono-protected carbazates (Boc/Cbz/Fmoc) using different solvents (EtOH/CH_3CN/DMF), with or without triethylamine as base under room temperature to reflux. Microwave reactions were also tried out, but unfortunately all the attempts were failed to get the target compound, only starting material peaks were observed in GC-MS analysis.



A series of reactions conducted using FK-123 with different reaction conditions are summarized in the following table:

Sr.No.	Lab code	Reaction conditions	R	Remarks
1	FK-129-1	DCM; RT;12 h	Boc	Only peaks corresponding to starting materials were observed in GC/MS analysis and no new peak was observed.
2	FK-129-2	CH_3CN; RT;12 h	Boc	
3	FK-130-1	DCM; RT;12 h	Cbz	
4	FK-130-2	CH_3CN; RT; 12 h	Cbz	
3	FK-131-1	DCM; RT; 12 h	Fmoc	
4	FK-131-2	CH_3CN; RT; 12 h	Fmoc	
5	FK-133-1	EtOH; RT-reflux; 24 h Et_3N; DMAP	Cbz	
6	FK-133-2	CH_3CN; RT-reflux; 24 h Et_3N; DMAP	Cbz	
7	FK-133-3	EtOH; RT-reflux; 24 h	Cbz	
8	FK-133-4	CH_3CN; RT-reflux;24 h	Cbz	
9	FK-134-1	EtOH; RT-reflux; 24 h Et_3N; DMAP	Boc	
10	FK-134-2	CH_3CN; RT-reflux; 24 h Et_3N; DMAP	Boc	
11	FK-134-3	EtOH; RT-reflux; 24 h	Boc	
12	FK-134-4	CH_3CN; RT-reflux; 24 h	Boc	
13	FK-142-1	EtOH; 130-150 °C; 60 min microwave	Cbz	
14	FK-142-2	CH_3CN; 130-150 °C; 60 min microwave	Cbz	
15	FK-142-3	DMF; 130-150 °C; 60 min microwave	Cbz	

4.1.1.4 *N,N*-Carbonyldiimidazole reactions

Displacement of imidazolyl moiety of *N,N*-carbonyldiimidazole by *tert*-butyl carbazate was reported [2000TL1159; 1992JACS3156; 2000OL19]; keeping this evidence in view, a series of parallel reactions were planned and performed in one pot fashion using Cbz-$NHNH_2$ (1.0 equiv.) and *N,N*-carbonyldiimidazole (1.0 equiv.) in solvents (DCM/CH_3CN/EtOH), with and without triethylamine (1.1 equiv.) as base and stirring at -15 °C to room temperature for 72 hrs. Reactions were constantly monitored with GCMS. It was observed that starting material was completely converted into product and base has no effect on reactivity.

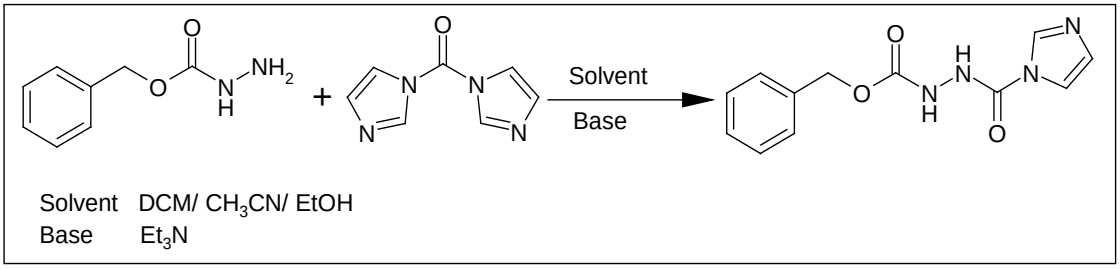
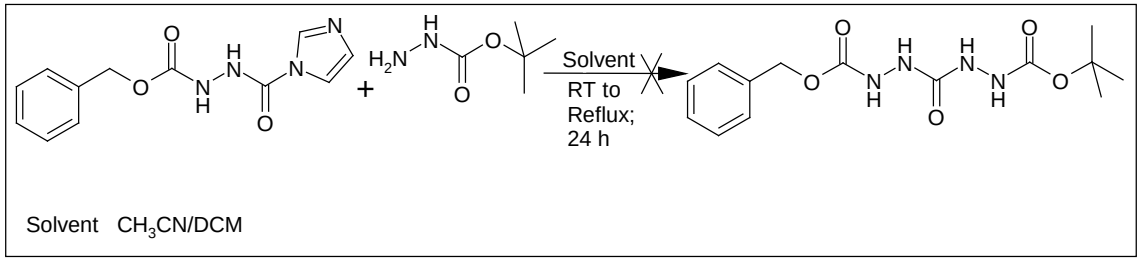


Fig: Reaction of *N,N*-carbonyldiimidazole with protected hydrazine.

Sr.No.	Lab code	Reaction conditions	Remarks
1	FK-152-1	DCM; Et3N -15 °C-RT; 72 hrs	GC-MS analysis showed complete consumption of reactants and a new species was observed.
2	FK-152-2	EtOH; Et3N -15 °C-RT; 72 hrs	A mixture was observed.
3	FK-152-3	CH3CN; Et3N -15 °C-RT; 72 hrs	GC-MS analysis showed complete consumption of reactants and a new species was observed.
4	FK-152-4	THF; Et3N -15 °C-RT;72 hrs	A mixture was observed.
5	FK-152-5	DCM; -15 °C-RT; 72 hrs	GC-MS analysis showed complete consumption of reactants and a new species was observed.
6	FK-152-6	EtOH; -15 °C-RT; 72 hrs	A mixture was observed.
7	FK-152-7	CH_3CN; -15 °C-RT; 72 hrs	GC-MS analysis showed complete consumption of reactants and a new species was observed.
8	FK-152-8	THF; -15 °C-RT; 72 hrs	A mixture was observed.

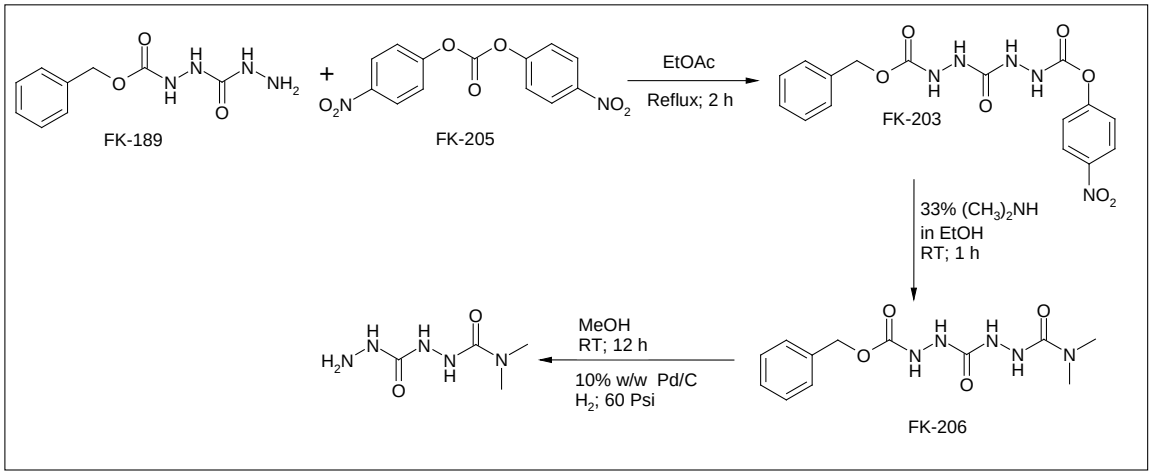
GC-MS analysis for reactions conducted in DCM or CH3CN and stirring at room temperature for 72 hrs showed a complete consumption of the starting materials. It was also observed that the base has no effect on these reactions.

These reaction mixtures were subjected to addition of second protected carbazate without isolation or purification of product. This addition was planned to carried out at elevated temperature so chloroform was used instead of DCM; *tert*-butyl carbazate was added to the reaction mixtures and were heated to reflux. GC-MS results showed a species formed whose mass peak was missing but characteristic peaks of *tert*-butyl and benzyl groups were observed. This species was isolated but it was not the target compound according to NMR and elemental anaylsis.



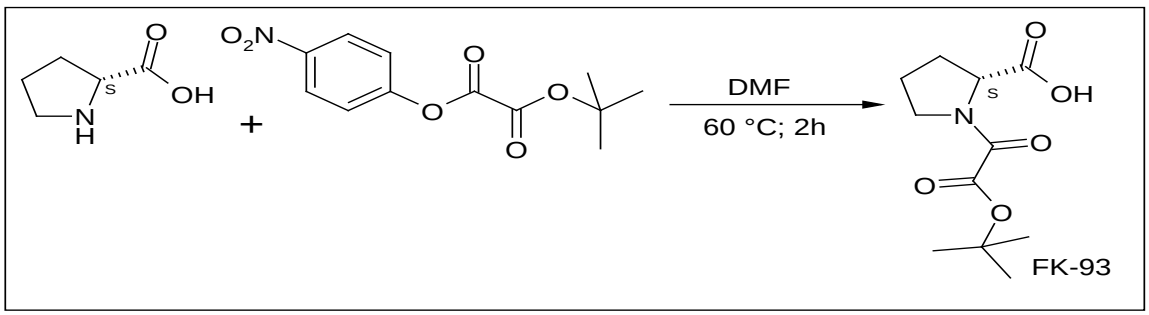
Synthesis of Benzyl *N*-[(dimethylcarbamoylamino)carbamoylamino]carbamate (FK-206)

A reaction of mono-protected carbohydrazide FK-189 with Bis-(*p*-nitrophenyl) carbonate to get FK-203 followed by stirring with 33% *N*,*N*-dimethylamine solution in ethanol should result in FK-206 but this reaction resulted into a inseparable mixture. Final step of this synthetic route was the deprotection of Cbz group using Pd /C; H2; 60 psi at room temperature. Detailed route is shown in (Scheme 3).



4.2.1 Synthesis of D and L-Proline derivatives with *N*-oxalyl functionality.

A proline compound having oxalyl group attached to nitrogen was the second part of the NXO beta-turn mimic with carbohydrazide substructure. A novel L-proline analogue (FK-93) was prepared which on subsequent Boc group deprotection followed by coupling with modified tripeptides (A) and (B) could result in a β-sheet mimic.



A second proline analogue FK-188 was synthesized to use in NXO beta-turn mimic with carbohydrazide substructure for comparison with beta-turn mimic FK-166.

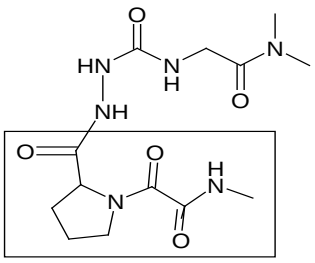


Fig: NXO β-turn mimic (FK-166)

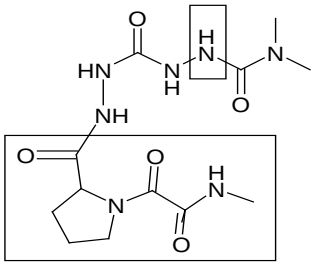
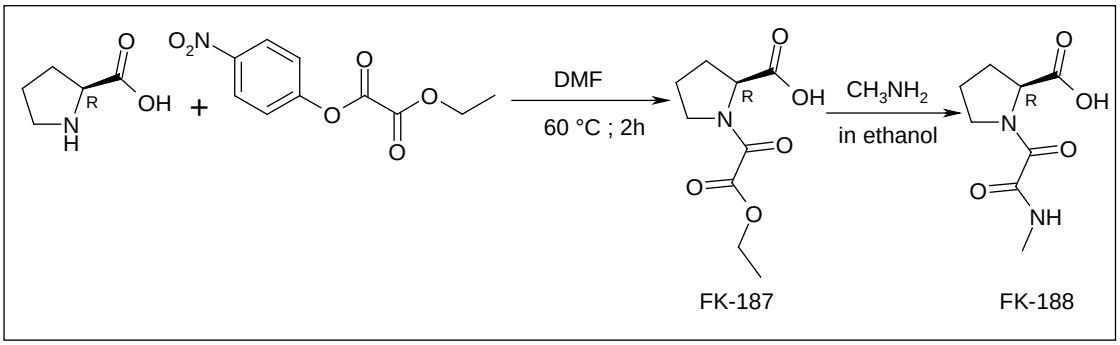


Fig: NXO β-turn mimic with carbohydrazide substructure

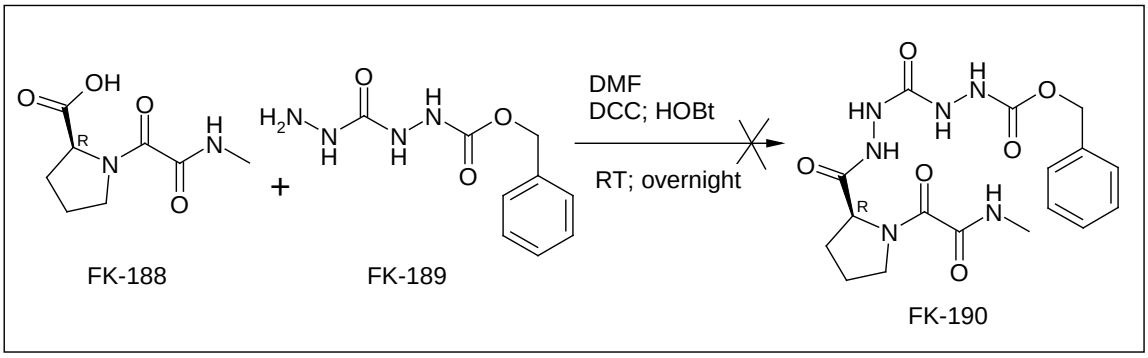
One novel D-proline derivative with oxalyl moiety attached to nitrogen (FK-187) was prepared and reacted with 33% methylamine solution in ethanol to get FK-188.



Scheme: Synthetic route for FK-188.

4.3 Coupling reaction of FK-188 and FK-189

Compounds FK-188 and FK-189 were subjected to DCC coupling conditions to get FK-190 but the target compound was not found in the reaction mixture.



In this reaction an intra-molecular coupling to form a six membered ring cannot be ruled out completely. In FK-188 a bond formation between hydroxyl group and NH group can possibly result in a six membered ring structure but this product was not present in the reaction mixture.

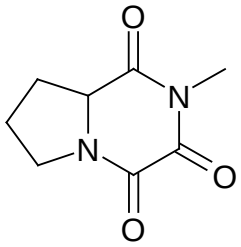


Figure: A proposed six membered ring structure in FK-190.

Unfortunately the NXO β-turn mimic with carbohydrazide substructure could not be synthesized.

Second part of NXO β-sheet mimic was synthesis of two modified tripeptides (A and B as shown in Scheme 6 and 7 respectively).

4.4 Modified tripeptide (A) [$AlaNH_2$-NTrpO-AlaN$(Me)_2$]

Modified tripeptide (A) was designed according to the modeled structure of NXO β-sheet mimic, containing two D-alanine and one L-tryptophan. Detailed synthetic route of this modified tripeptide (A) is shown in (Scheme 6).

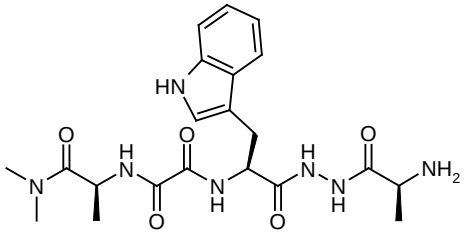
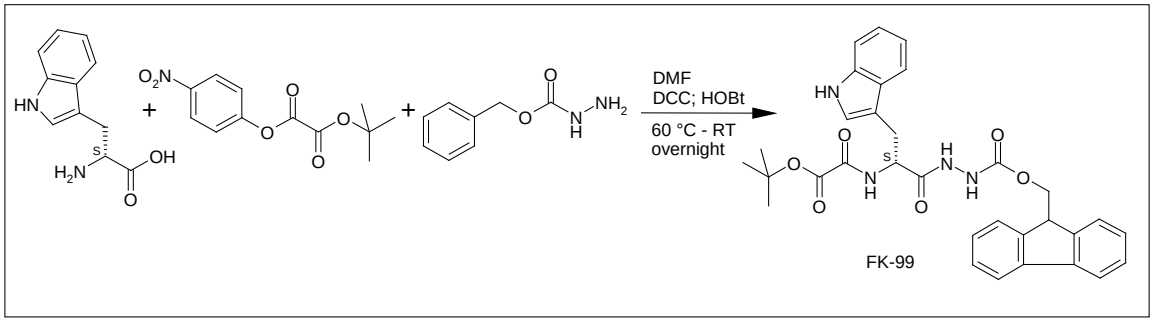
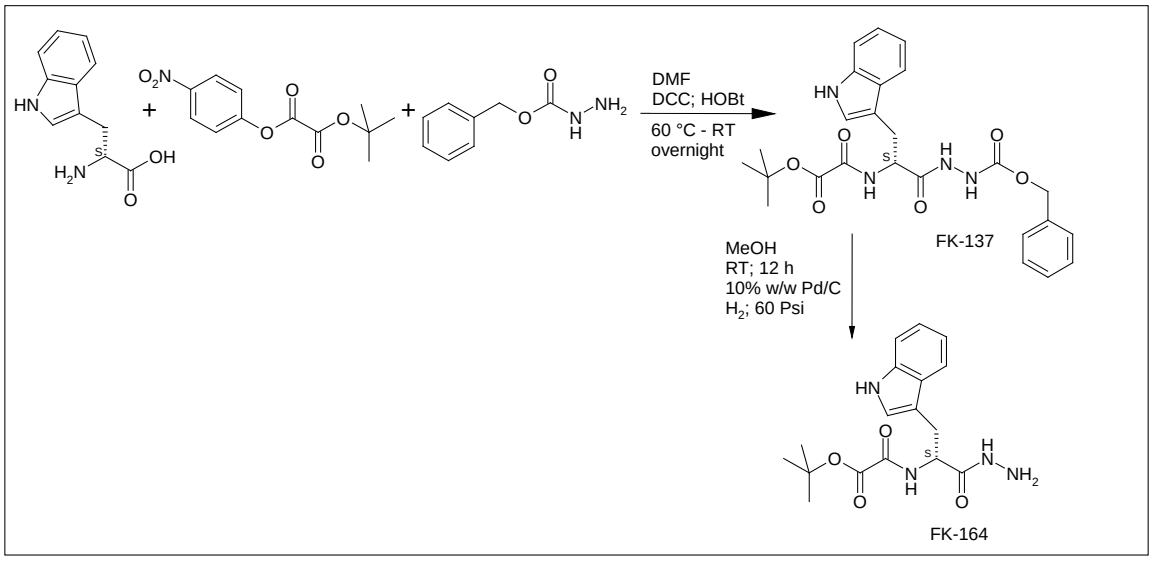


Figure: Modified tripeptide (A) [$AlaNH_2$-NTrpO-AlaN$(Me)_2$].

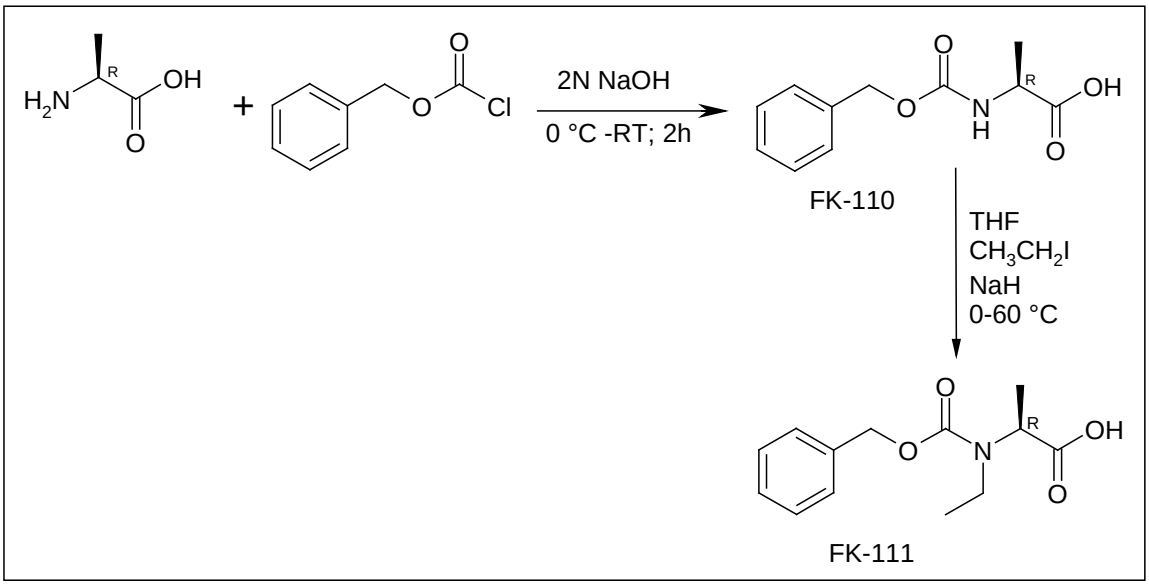
Initially an NXO building block of L-tryptophan (FK-99) was prepared with Fmoc and *tert*-butyl ester on ends. But due to two limitations it was not used in next steps of synthetic route. First was a deprotection reaction of Fmoc group which resulted in a mixture with many impurities, second was bulky Fmoc group itself and its deprotection was a major loss in the total amount of the product.



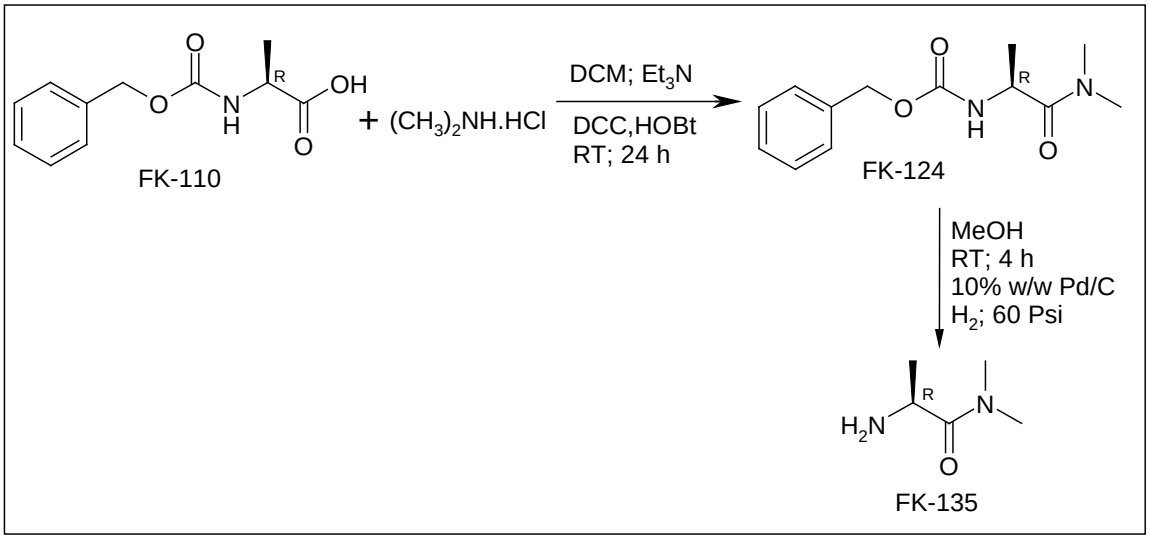
An NXO building block FK-137 was prepared with Cbz and *tert*-butyl ester on ends. Cbz group was later deprotected according to the reported method [2003BMC2715] and FK-164 was obtained in 93% yield.



Z-D-Ala-OH (FK-110) was prepared according to reported procedure in [2008JMC6371] and product was used to prepare an *N*-ethyl derivative (FK-111) according to [2008TA970]; pH 4 was critical in the extraction of the product (FK-111) which was maintained by adding aqueous citric acid, this reaction gave only 36 % yield in my hands.

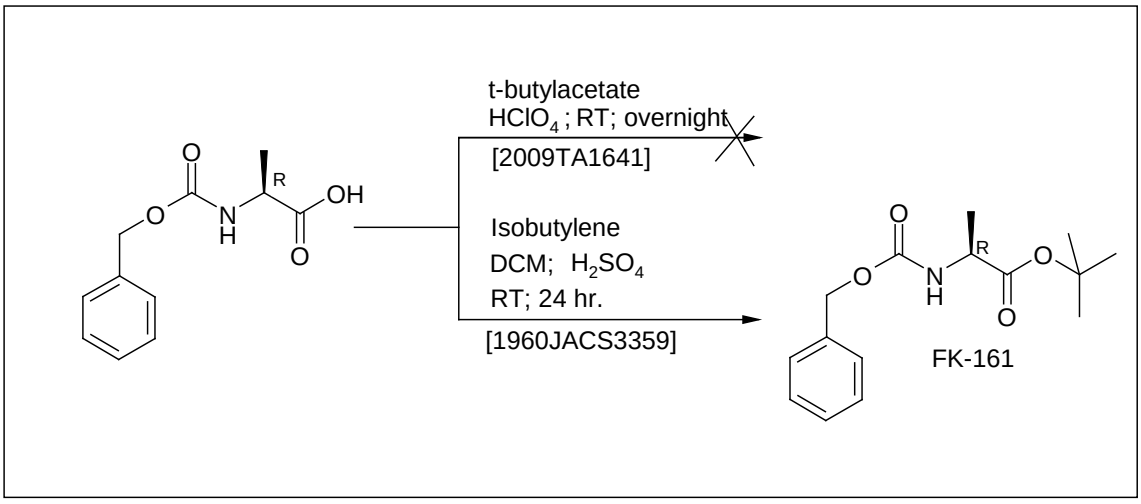


Z-D-Ala-OH was used to synthesize an *N*,*N*-dimethyl derivative (FK-124) according to reported procedure in [2003OBC965] followed by Cbz deprotection using Pd/C hydrogenation method and resulted in FK-135.



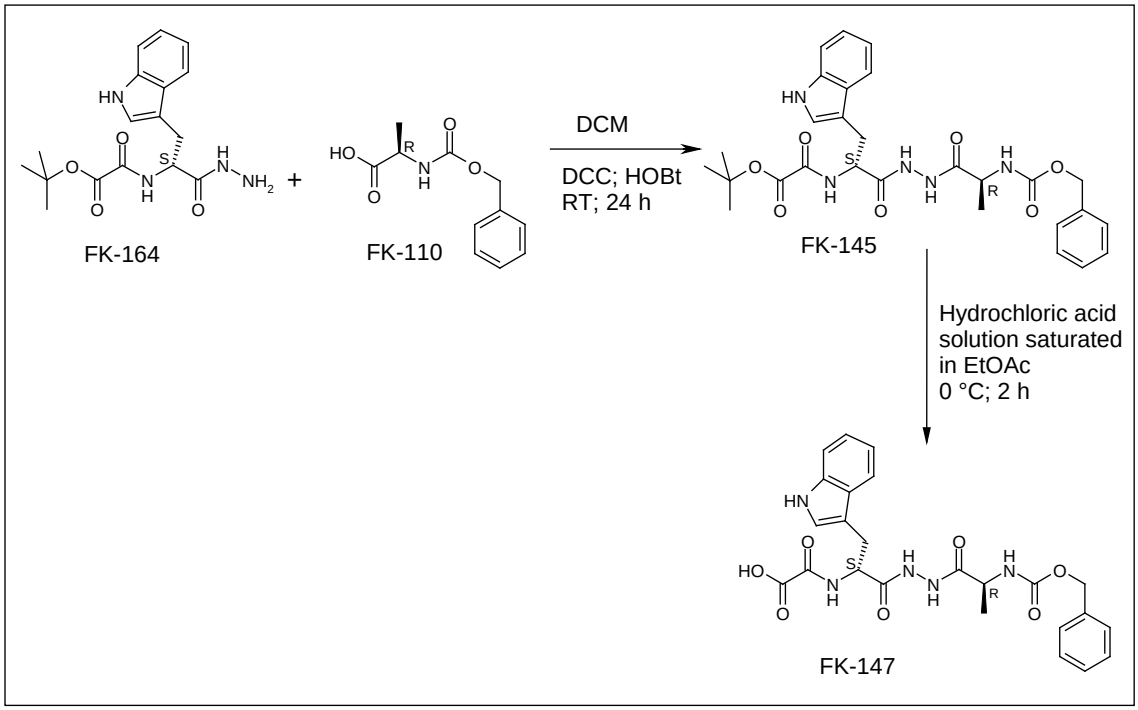
4.4.1 Synthesis of Cbz-Ala-Bu^t (FK-161)

In an attempt to synthesize *tert*-butyl ester of Z-D-Alanine following literature methods were tried out [2006JMC7215; 2009TA1641;1988US4730006], but they did not work in my hands. Finally a method reported in [1960JACS3395] was used and successfully resulted in FK-161.

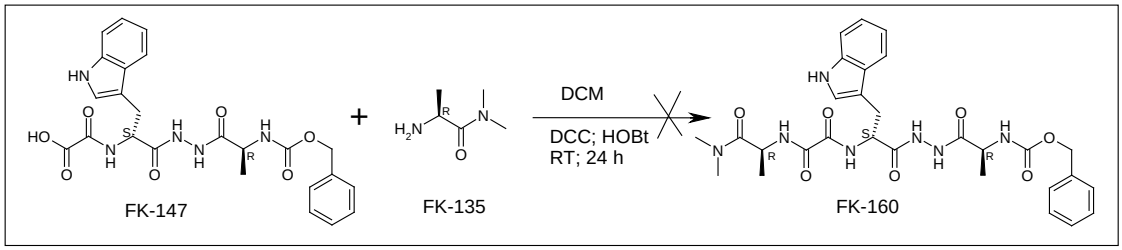


4.4.2 Coupling reactions for synthesis of modified tripeptide A

In an attempt to synthesize a dipeptide NXO building block, FK-164 and FK-110 were coupled using DCC coupling method and resulted in FK-145 with only 25% yield. Subsequent deprotection of *tert*-butyl ester using hydrochloric acid solution saturated in EtOAc gave compound FK-147 in quantitative yield.

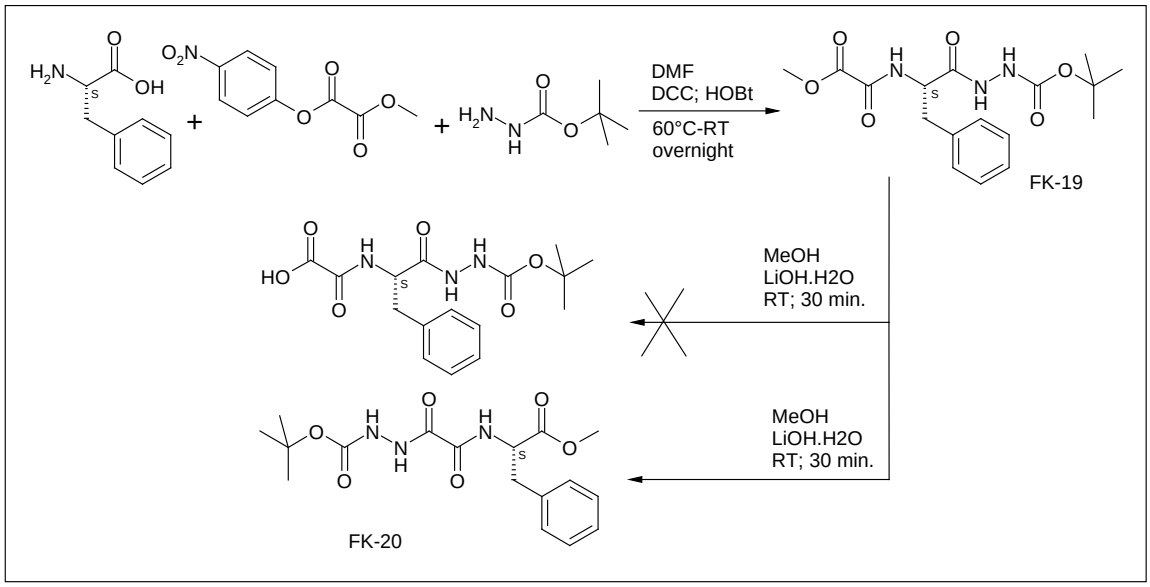


In order to synthesize a modified peptide (A), FK-147 was coupled with FK-135 but the reaction resulted in an unidentified solid product which was insoluble in most of the common NMR solvents (CDCl3, MeOD, DMSO and CF3COOD) and a mass peak on high resolution mass was not found.

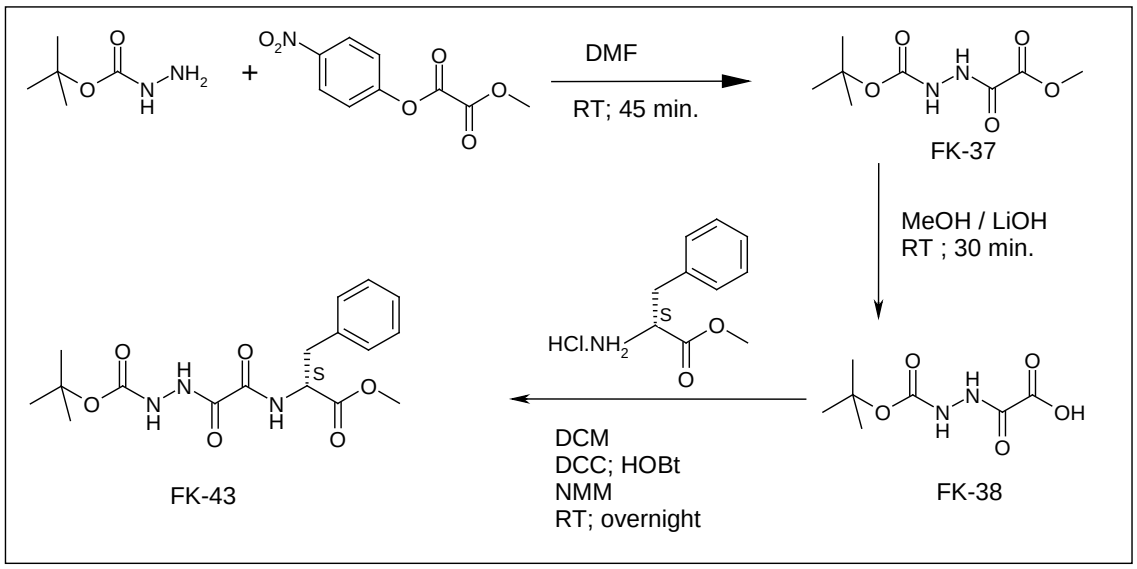


5. Rearranged Product

During a methyl ester hydrolysis reaction of Boc-NPheO-OMe (FK-19) using lithium hydroxide as mild base and methanol as solvent, a side product (FK-20; 57%) was observed and isolated; its structure was established by NMR analysis.



An alternate synthetic route was followed to confirm the structure, details are as shown below:



This new species was called “Rearranged Product” and was published in a poster [2009ECSOC1]. The exact mechanism is still not known but some new facts have been observed.

To understand the mechanism of the reaction NXO building blocks of L-phenylalanine were prepared with different protecting groups as shown in figure:

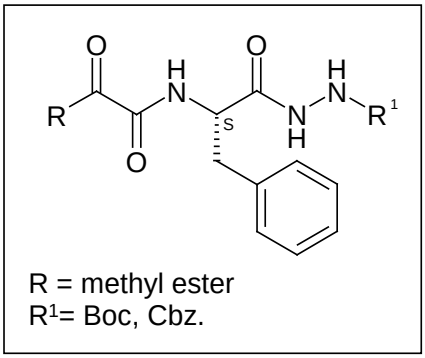
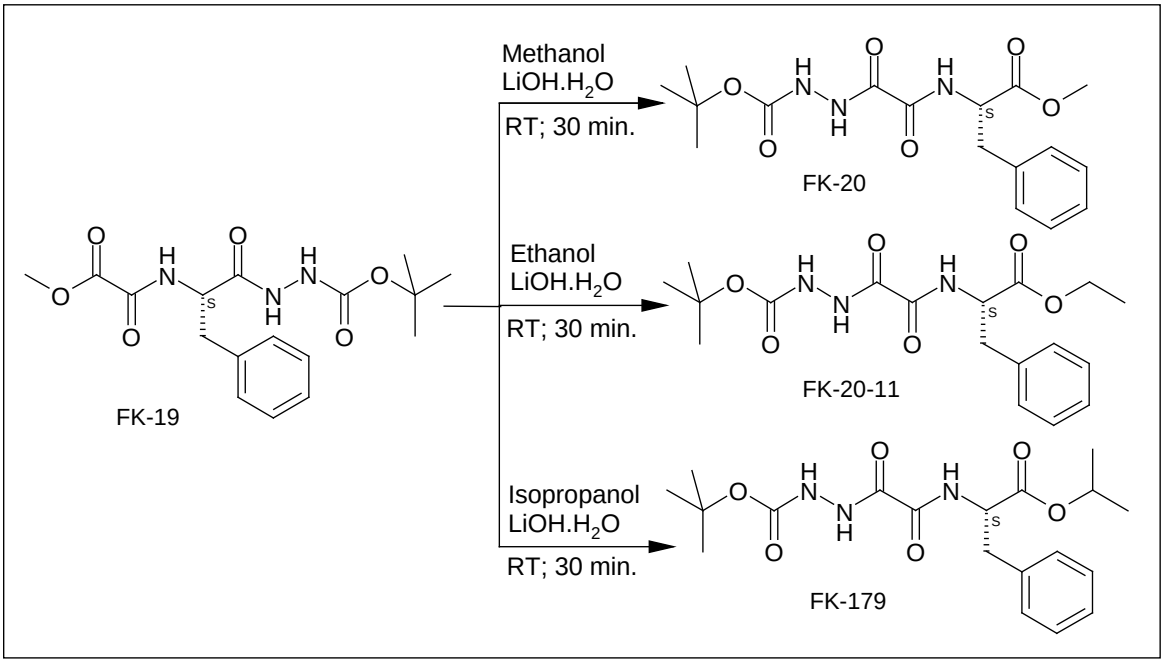
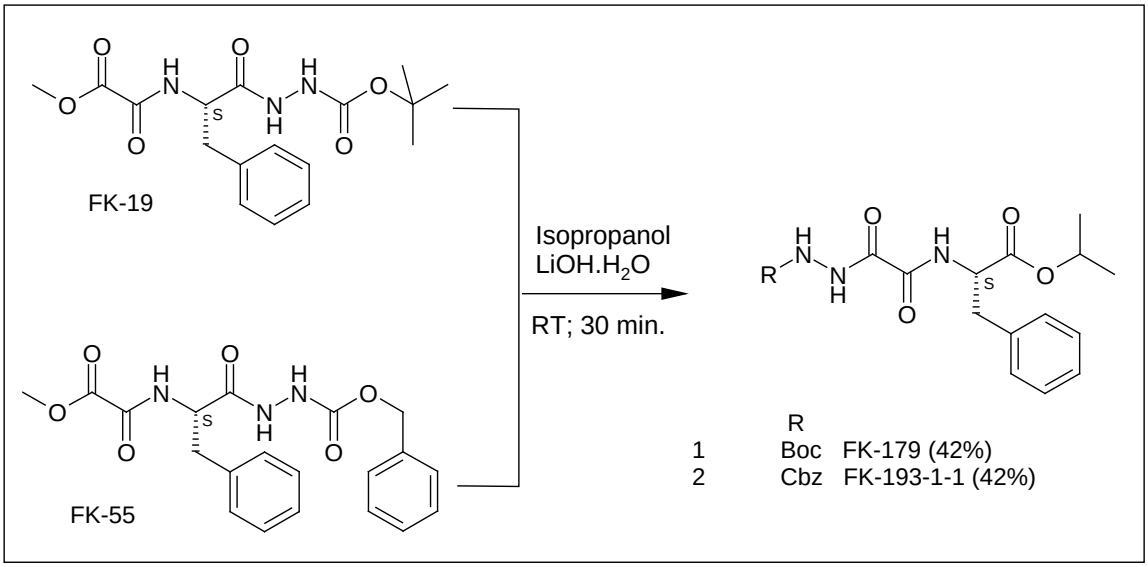


Figure: NXO building blocks of L-phenylalanine

FK-19 was hydrolyzed using different solvents. In a hydrolysis reaction using ethanol as a solvent a product FK-20-11 was isolated and in 13C NMR carbon signals of ethyl ester were observed, in another hydrolysis reaction using isopropanol as solvent a product FK-179 was isolated with carbon signals of isopropyl ester in 13C NMR.

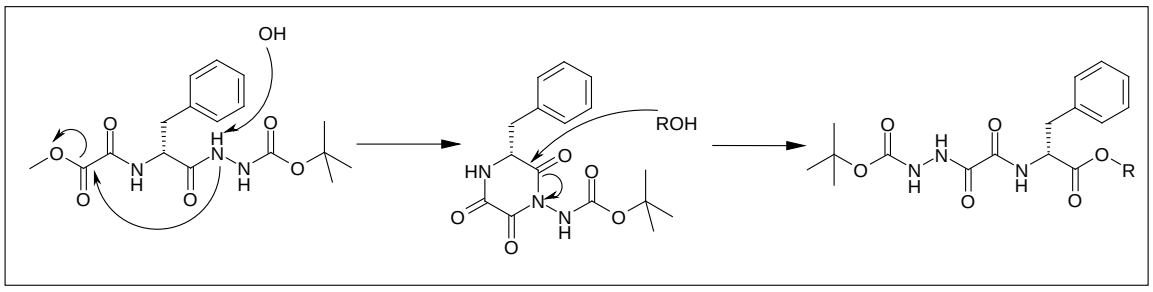


To check the effect of protecting groups on this rearrangement, two hydrolysis reactions were performed with different NXO building blocks of L-phenylalanine using isopropanol as solvent, results of these reactions are summarized in following scheme:



From these experiments it is evident that the ester part in the product came from the alcohol used as solvent during hydrolysis reaction, secondly different protecting groups has no effect on this rearrangement in terms of yields.

A proposed mechanism is shown for this rearrangement:



Experimental Part

All solvents used in reactions were dried over molsieves and are of analytical grade. Melting points were measured on a Büchi B-545 automated melting point apparatus, sometimes with visual determination. NMR spectra was measured on (1) a BrukerAC-200 (200MHz), or (2) Bruker Spectrospin 400 Ultrashield^{TM} 400MHz, pulsed Fourier-transform NMR spectrometer in $CDCl_3$, DMSO-d_6 or MeOD. Chemical shifts were reported relative to the resonance of tetramethylsilane (TMS).
HPLC was performed on Waters Alliance 2695 instrument with Merck Chromolith RP-18e 100-3 column, 100 x 3.0mm (UM6038/017). The two solvent systems employed in all HPLC measurements was:

A (97% water and 3% acetonitrile + 0.1 formic acid (added to the water/acetonitrile solution).
B (97% acetonitrile and 3% water + 0.1 formic acid (added to the water/acetonitrile solution).

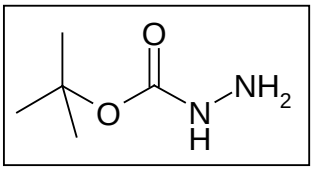
HPLC samples were detected with a Waters 996 Photodiode Array detector at the wavelength 254 $\pm$ 15 nm or a PL-ELS 1900 evaporative light-scattering detector. HPLC purity reported is for the number generated for the peak area as calculated using the Waters Millennium software with the Maxplot option for the UV maximum of the corresponding peak.

Chromatography was performed on a Büchi MPLC with a C-630 UV monitor, using 40-63µm flash silica columns. For thin layer chromatography (TLC) Merck TLC aluminium sheets (Silica 60 F_{254}, 0.25 mm) were used. TLC plates were visualized by UV light at 254 and 366 nm or with spray reagents (molybdophosporic acid, ninhydrin, anisaldehyde in sulphuric acid, iodine on silica and concentrated sulphuric acid in ethanol and with or without heating).
Hydrogenation was carried with a Parr Hydrogenation apparatus using 500 mL glass Parr bottle.

Trifluoroacetic acid and oxalyl chloride were purchased from ACROS ORGANICS. Amino acids and coupling reagents were mainly purchased from IRIS Biotech. I would like to thank Senn Chemicals for gift of D and L-Proline, D and L-Boc-Pro-OH and D-Alanine, for beta-turn and beta-sheet mimic project.

1. Synthesis of NXO building blocks

tert-Butyl carbazate (FK-148)

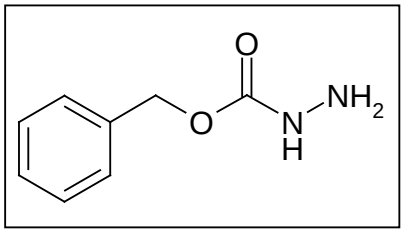


Hydrazine monohydrate (75%, 201.80 g; 4032 mmol) was mixed with isopropanol (800 mL) at 0 °C, then a solution of Boc_2O (400.00 g; 1832 mmol) in isopropanol (600 mL) was added drop wise. The reaction mixture turned cloudy upon addition and stirring was continued at room temperature for 2 hrs. The solvent was removed and the residue was dissolved in DCM and dried over $MgSO_4$. Solvent was removed under reduced pressure and the residue was re-crystallized from hexane, resulting in the title compound as 179 g (74%) of colourless crystals.

m.p.	38-40°C	Lit. m.p. 36-37 °C
		Lit. Yield 75%

Ref: [2008T6788]

Benyzl carbazate (FK-36)



To a mixture of hydrazine monohydrate (75%, 102.7 g; 2050 mmol) and diethyl ether (1L) was added benzyl chloroformate (58.58 mL; 410 mmol) in diethyl ether (400 mL) at 0 °C with stirring over a period of 2 hrs. The mixture was stirred overnight and solvent was evaporated under reduced pressure. Water (500 mL) was added to the residue. The aqueous phase was extracted with EtOAc. The combined organic layer was washed with brine and dried over anhydrous sodium sulphate. Solvent was removed to give crude compound which was purified by column chromatography to give 40 g (59%) of the target compound as a white solid with HPLC-purity 99%.

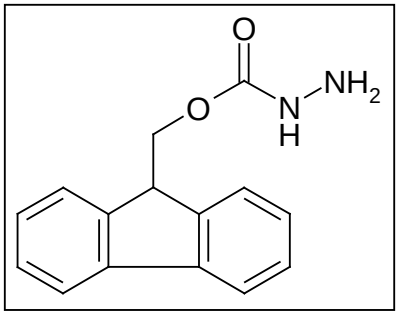
m.p. 68-70 °C Lit. m.p 69-70 °C

Ref: [2000CJC942]

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 7.35 (s, 5H), 6.38 (br.s, 1H), 5.14 (s, 2H), 3.76 (br.s, 2H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 158.62, 136.05, 128.55, 128.31128.21, 67.27.

Fmoc-carbazate (FK-100)

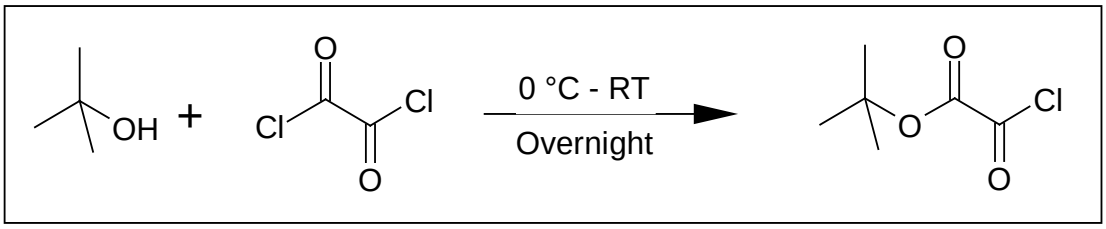


To a mixture of $N_2H_4.H_2O$ (75%, 48.38 g, 966 mmol) and diethyl ether (1500 mL) was added drop wise with stirring and ice-bath cooling a solution of 9*H*-fluoren-9-ylmethyl chloroformate (50.00 g, 193 mmol) in ether (500 mL) over a period of 5 hrs. The mixture was stirred at room temperature for 12 hrs under argon. Solvent was evaporated under reduced pressure, and water (1000 mL) was added to the mixture. The residual solid was filtered and washed with water. The crude compound was crystallised from EtOAc to give 45.00 g (91%) of product as a white solid with HPLC-purity 95%.

m.p. 174-175 °C Lit. m.p. 172-173 °C

Ref: [1972JOC3404]

***tert*-Butyl oxalyl chloride (FK-16)**



tert-Butanol (150.0 g; 2024 mmol) was added drop wise over 60 min into an excess of oxalyl chloride (513.8 g; 4048 mmol) at 0 °C under argon. After the completion of addition, ice cooling was removed. The reaction mixture was allowed to rise to room temperature and stirred it overnight. The excess oxalyl chloride was removed by distillation without vacuum and the product was distilled at 52 °C / 22 Torr to get 266.5 g (80%) and product was stored in the dark, in a refrigerator.

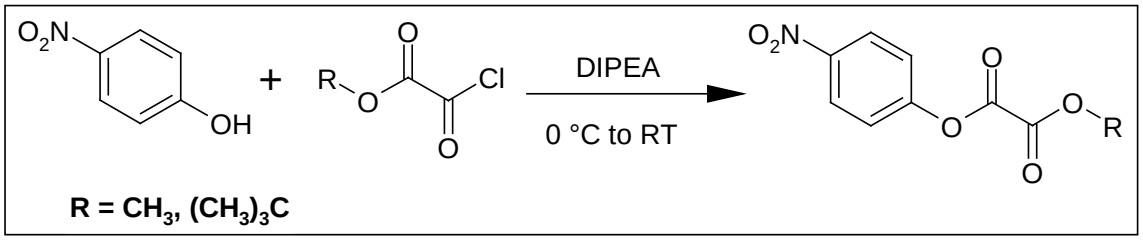
Ref: [WO2007095980]

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 1.5 (s, 9H).

13C NMR (200 MHz, $CDCl_3$) δ(ppm) 161.39, 154.36, 87.52, 27.40.

General Procedure for Compounds (FK-21 & FK-12)

To a solution of 4-nitrophenol and DIPEA (or pyridine) (1.5 equiv.) in DCM was added oxalyl chloride mono ester (1.05 equiv.) drop-wise with stirring at 0 ^{o}C under argon. The ice-bath was removed and temperature of the mixture was allowed to increase. The reaction mixture was further stirred for 30 min at room temperature. The reaction mixture was washed with water followed by brine solution. The organic phase was dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure. The crude compound was crystallized from EtOAc and pet-ether to get the pure product.

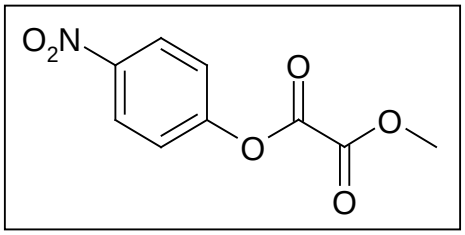


Oxalic acid 4-nitro-phenyl ester-methyl ester (FK-21)

Off-white solid; Yield 90%; HPLC 95%;

m.p. 110-112 °C

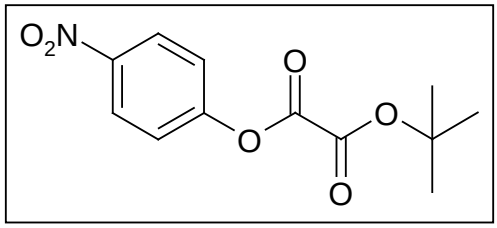
Lit. m.p. 114-115 °C



1H NMR (200 MHz, $CDCl_3$) δ (ppm) 8.22-8.29 (m, 2H), 7.31-7.39 (m, 2H), 3.95 (s, 3H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 156.90, 154.79, 154.21, 146.05, 125.47, 122.03, 54.22.

Oxalic acid 4-nitro-phenyl ester-*tert*-butyl ester (FK-12)

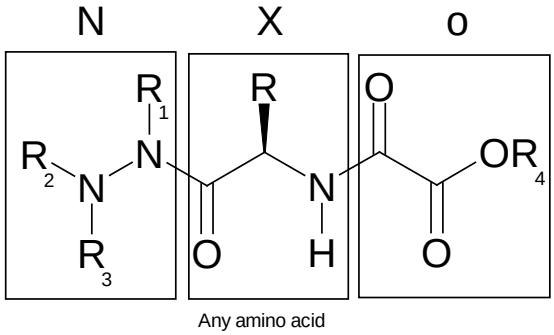


Off-white solid; Yield 81%; HPLC 98%;
m.p. 54-56 °C.

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 8.21-8.30 (m, 2H), 7.28-7.38 (m, 2H), 1.56 (s, 9H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 155.74, 155.53, 154.5, 145.92, 125.39, 122.12, 86.38, 27.71.

Some novel NXO building blocks prepared are shown in the following table.



Sr.No.	R2	R4	R3,R1	X= amino acid	NXO-name	Lab Journal number	Isolated yield(%)
1	Fmoc	Bu^t	H,H	Met	Fmoc-NMetO-OBu^t	FK-29	78
2	Cbz	Bu^t	H,H	Met	Cbz-NMetO-OBu^t	FK-32	66
3	Boc	Me	H,H	Met	Boc-NMetO-OMe	FK-76	60
4	Fmoc	Bu^t	H,H	Thr	Fmoc-NThrO-O Bu^t	FK-78	45
5	Fmoc	Bu^t	H,H	Thr(D)	Fmoc-NThrO-O Bu^t	FK-92	63
6	Fmoc	Bu^t	H,H	Phg(D)	Fmoc-NPhgO-OBu^t	FK-81	56
7	Fmoc	Bu^t	H,H	Sar	Fmoc-NSarO-OBu^t	FK-89	70
8	Fmoc	Bu^t	H,H	β-Ala	Fmoc-N beta-AlaO-OBu^t	FK-91	78
9	Cbz	Bu^t	H,H	β-Ala	Cbz-N beta-AlaO-OBu^t	FK-113	62
10	Fmoc	Bu^t	H,H	Dopa	Fmoc-NDopaO-OBu^t	FK-86	57
11	Fmoc	Bu^t	H,H	Aib	Fmoc-NAibO-OBu^t	FK-13	41
12	Fmoc	Bu^t	H,H	Glu(OMe)	Fmoc-NGlu(OMe)O-OBu^t	FK-80	54
13	Boc	Me	H,H	Ala	Boc-NAlaO-OBu^t	FK-72	2.5

One pot synthesis of NXO-compounds

A one pot synthesis of NXO-compounds was established in our group and reported [2010JOC2492]. The same methodology was adopted to produce some novel NXO building blocks.

General procedure

To a solution of the amino acid in DMF, was added oxalic acid *tert*-butylester-4-nitrophenyl ester (or oxalic acid methylester-4-nitrophenyl ester) (1.0 equiv.) and stirred at 60°C for 1.5 h under argon. The reaction mixture was cooled to room temperature. HOBt (1.05 equiv.), R-NHNH2 (1.0 equiv.) and DCC (1.05 equiv.) was added to the reaction mixture and stirred overnight at room temperature. DMF was removed under reduced pressure. Residue was diluted with EtOAc and the mixture was cooled in freezer for 4 hrs to completely separate the DCHU formed and was removed by filtration. The EtOAc solution was washed with 1N HCl, 10% $NaHCO_3$ followed by brine (3x) and dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure.The crude compound was purified by column chromatography to get the desired product.

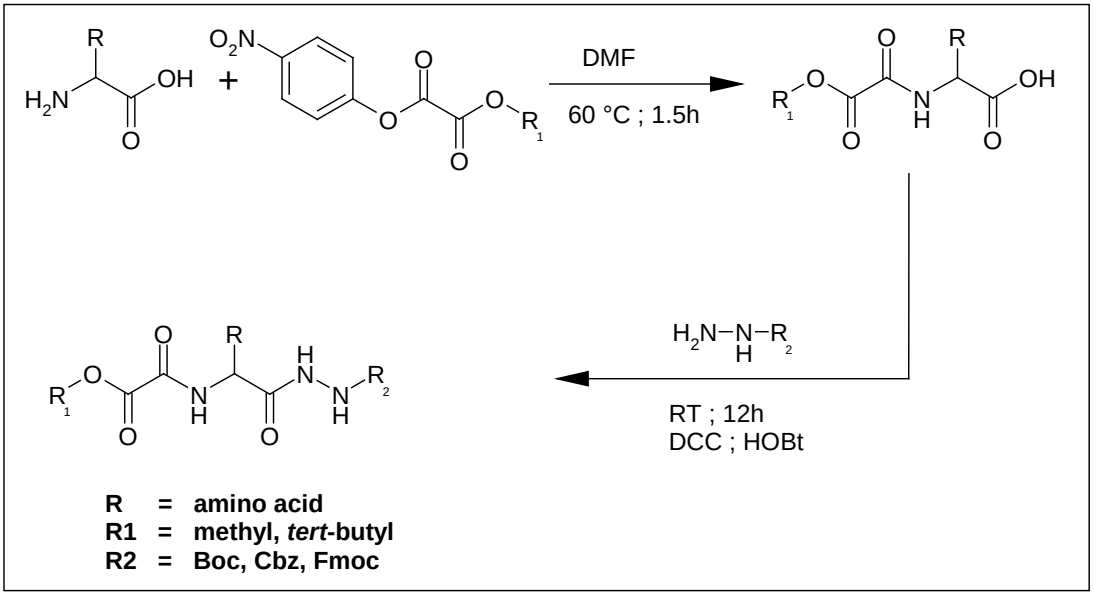
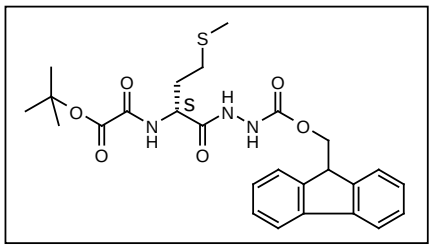


Figure: One pot synthesis of NXO building blocks.

***tert*-Butyl 2-[[(1S)-1-[(9H-fluoren-9-ylmethoxycarbonylamino) carbamoyl]-3-methylsulfanyl-propyl]amino]-2-oxo-acetate (FK-29; Fmoc-NMetO-OBu^t)**

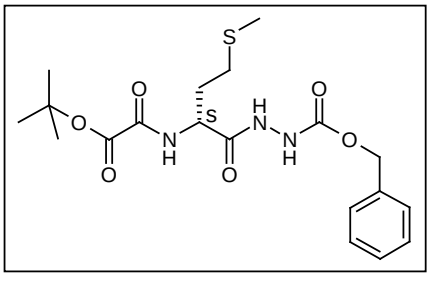


White Solid; Yield 78%; HPLC 95%;
m.p. 72-74 °C.

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 8.1 (br.s, 1 H), 7.7 (d, *J*=7.2 Hz, 2 H), 7.6 (d, *J*=7.2 Hz, 2 H), 7.24-7.38 (m, 4 H), 4.70-4.84 (s, 1 H), 4.4 (d, 2 H), 4.2 (t, 1 H), 2.49-2.70 (m, 2 H), 2.10-2.34 (m, 2 H), 2.1 (s, 3 H), 1.5 (s, 9 H).

13C NMR (200MHz, $CDCl_3$) δ (ppm) 170.43, 158.57, 157.91, 156.20, 143.45, 141.21, 127.79, 127.15, 125.18, 112.97, 85.15, 68.16, 51.27, 46.78, 31.05, 29.80, 27.65, 15.17.

***tert*-Butyl 2-[[(1S)-1-(benzyloxycarbonylaminocarbamoyl)-3-methylsulfanyl-propyl]amino]-2-oxo-acetate (FK-32; Cbz-NMetO-OBu^t)**



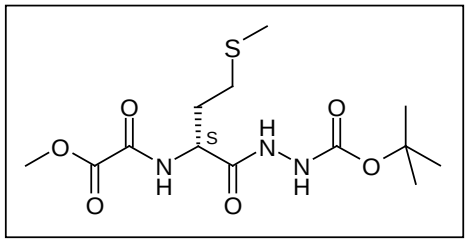
White Solid; Yield 66%; HPLC 95%;
m.p. 49-50 °C.

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 8.8 (br.s, 1 H) 8.0 (br.s, 1 H), 7.27-7.36 (m, 5 H), 7.1 (br.s, 1 H), 5.08-5.71 (m, 2 H), 2.6 (t, 2 H), 2.1 (s, 3 H), 2.0-2.06 (m, 2 H), 1.5 (s, 9 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 170.29, 170.3, 158.56, 157.78, 156.08, 135.46, 128.54, 128.38, 128.19, 85.07, 67.87, 51.14, 30.94, 29.71, 27.64, 15.11.

Methyl2-[[(1S)-1-[(*tert*-butoxycarbonylamino)carbamoyl]-3-methyl sulfanyl-propyl]amino]-2-oxo-acetate (FK-76; Boc-NMetO-OMe)

White Solid; Yield 60%; HPLC 98%;
m.p. 125-128 °C.

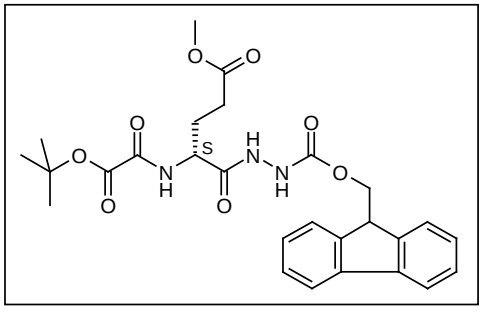


1H NMR (200 MHz, $CDCl_3$) δ (ppm) 8.0 (br.s, 1 H), 6.8 (br.s, 1 H), 4.7 (br.s, 1 H), 3.8 (s, 3 H), 2.6 (t, *J*=6.8 Hz, 2 H), 2.1 (m, 2 H), 2.0 (s, 3 H), 1.4 (s, 1 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 169.89, 160.32, 156.57, 155.28, 82.02, 53.77, 51.03, 30.96, 29.80, 28.09, 15.16.

Methyl (4R)-4-[(2-*tert*-butoxy-2-oxo-acetyl)amino]-5-[2-(9H-fluoren-9-ylmethoxycarbonyl)hydrazino]-5-oxo-pentanoate (FK-80; Fmoc-NGlu(OMe)O-OBu^t)

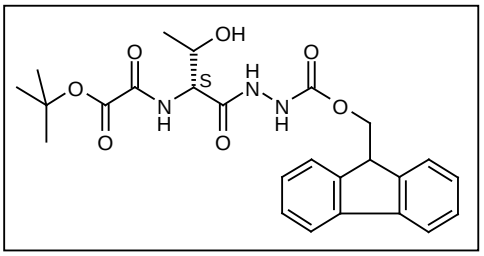
White Solid; Yield 54%; HPLC 99%;
m.p. 64-66 °C.



1H NMR (200 MHz, $CDCl_3$) δ (ppm) 8.83 (br.s, 1 H), 8.01 (br.s, 1 H), 7.64 (d, *J*=7.24 Hz, 2 H), 7.49 (d, *J*=7.24 Hz, 2 H), 7.12.7.36 (m, 5 H), 4.46-4.61 (m, 1 H), 4.24-4.42 (m, 2 H), 4.03-4.19 (m, 1 H), 3.57 (s, 3 H), 2.40-2.59 (m, 2 H), 1.98-2.22 (m, 2 H), 1.44 (s, 9 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 173.98, 170.31, 158.47, 157.96, 156.13, 143.48, 141.22, 127.77, 127.14, 125.15, 119.95, 85.06, 68.10, 52.01, 51.47, 46.80, 29.95, 27.64, 27.26.

***tert*-Butyl 2-[[1-[(9H-fluoren-9-ylmethoxycarbonylamino)carbamoyl]-2-hydroxy-propyl]amino]-2-oxo-acetate (FK-78; Fmoc-NThrO-OBu^t)**

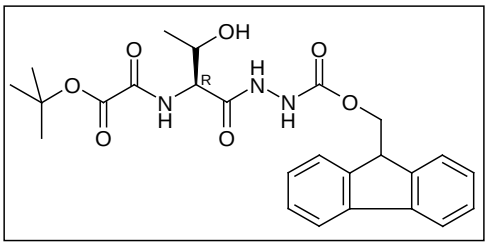


White Solid; Yield 45%; HPLC 98%;
m.p. 93-95 °C.

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 8.78 (br.s, 1 H), 7.94 (br.s, 1 H), 7.64 (d, *J*=7.4 Hz, 2 H), 7.46 (d, *J*=7.2 Hz, 2 H), 7.12-7.35 (m, 5 H), 4.39-4.52 (m, 1 H), 4.29-4.36 (m, 3 H), 4.04-4.15 (m, 1 H), 3.49 (br.s, 1 H), 1.43 (s, 9 H), 1.14 (d, *J*=6.3 Hz, 3 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 169.92, 158.44, 158.42, 156.45, 143.39, 141.21, 127.80, 127.16, 125.14, 119.98, 85.31, 68.25, 66.75, 57.07, 47.76, 27.64, 18.34.

***tert*-Butyl 2-[[(1S)-1-[(9H-fluoren-9-ylmethoxycarbonylamino) carbamoyl]-2-hydroxy-propyl]amino]-2-oxo-acetate (D-Threonine) (FK-92; Fmoc-NThrO-OBu^t)**

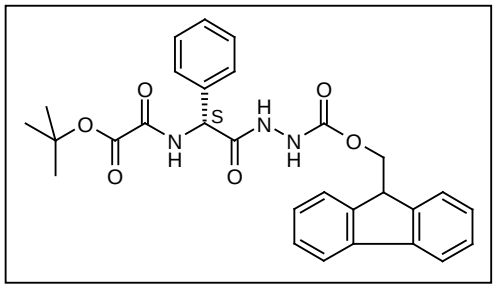


White Solid; Yield 63%; HPLC 99%;
m.p. 92-94 °C.

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 8.92 (br.s, 1 H), 7.97 (br.s, 1 H), 7.61 (d, *J*=7.2 Hz, 2 H), 7.39-7.49 (m, 3 H), 7.06-7.33 (m, 4 H), 4.39-4.53 (m, 1 H), 4.20-4.37 (m, 3 H), 4.02-4.13 (m, 1 H), 3.66 (br.s, 1 H), 1.41 (s, 9 H), 1.12 (d, *J*=6.3 Hz, 3 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 169.90, 158.48, 158.42, 156.54, 143.40, 141.19, 127.80, 127.16, 125.16, 119.96, 85.30, 68.25, 66.86, 57.28, 46.75, 27.64, 18.42.

***tert*-Butyl 2-[[(1S)-2-[2-(9H-fluoren-9-ylmethoxycarbonyl)hydrazino]-2-oxo-1-phenyl-ethyl]amino]-2-oxo-acetate (FK-81; Fmoc-NphgO-OBu^t)**

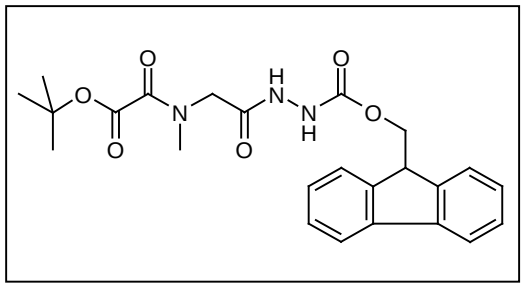


White Solid; Yield 56%; HPLC 98%;
m.p. 84-86 °C.

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 8.88 (br.s, 1 H), 8.16-8.24 (m, 1 H), 7.63 (d, *J*=7.4 Hz, 2 H), 6.99-7.46 (m, 12 H), 5.64-5.73 (m, 1 H), 4.16-4.30 (m, 2 H), 3.96-4.05 (m, 1 H), 1.40 (s, 9 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 169.33, 158.57, 157.20, 156.05, 143.46 (appear as doublet), 141.19, 135.94, 129.06, 128.85, 127.77, 127.62, 127.15, 125.19 (appear as doublet), 119.93, 85.02, 68.17, 55.64, 46.72, 27.63.

***tert*-Butyl 2-[[2-[2-(9H-fluoren-9-ylmethoxycarbonyl)hydrazino]-2-oxo-ethyl]-methyl-amino]-2-oxo-acetate (FK-89; Fmoc-NSarO-OBu^t)**

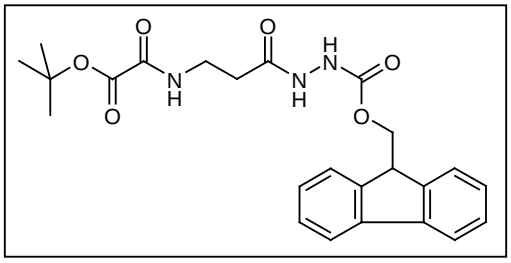


White Solid; Yield 70%; HPLC 98%;
m.p. 72-73 °C.

1H NMR (200 MHz, $CDCl_3$) δ (ppm)
8.61 (br.s, 1 H), 7.64 (d, *J*=7.4 Hz, 2 H), 7.47 (d, *J*=7.2 Hz, 2 H), 7.08-7.35 (m, 5 H), 3.87-4.33 (m, 5 H), 2.84-3.07 (m, 3 H), 1.45 (s, 9 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 167.67, 162.89, 161.54, 156.32, 143.50, 141.20, 127.77, 127.17, 125.21, 119.94, 85.17, 68.06, 48.59, 46.80, 36.73, 27.89.

***tert*-Butyl 2-[[3-[2-(9H-fluoren-9-ylmethoxycarbonyl)hydrazino]-3-oxo-propyl]amino]-2-oxo-acetate (FK-91; Fmoc-N beta-AlaO-OBu^t)**

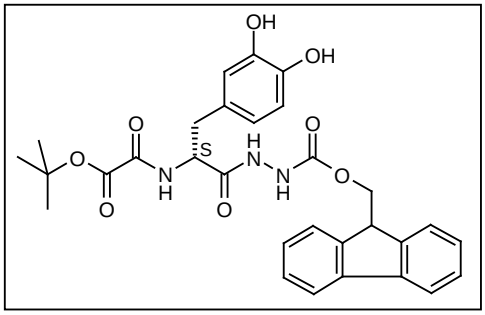


White Solid; Yield 78%; HPLC 99%;
m.p. 78-80 °C.

1H NMR (200 MHz, $CDCl_3$) δ (ppm)
7.85 (br.s, 1 H), 7.62 (d, *J*=7.2 Hz, 2 H),
7.45 (d, *J*=7.2 Hz, 2 H), 7.06-7.40 (m, 6 H), 4.00-4.41 (m, 3 H), 3.40-3.58 (m, 2 H),
2.31-2.53 (m, 2 H), 1.39 (s, 9 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 171.57, 159.05, 158.19, 156.50. 143.46, 141.20, 127.80, 127.12, 125.12, 119.98, 84.65, 67.96, 46.79, 36.00, 32.84, 27.64.

***tert*-Butyl 2-[[1-[(3,4-dihydroxyphenyl)methyl]-2-[2-(9H-fluoren-9-ylmethoxycarbonyl)hydrazino]-2-oxo-ethyl]amino]-2-oxo-acetate (FK-86; Fmoc-NDopaO-OBu^t)**



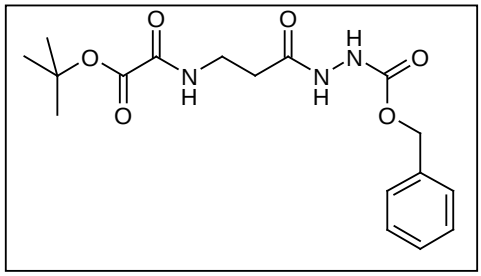
325 mg; Yield 57%; HPLC 99 %;
m.p. 112-114 °C.

1H NMR *(200 MHz, $CDCl_3$) δ (ppm)*
8.95 (br.s, 1 H), 7.80 (br.s, 1 H), 7.48-7.63 (m, 3 H),
7.36 (d, *J*=7.0 Hz, 2 H), 7.01-7.21 (m, 4 H),
6.40-6.74 (m, 3 H), 4.60-4.80 (m, 1 H), 3.94-4.23 (m, 3 H), 2.67-3.08 (m, 2 H),
1.28 (s, 9 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 170.8, 158.2, 157.9, 156.5, 144.1, 143.7, 143.3, 143.3, 141.1, 127.7, 127.3, 127.1, 125.1, 121.6, 119.9, 116.3, 115.5, 85.6, 68.3, 53.7, 46.6, 37.5, 27.4.

***tert*-Butyl 2-[[3-(2-benzyloxycarbonylhydrazino)-3-oxo-propyl]amino]-2-oxo-acetate (FK-113; Fmoc-N beta-AlaO-OBu^t)**

White Solid; Yield 62 %; HPLC 99 %;
m.p. 49-51 °C.

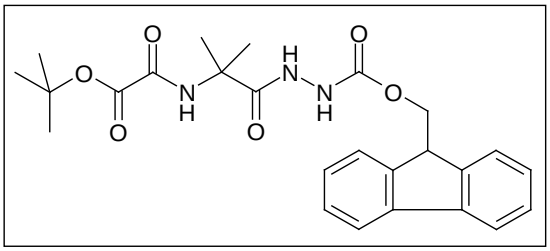


1H NMR (200 MHz, $CDCl_3$) δ (ppm) 8.67(br.s, 1 H).
7.81 (br.s, 1 H), 7.32 (br.s, 1 H), 7.20-7.27 (m, 5 H),
5.00-5.07 (m, 2 H), 3.5 (t, 2 H), 2.4 (t, 2 H), 1.4 (s, 9 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 171.55, 159.11, 158.09, 156.47, 135.58, 128.54, 128.36, 128.09, 84.59, 67.71, 35.91, 32.76, 27.64.

***tert*-Butyl 2-[[2-[2-(9H-fluoren-9-ylmethoxycarbonyl)hydrazino]-1,1-dimethyl-2-oxo-ethyl]amino]-2-oxo-acetate (FK-13; Fmoc-NAibO-OBu^t)**

Off-white solid; Yield 41%; HPLC 99%;
m.p. 143-145 °C.

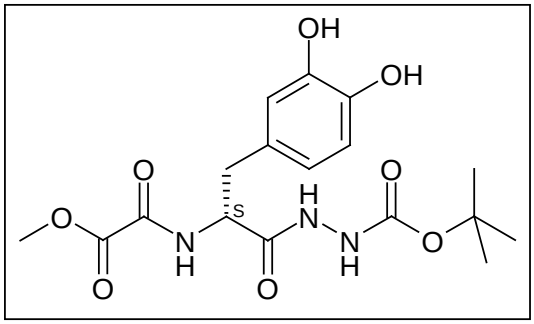


1H NMR (200 MHz, DMSO-d6) δ (ppm)
9.79 (br.s, 1 H), 9.34 (br.s, 1 H), 8.43 (br.s, 1 H),
7.83-7.93 (m, 2 H), 7.63-7.77 (m, 2 H),
7.23-7.48 (m, 4 H), 4.16...4.37 (m, 3 H), 1.48.(s, 15 H).

13C NMR (200 MHz, DMSO-d6) δ (ppm) 173.1, 159.4, 156.4, 156.0, 143.6, 140.7, 127.6, 127.0, 125.3, 120.1, 83.0, 66.1, 55.8, 46.4, 27.3, 24.1.

Methyl 2-[[2-(2-*tert*-butoxycarbonylhydrazino)-1-[(3,4dihydroxyphenyl) methyl]-2-oxo-ethyl]amino]-2-oxo-acetate
(FK-87; Boc-NDopaO-OBu^t)

Off-white solid; Yield 3%; HPLC 99%.

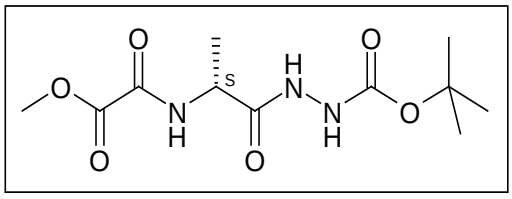


1H NMR (200 MHz, DMSO-d6) δ (ppm)
9.90 (br.s, 1 H), 8.59-9.01(m, 4 H),
6.40-6.73(m, 3 H), 4.37-4.58(m, 1 H),
3.77(s, 3 H), 2.72-3.05(m, 2 H), 1.39 (s, 9 H).

13C NMR (200 MHz, DMSO-d6) δ (ppm) 170.0, 160.7, 156.6, 155.1, 144.8, 143.7, 128.2, 119.8, 116.4, 115.2, 79.2, 53.4, 52.8, 36.2, 28.0.

Methyl 2-[[(1S)-2-(N'-*tert*-butoxycarbonylhydrazino)-1-methyl-2-oxo-ethyl]amino]-2-oxo-acetate (FK-72; Boc-NAlaO-OBu^t)

White solid; Yield 2.5%; HPLC 98%.

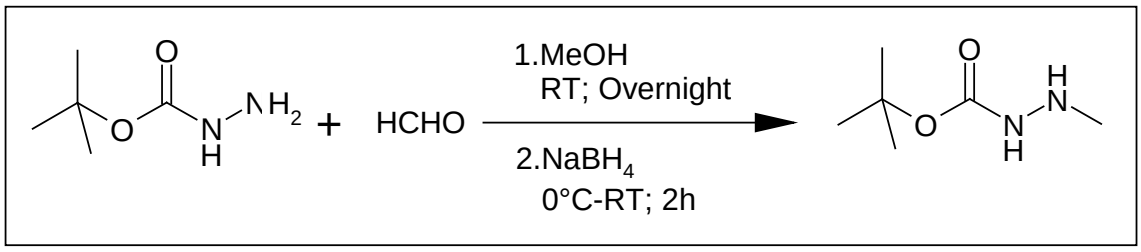


1H NMR (200 MHz, $CDCl_3$) δ (ppm)
8.83 (br.s, 1 H), 8.01 (br.s, 1 H),
6.90 (br.s, 1 H), 4.51-4.68 (m, 1 H),
3.81 (s, 3 H), 1.43 (d, *J*=7.04 Hz, 3 H), 1.37 (s, 9 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 171.1, 160.4, 156.3, 155.5, 81.9, 53.7, 47.8, 28.1, 17.7.

2: Synthesis of *N*-substituted NXO-compounds

tert-Butyl *N*-(methylamino) carbamate (FK-47)



To a solution of *tert*-butyl carbazate (5.00 g; 38.0 mmol) in MeOH (50 mL) was added paraformaldehyde (5.70 g; 190.0 mmol) at room temperature and stirred overnight under argon. The reaction mixture was cooled and $NaBH_4$ (12.93 g; 340.0 mmol) was added to it in portions then stirred it for 2 hrs. Excess of sodium borohydride was quenched by adding saturated ammonium chloride solution and water (1:1 :: NH_4Cl : H_2O). The reaction mixture was extracted with EtOAc (3x), dried over anhydrous sodium sulphate and evaporated to get the crude compound which was purified by column chromatography to get 3.5 g (63%) of the title compound as colourless solid with HPLC-purity 97%.

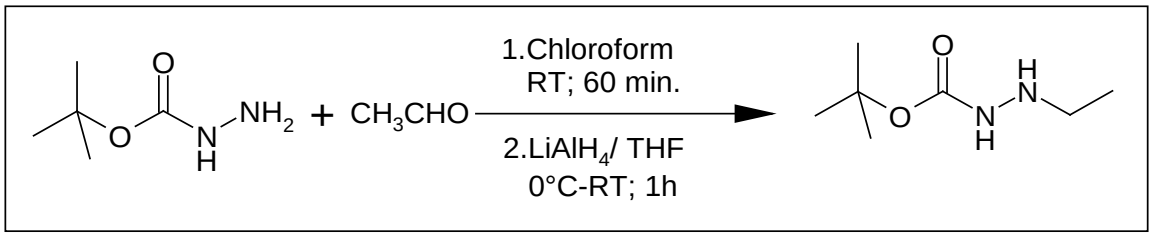
m.p. 48-50 °C
Lit. m.p. 49-51 °C

Ref: [2004JACS6759]

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 2.52-2.65 (m, 3 H), 1.4 (s, 9 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 156.56, 80.44, 39.10, 28.29.

***tert*-Butyl *N*-(ethylamino)carbamate (FK-57)**



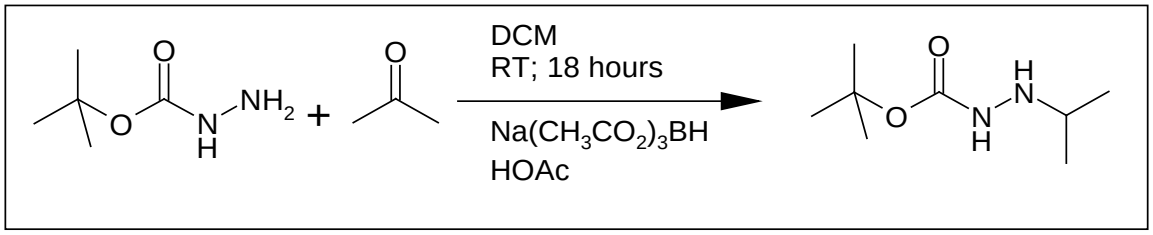
To a solution of *tert*-butyl carbazate (10.00 g; 75.6 mmol) in DCM (100 mL) was added freshly distilled CH_3CHO (4.22 mL, 75.6 mmol). After 30 min the reaction was mainly completed. Then another portion of CH_3CHO (0.42 mL) and $MgSO_4$ were added and stirred for 30 min the mixture was filtered and evaporated to obtain the *tert*-butyl 2-ethylidenehydrazinecarboxylate as colourless liquid. $LiAlH_4$ (2.91 g; 76.7 mmol) was suspended in dry THF (75 mL) under argon. Then a solution of *tert*-butyl 2-ethylidenehydrazinecarboxylate (12.00 g; 75.8 mmol) in THF (75 mL) was slowly added to the ice cooled reaction mixture. After 1h of stirring, the reaction was completed and volatiles were removed under reduced pressure. Et_2O (100 mL) and 3 M NH_4Cl aqueous solution (50 mL) were added to the residue. The organic layer was separated and aqueous fraction was additionally extracted with Et_2O (2 * 100 mL). The organic layers were combined and evaporated. The residue was purified by column chromatography to afford the title compound to get 7.9 g (60%) of the title compound as colourless oil with HPLC-purity 94%.

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 2.8 (q, *J*=7.1 Hz, 1 H), 1.4 (s, 9 H), 1.0 (t, *J*=7.1 Hz, 3 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 156.74, 80.29, 46.19, 28.28, 12.65.

Ref: [2008T6788]

***tert*-Butyl *N*-(isopropylamino)carbamate (FK-50)**



To a pre-stirred solution of the Acetone (2.42 g ; 41.0 mmol) and *tert*-butylcarbazate (5.00 g; 38.0 mmol) in DCE (200 mL) was added sequentially sodium triacetoxy borohydride (13.61 g ; 64.0 mmol) and HOAc (4.54 g ; 75.0 mmol). The resultant mixture was further stirred at ambient temperature for 18h. The mixture was treated with 10% K_2CO_3 (200 mL) and diluted with DCM (200 mL). The organic layer was separated, washed with brine (300 mL* 2x) and dried over $MgSO_4$. Filtration and evaporation of the solvent gave crude compound which was purified by column chromatography (EtOAc : DCM) to get 2.76 g (42%) of the title compound as a white solid.

m.p. 52-54 °C

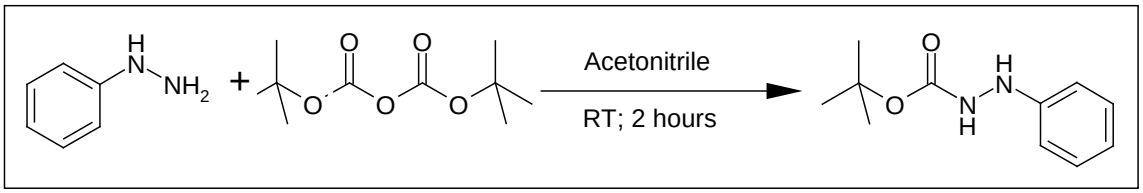
Lit. m.p. 47-51 °C

Ref: [2004JACS6759; 2007JMC4789]

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 5.27 (br.s, 1 H), 3.04-3.18 (m, 1 H), 1.40 (s, 9 H), 0.98 (d, *J*=6.3 Hz, 6 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 156.8, 80.4, 50.9, 28.3, 20.4.

***tert*-Butyl *N*-anilinocarbamate (FK-58)**



To a solution of Phenyl hydrazine (5.40 g, 50.0 mmol) in acetonitrile (20 mL) was added Boc_2O (11.50 mL, 50.0 mmol). The reaction was stirred for 2 hrs at room temperature, under argon. Volatiles were removed from the reaction mixture and residue was crystallised from n-hexane to give 8.40 g (80%) of the title compound as yellow solid with HPLC-purity 98%.

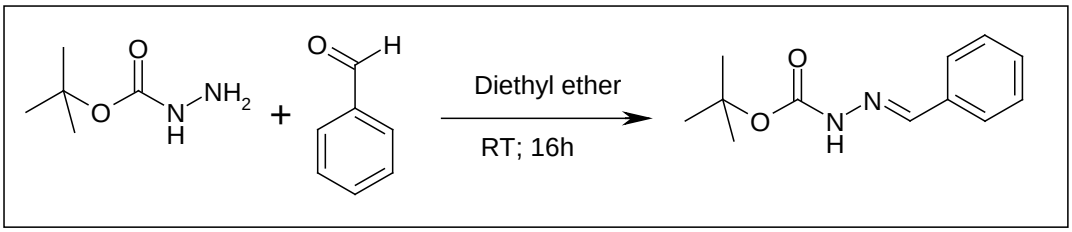
m.p. 85-87 °C
Lit. m.p. 89-90 °C

Ref: [2008T6788]

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 7.12-7.29 (m, 2 H), 6.77-6.97 (m, 3 H), 6.58 (br.s, 1 H), 5.76 (br.s, 1 H), 1.47 (s, 9 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 156.29, 148.19, 129.13, 120.86, 113.12, 81.24, 28.24.

***tert*-Butyl *N*-(benzylideneamino)carbamate (FK-45)**



To a solution of *tert*-butyl carbazate (5.00 g; 38.0 mmol) in diethyl ether (50 mL) was added benzaldehyde (11.12 g; 49.0 mmol) and stirred at room temperature for 16 hrs under argon. The white precipitates formed were collected by filtration, washed with cold diethyl ether and dried under reduced pressure to get the title hydrazone as a white solid 7.6 g (90%) with HPLC-purity 99%.

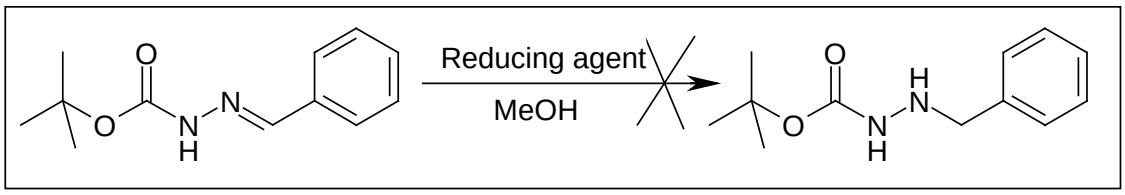
m.p. 188-189 °C
Lit. m.p.: 187-189 °C

Ref: [1976JOC3805]

1H NMR (200 MHz, MeOD) δ ppm 7.92 (br.s, 1 H), 7.69 (m, 2 H), 7.37 (m, 3 H), 3.31 (s, 1 H), 1.53 (s, 9 H).

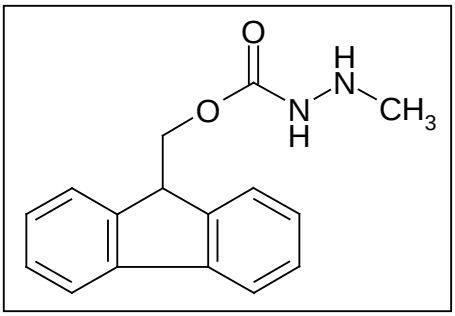
13C NMR (200 MHz, MeOD) δ ppm 155.7, 145.6, 136.0, 130.8, 129.7, 128.1, 81.9, 28.6.

***tert*-Butyl 2-benzylhydrazinecarboxylate (FK-46)**



Three reducing methods were tried to reduce the double bond (1) Hydrogenation using 10% w/w Pd/C in MeOH, (2) $NaBH_4$ in MeOH, (3) $NaBH(OAc)_3$ in MeOH but only the starting material recovered at the end of all three reactions.

Fmoc-2-methylhydrazine (FK-75)



To a solution of Boc_2O (5.00 g; 22.92 mmol) in anhydrous chloroform (30 mL) methyl hydrazine (1.00 g; 21.70 mmol) was added at -78 °C with stirring. The combined mixture was allowed to warm to room temperature and was further stirred for 30 min. To this reaction mixture 9*H*-fluoren-9-ylmethylchloroformate (5.62 g; 21.71 mmol) was added and DIPEA (4.60 mL; 26.00 mmol) was added drop wise over a period of 10 min. After 8 hrs, TFA (10 mL) was added and stirred the mixture for 2 hrs. The mixture was concentrated under reduced pressure. The residue was re-dissolved in EtOAc and washed with aq. $NaHCO_3$ (3x) and brine (1x). The organic layer was dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure to give a yellow solid, which was crystallized from pet-ether and EtOAc to get the title compound 3.50 g (74%) as a white crystalline solid with HPLC-purity 99%.

m.p. 154-155 °C
Lit. m.p. 155-156 °C

Ref: [1999JOC7388]

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 8.66 (s, 1H), 7.87 (d, *J*=7.24 Hz, 2H), 7.68 (d, *J* = 7.24 Hz, 2H), 7.28-7.44(m, 4H), 4.21-4.32(m, 4H), 2.42 (s, 3H).

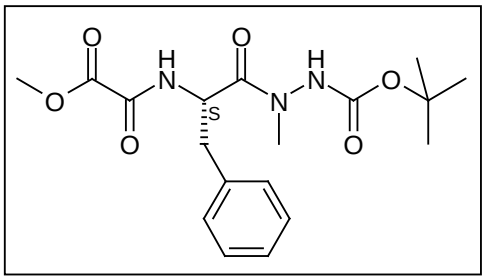
13C NMR (200 MHz, $CDCl_3$) δ (ppm) 156.69, 143.75, 140.67, 127.58, 127.01, 125.15, 120.04, 65.39, 46.63.

General method for the preparation of *N*-substituted NXO compounds

To a solution of amino acid in DMF was added oxalic acid *tert*-butylester 4-nitrophenyl ester (or oxalic acid methyl ester 4-nitrophenyl ester) (1.0 equiv.) and stirred at 60°C for 1.5 h under argon. The reaction mixture was cooled to room temperature. HOBt (1.05 equiv.), mono-protected *N*-substituted hydrazine derivative (1.0 equiv.) and DCC (1.05 equiv.) were added to the reaction mixture and stirred overnight at room temperature under argon. DMF was removed under reduced pressure. The residue was diluted with EtOAc and cooled in freezer for 4 hrs to completely separate the DCHU formed and was removed by filtration. The EtOAc solution was washed with 1N HCl, 10% $NaHCO_3$ followed by brine (3x) and dried over anhydrous sodium sulphate. After removing the solvent, crude compound was purified by column chromatography to get the desired product.

Methyl 2-[[1-benzyl-2-[(tert-butoxycarbonylamino)-methyl-amino]-2-oxo-ethyl]amino]-2-oxo-acetate (FK-62; Boc-N(Me)PheO-OMe)

White Solid; Yield 37%; HPLC-purity 98 %



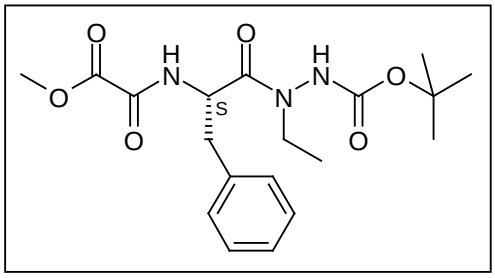
1H NMR (200 MHz, $CDCl_3$) δ (ppm) 7.7 (br.s, 1 H), 7.05-7.39 (m, 6 H), 5.01-5.31 (m, 1 H), 3.8 (s, 3 H), 2.79-3.19 (m, 5 H), 1.4 (s, 9 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 172.51, 160.17, 155.69, 153.93, 135.77, 129.33, 128.56, 127.21, 82.57, 53.57, 50.79, 35.81, 28.12.

Methyl 2-[[1-benzyl-2-[(tert-butoxycarbonylamino)-ethyl-amino]-2-oxo-ethyl]amino]-2-oxo-acetate (FK-63; Boc-N(Et)PheO-OMe)

White Solid; Yield 37%; HPLC-purity 97 %;

m.p. 178-180 °C.



1H NMR (200 MHz, $CDCl_3$) δ ppm

7.8 (br.s, 1 H), 7.09-7.41 (m, 6 H),

4.99-5.30 (m, 1 H), 3.9 (s, 3 H),

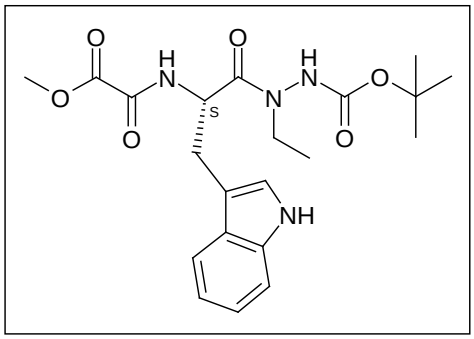
2.59-3.75 (m, 4 H), 1.5 (s, 9 H), 0.88-1.19 (m, 3 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 172.52, 160.44, 155.63, 151.62, 135.52, 129.30, 128.56, 127.23, 84.96, 53.95, 53.60, 45.61, 38.42, 27.96, 12.64.

Methyl 2-[[2-[(tert-butoxycarbonylamino)-ethyl-amino]-1-(1H-indol-3-ylmethyl)-2-oxo-ethyl]amino]-2-oxo-acetate
(FK-154, Boc-N(Et)TrpO-OMe)

Yellowish solid; Yield 24%; HPLC-purity 99%;

m.p. 70-72 °C.



13C NMR (200 MHz, $CDCl_3$) δ (ppm) 172.8,

160.2, 155.8, 154.1, 136.2, 127.2, 123.2, 122.4,

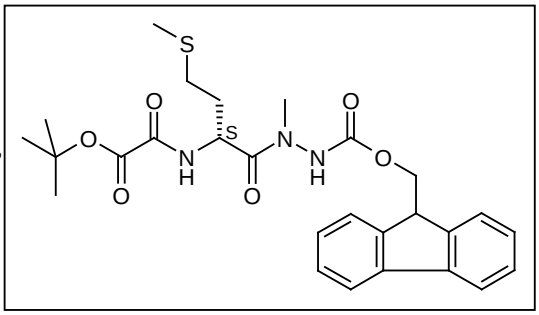
119.7, 118.7, 111.5, 109.7, 82.4, 53.5, 50.2, 43.0,

28.0, 11.4.

***tert*-Butyl 2-[[(1S)-1-[(9H-fluoren-9-ylmethoxycarbonylamino)-methyl-carbamoyl]-3-methylsulfanyl-propyl]amino]-2-oxo-acetate**
(FK-79; Fmoc-N(Me)MetO-OBu^t)

White Solid; Yield 46%; HPLC-purity 97%;

m.p. 117-119 °C.

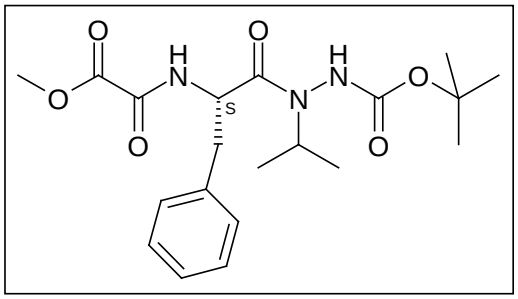


1H NMR (200 MHz, $CDCl_3$) δ(ppm) 8.12 (br.s, 1 H),
7.68 (d, *J*=7.2 Hz, 2 H), 7.51 (m, 2 H),
7.19-7.38 (m, 4 H), 4.95-5.14 (m, 1 H),
4.32-4.51 (m, 2 H), 4.16 (t, *J*=6.4 Hz, 1 H),
3.06 (s, 3 H), 2.10-2.69 (m, 2 H), 1.92-2.10 (m, 2 H), 1.88 (s, 3 H), 1.46 (s, 9 H).

13C NMR (200 MHz, $CDCl_3$) δ(ppm) 171.34, 172.8, 158.56, 157.56, 155.10, 143.32, 141.33, 127.90, 127.18, 124.98, 120.08, 84.99, 67.92, 48.53, 46.96, 35.84, 30.61, 29.94, 27.69, 15.31.

Methyl 2-[[(1S)-1-benzyl-2-[(tert-butoxycarbonylamino)-isopropyl amino]-2-oxo-ethyl]amino]-2-oxo-acetate
(FK-52; Boc-N(iPr)PheO-OMe)

White solid; Yield 35%; HPLC-purity 95%.

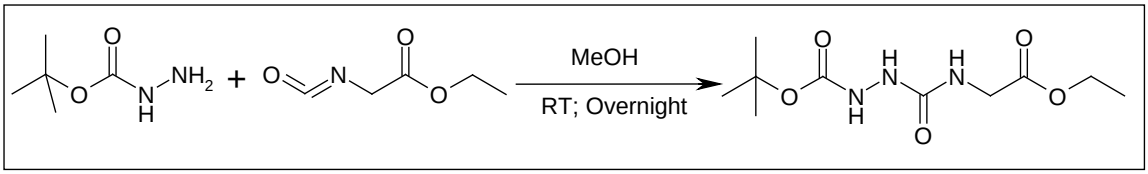


1H NMR (200 MHz, $CDCl_3$) δ (ppm)
8.17 (br.s, 1H), 7.59 (br.s, 1 H),
7.04-7.22 (m, 5 H), 5.81-5.93 (m, 1 H),
3.77 (s, 3 H), 3.23-3.48 (m, 1 H),
2.81-3.07 (m, 2 H), 1.49 (s, 9 H), 0.97 (d, *J*=6.4 Hz, 6 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 173.1, 160.4, 155.5, 152.0, 135.5, 129.3, 129.1, 128.5, 127.2, 84.8, 54.0, 53.6, 51.1, 38.5, 27.9, 20.7, 20.3.

3. Synthesis of NXO β-turn mimic

Ethyl 2-[(*tert*-butoxycarbonylamino)carbamoylamino]acetate (FK-61)



To a solution of *tert*-butyl carbazate (30.0 g; 227.0 mmol) in MeOH (300 mL) was added ethyl isocyanatoacetate (29.3 g; 227.0 mmol) and stirred overnight at room temperature under argon. The solvent was removed under reduced pressure to get 59.3 g (quant.) of the title compound as a white solid with HPLC-purity 99%.

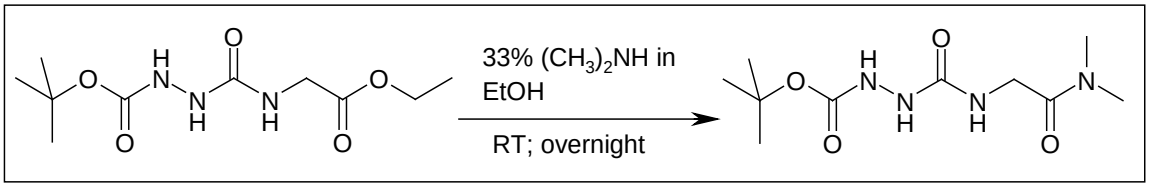
m.p. 116-118 °C
Lit. m.p. 72-74 °C

Ref: [1988JMC374; WO2007095980]

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 7.21 (br.s, 1 H), 7.07 (br.s, 1 H), 6.21 (br.s, 1 H), 4.08-4.22 (m, 2 H), 3.96 (d, *J*=5.7 Hz, 2 H), 1.42 (s, 9 H), 1.30 (t, *J*=11.82 Hz, 3 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 171.1, 158.9, 156.4, 81.6, 61.3, 41.8, 28.1, 14.1.

***tert*-Butyl *N*-[[2-(dimethylamino)-2-oxo-ethyl]carbamoylamino] carbamate (FK-65)**



To a solution of FK-61 (10.0 g; 38.3 mmol) in EtOH (50 mL) was added 33% dimethylamine solution in ethanol (50 mL). The reaction mixture was stirred overnight at room temperature under argon. Volatiles were removed under reduced pressure and the residual solid was well dried under high vacuum to get 9.9 g (quant.) of the target compound as a white solid.

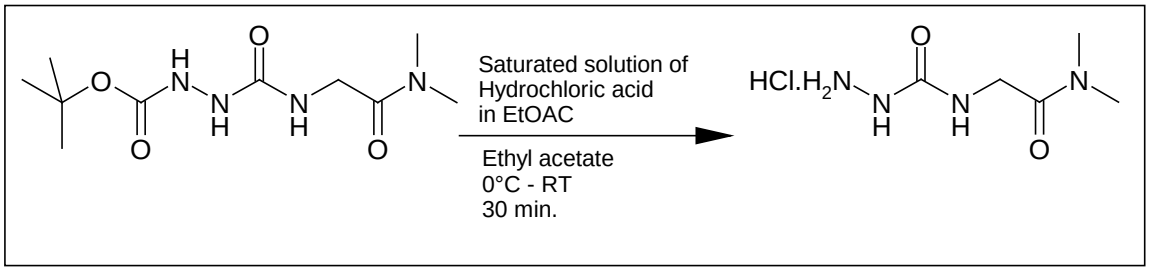
m.p. 136-141 °C.

Ref: [WO2007095980]

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 7.40 (br.s, 1 H), 7.12 (br.s, 1 H), 6.47 (br.s, 1 H), 3.94-4.06 (m, 2 H), 2.91 (d, *J*=7.04 Hz,4 H), 1.36 (s, 9 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 169.2, 158.8, 156.4, 81.0, 41.8, 36.0, 35.6, 28.1.

***N,N*-Dimethyl-2-(methylcarbamoylamino)acetamide hydrochloride (FK-66)**



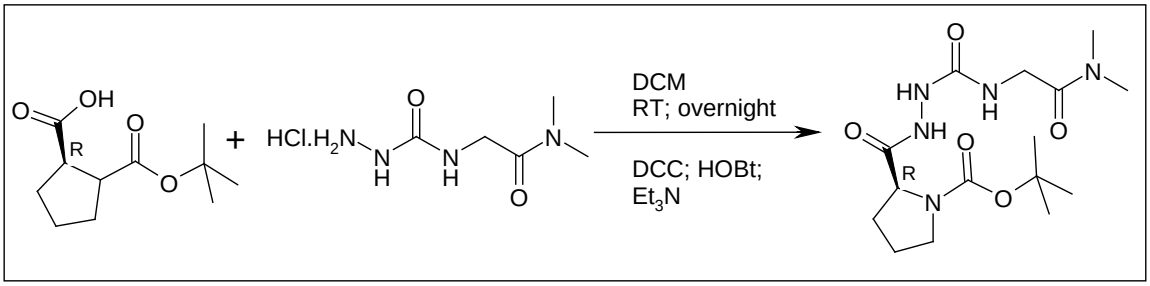
To a solution of FK-65 (5.00 g; 19.2 mmol) in EtOAc (100 mL), a saturated solution of hydrochloric acid in EtOAc (150 mL) was added slowly with stirring at 0 °C. The reaction mixture was further stirred at room temperature for 30 min. Volatiles were removed under reduced pressure and the residual solid was dried under high vacuum to get 3.80 g (quant.) of the target compound as a white solid with HPLC-purity 99%.

m.p. 155-157 °C

1H NMR (*200 MHz, DMSO-d6*) δ (ppm) 10.11 (br.s, 2H), 9.02 (br.s, 1H), 6.87 (br.s, 1H), 3.91 (s, 2H), 2.91 (s, 3H), 2.80 (s, 3H).

13C NMR (*200 MHz, DMSO-d6*) δ (ppm) 168.02, 156.83, 41.23, 35.51, 35.03.

***tert*-Butyl 2-[[[2-(dimethylamino)-2-oxo-ethyl]carbamoylamino] carbamoyl]pyrrolidine-1-carboxylate (FK-67)**



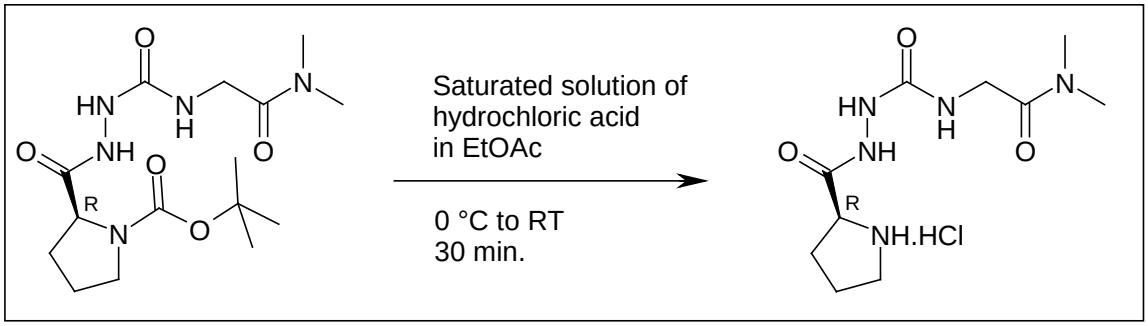
To a solution of Boc-D-Proline (2.19 g; 10.2 mmol) in DCM (200 mL) was added HOBt (1.44 g; 10.6 mmol) and DCC (2.19 g; 10.6 mmol) and stirred for 15 min at room temperature. FK-66 (2.00 g; 10.2 mmol) and triethylamine (1.13 g; 11.1 mmol) were mixed in minimum amount of DCM and added to the reaction mixture with overnight stirring under argon at room temperature. The solvent was evaporated under reduced pressure. The residue was diluted with EtOAc and cooled in freezer for 4 hrs to completely separate the DCHU formed and was removed by filtration. Ethyl acetate was removed under reduced pressure and the crude product was purified by column chromatography (MeOH : EtOAc) to afford 2.5 g (69%) of the title compound as a white solid with HPLC-purity 96%.

m.p. 72-74 °C
Ref: [2007DISS1]

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 9.1 (br.s, 1 H), 7.8 (br.s, 1 H), 6.51 (br.s, 1 H), 4.18-4.33 (m, 1 H), 4.0 (s, 2 H), 3.23-3.53 (m, 2 H), 2.9 (d, *J*=6.7 Hz, 6 H), 1.71-2.22 (m, 4 H), 1.4 (s, 9 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 172.4, 169.98, 158.15, 155.47, 80.46, 58.73, 47.11, 41.88, 36.06, 35.74, 28.97, 28.37, 24.58.

2-[(Cyclopentanecarbonylamino)carbamoylamino]-*N*,*N*-dimethyl-acetamide hydrochloride (FK-68)



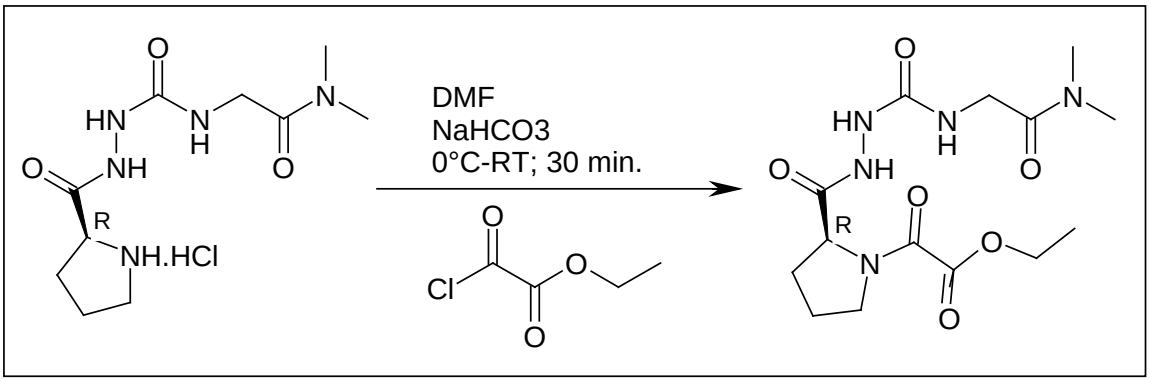
To a solution of FK-67 (1.20 g; 3.4 mmol) in EtOAc (30 mL), a solution of hydrochloric acid saturated in EtOAc (30 mL) was added drop-wise under ice bath cooling. The reaction mixture was stirred further at room temperature for 30 min. Volatiles were removed under reduced pressure and the residue was dried under high vacuum to get 937 mg (quant.) of the title compound as an off-white solid.

m.p. 63-65 °C

1H NMR (200 MHz, MeOD) δ (ppm) 10.11 (br.s, 2H), 9.02 (br.s, 1H), 6.78 (br.s, 1H), 3.91 (s, 2H), 2.91 (s, 3H), 2.80 (s, 3H).

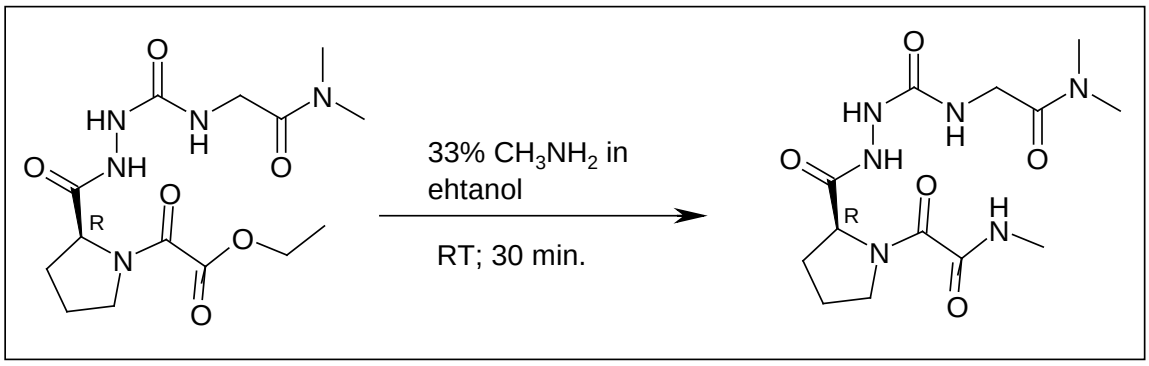
13C NMR (200 MHz, MeOD) δ (ppm) 173.4, 171.0, 160.3, 58.3, 47.6, 42.5, 36.5, 36.1, 30.8, 25.1.

2-[[[1-(2-Ethoxy-2-oxo-propanoyl)pyrrolidine-2-carbonyl]amino] carbamoylamino]-*N*,*N*-dimethyl-acetamide (FK-69)



To a solution of compound FK-68 (937 mg; 3.35 mmol) in DMF (30 mL), sodium bicarbonate (1407 mg; 16.7 mmol) was added and stirred. A solution of oxalyl chloride ethyl ester (777 mg; 5.69 mmol) in DMF (20 mL) was added drop-wise to this pre-stirred mixture at 0°C in 15 min. The reaction mixture was further stirred for 30 min at room temperature. The solvent was evaporated under reduced pressure and the residue obtained was re-dissolved in EtOAc (50 mL * 2x) and filtered through Celite to remove the salt and excess of sodium bicarbonate. The filtrate was concentrated to give the crude product 848 mg (crude yield 68%). This compound was reported [2007DISS1] unstable so it was used in the next step without further purification.

***N,N*-Dimethyl-2-[[[1-[2-(methylamino)-2-oxo-propanoyl]pyrrolidine-2-carbonyl]amino]carbamoylamino]acetamide (FK-166)**



To a solution of crude FK-69 (848 mg; 2.27 mmol) in ethanol (10 mL) was added 33% methylamine solution in ethanol (10 mL) and stirred for 30 min at room temperature under argon. The solvent was removed under reduced pressure to get the crude compound which was further purified by column chromatography (MeOH : DCM) to get 150 mg (18%) of the title compound as a white solid with HPLC-purity 99%.

m.p. 202-203 °C

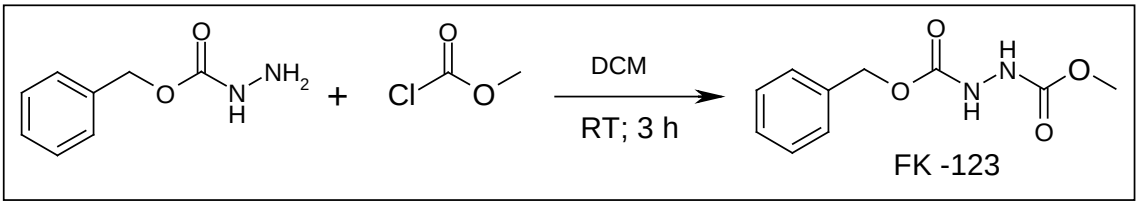
1H NMR (200 MHz, DMSO-d6) δ (ppm) 9.8 (br.s, 1 H), 8.48-8.68 (m, 1 H), 8.06 (d, *J*=10.0 Hz, 1 H), 6.20-6.38 (m, 1 H), 4.26-4.99 (m, 1 H), 3.52-4.00 (m, 4 H), 2.87-2.95 (m, 3 H), 2.81 (d, *J*=3.9 Hz, 3 H), 2.61 (dd, *J*=4.7, 3.9 Hz, 3 H), 1.94 (m, 4 H).

13C NMR (200 MHz, DMSO-d6) δ (ppm) 172.5, 171.4, 169.1, 169.0, 162.3, 161.6, 161.1, 160.5, 158.2, 158.0, 60.2, 59.9, 49.0, 48.5, 41.7, 41.6, 36.0, 35.5, 32.4, 29.1, 25.5, 25.4, 24.7, 21.9.

Note: (all carbon signals appeared twice in this 13C NMR spectrum.)

4. Synthesis of NXO β-turn mimic with carbohydrazide substructure

Methyl *N*-(benzyloxycarbonylamino)carbamate (FK-123)



To a solution of Cbz-$NHNH_2$ (5.0 g; 30.08 mmol) in DCM (100 mL), methyl chloroformate (2.8 g; 30.08 mmol) was added at room temperature and stirred for 3 h. The reaction mixture was filtered and the solvent was removed under reduced pressure to get the crude compound which was further purified by column chromatography (pet-ether : EtOAc) to get 3.2 g (48%) of the product as an off-white solid with HPLC-purity 99%.

m.p. 78-79 °C

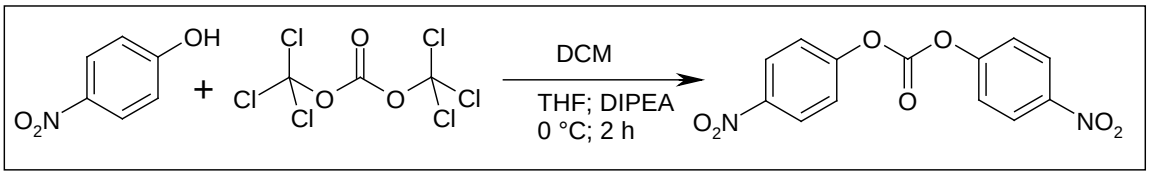
Ref: [1912BER3018; 2002TJC159]

Anal. Calc. for $C_{10}H_{12}N_2O_4$: C, 53.57%; H, 5.39%; N, 12.49%.
Found: C, 53.68%; H, 5.16%; N, 12.35%.

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 6.97-7.28 (m, 7H), 5.03 (s, 2 H), 3.59 (s, 3 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 157.5, 156.9, 135.6, 128.5, 128.4, 128.2, 67.8, 53.1.

Bis(4-nitrophenyl) carbonate (FK-205)



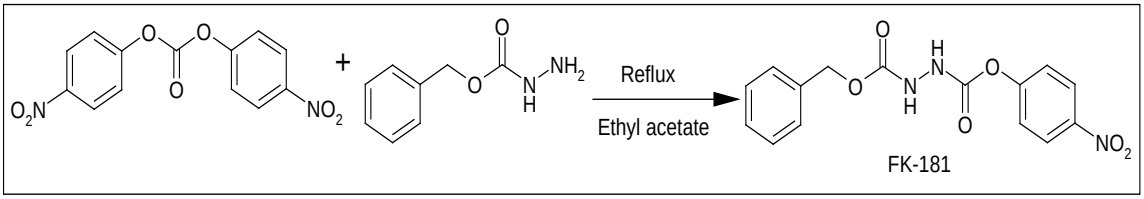
A mixture of *p*-nitrophenol (59.70 g, 429.3 mmol) and Hunig's base (DIPEA) (198.14 mL, 1533 mmol) in THF (500 mL) was stirred and cooled to 0 °C, a solution of triphosgene (91.00 g, 306.6 mmol) in DCM (300 mL) was added drop-wise over 2 hrs. The reaction mixture was kept stirring for further 2 hrs and was poured into 1N HCl (1.5 L). The crude compound was extracted with DCM (500mL * 3x). The combined organic extracts were washed with brine, dried over anhydrous sodium sulphate, concentrated and purified by flash chromatography (eluent: DCM), to give 90.00 g (96%) of the title compound as a white solid with HPLC-purity 95%.

m.p. 141-142 °C

Lit. m.p. 140-141 °C

Ref: [2010AG(E)3049]

(4-Nitrophenyl) *N*-(benzyloxycarbonylamino)carbamate (FK-181)



To a solution of bis (p-nitro-phenyl)-carbonate (2.0 g; 6.6 mmol) in EtOAc (20 mL), a solution of Cbz-NHNH2 (1.1 g; 6.6 mmol) in EtOAc (16 mL) was added drop-wise within 20 min under reflux and stirring. Heating was continued for 15 min and later the reaction mixture was kept at room temperature for 2.5 hrs. Volatiles were removed under reduced pressure and the crude compound was purified by column chromatography (pet-ether : EtOAc) to get 1.4 g (65%) of the title compound as a white solid with HPLC-purity 99%.

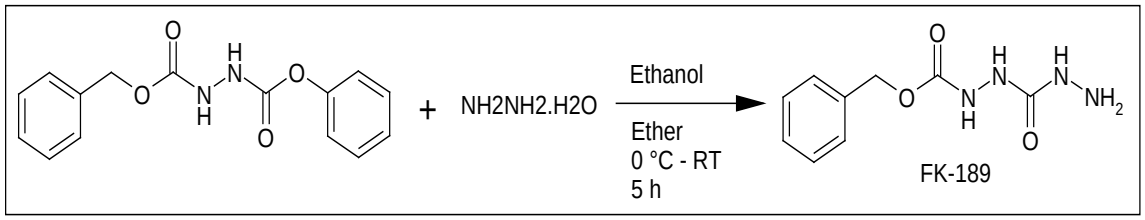
m.p. 137-139 °C
Lit. m.p. 138-139 °C

Ref: [1965CB3340]

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 10.07 (br.s, 1 H), 9.54 (br.s, 1 H), 8.20-8.35 (m, 2 H), 8.04-8.16 (m, 1 H), 7.23-7.42 (m, 5 H), 6.85-6.99 (m, 1 H), 4.99-5.44 (m, 2 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 163.8, 157.9, 156.6, 139.5, 136.6, 128.3, 127.8, 126.1, 115.7, 65.7.

Benzyl *N*-(hydrazinecarbonylamino)carbamate (FK-189)



To a solution of hydrazine monohydrate (64%) (12.4 g; 246.9 mmol) in ethanol (200 mL) was added azaglycin Z-(p-nitro-phenyl ester) (8.2 g; 24.7 mmol) in dry ether (600 mL), drop-wise with stirring in 120 min at ice cooling temperature. The reaction mixture was kept at room temperature for 5 hrs. Precipitated solid was removed and dried under vacuum to get 2.6 g (46%) of the title compound as an off-white solid with HPLC-purity 99%.

m.p. 141-143 °C
Lit. m.p. 140-141 °C

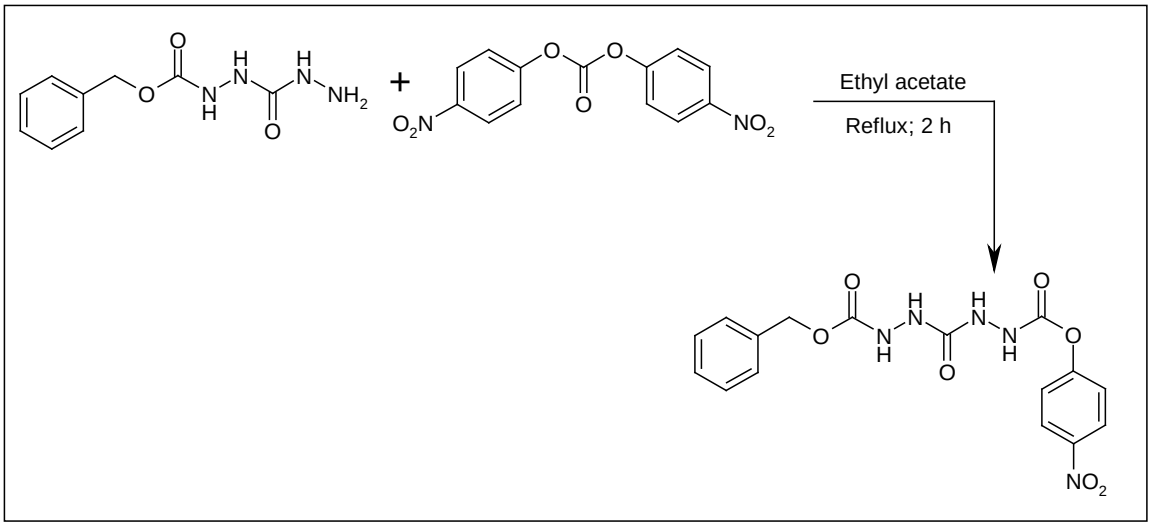
Ref: [1965CB3340]

Elemental analysis
Calculated for $C_9H_{12}N_4O_3$: C, 48.21; H, 5.39; N, 24.99
Found C, 48.02; H, 5.18; N, 24.43

1H NMR (200 MHz, DMSO-d6) δ (ppm) 8.86 (br.s, 1 H), 8.03 (br.s, 1 H), 7.21-7.40 (m, 2 H), 4.98-5.11 (m, 2 H), 4.03 (br.s, 2 H).

13C NMR (200 MHz, DMSO-d6) δ (ppm) 159.7, 156.7, 136.7, 128.3, 127.8, 65.7.

(4-Nitrophenyl) *N*-(benzyloxycarbonylaminocarbamoylamino) Carbamate (FK-203)



To a solution Cbz-carbohydrazide (340 mg; 1.52 mmol) in EtOAc (10 mL) was added bis-[p-nitro-phenyl]-carbonat (461 mg; 1.52 mmol) and stirred for 2 hrs under reflux. To the oil formed pet-ether (10 mL) was added and let the mixture stand for 24 hrs for crystallization. Impurities were filtered off to get 450 mg (76%) of the title compound as a white solid with HPLC-purity 99%.

m.p. 156-157°C

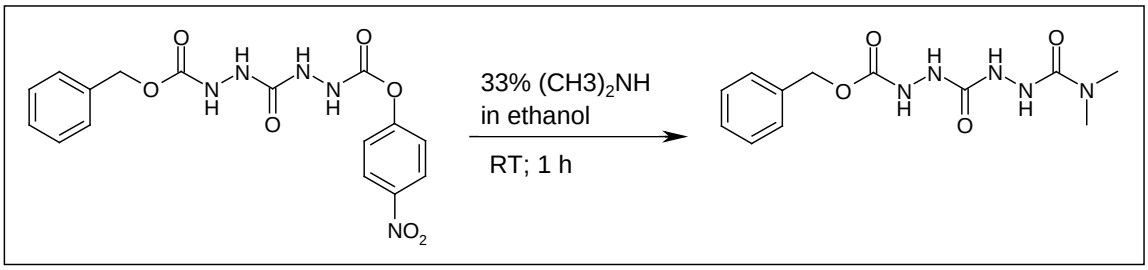
Lit. m.p. 157°C

Ref: [1965CB3340]

1H NMR (200 MHz, $CDCl_3$) δ (ppm)

13C NMR (200 MHz, $CDCl_3$) δ (ppm)

Benzyl *N*-[(dimethylcarbamoylamino)carbamoylamino]carbamate (FK-206)

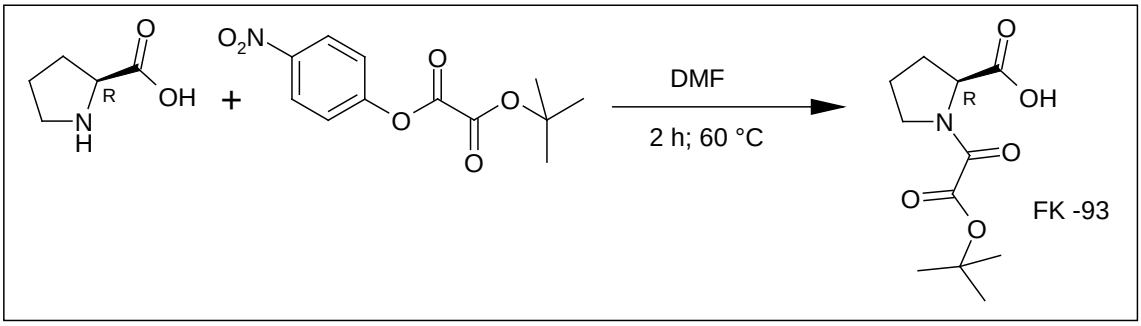


To a solution of FK-203 (350 mg; 0.899 mmol) in ethanol (5 mL) was added 33% dimethylamine solution in ethanol (5 mL) and was stirred for 1 h at room temperature under argon. Volatiles were evaporated and the residue was dried under high vacuum to get crude product, which was further purified by column chromatography.

Remarks

The reaction resulted in an inseparable mixture.

1-(2-*tert*-Butoxy-2-oxo-acetyl)pyrrolidine-2-carboxylic acid (FK-93)



To a solution of D-Proline (3.0 g; 26.0 mmol) in DMF (50 mL) was added oxalic acid *tert*-butyl ester 4-nitophenyl ester (7.7 g; 28.7 mmol) and stirred for 2 hrs at 60 °C under argon. The solvent was removed under reduced pressure to get the crude compound which was further purified by column chromatography (DCM : MeOH) to get 3.0 g (48%) of the title compound as a white solid with HPLC-purity 99%.

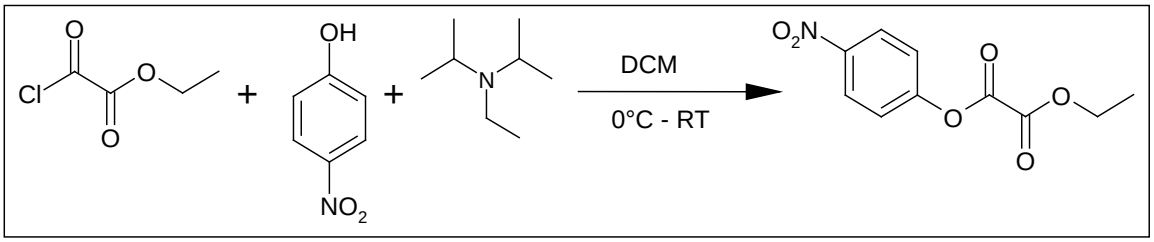
m.p. 92-94 °C

$[\alpha]_D^{20}$ = -76.901° (c 1.0, MeOH)

1H NMR (400 MHz, DMSO-d6) δ (ppm) 8.62 (br.s, 1 H), 4.43-4.81 (m, 1 H), 3.48-3.79 (m, 2 H), 1.78-2.34 (m, 4 H), 1.47 (d, *J*=9.6 Hz, 9 H).

13C NMR (400 MHz, DMSO-d6) δ (ppm) 173.6, 172.8, 162.1, 161.1, 159.5, 159.3, 84.3, 83.9, 60.1, 58.6, 47.6, 47.2, 31.3, 29.1, 27.9, 27.7, 24.7, 22.4.

O2-ethyl O1-(4-nitrophenyl) oxalate (FK-185)



To a solution of 4-nitrophenol (10.0 g; 71.9 mmol) and DIPEA (11.2 g; 86.3 mmol) in DCM (100 mL) was added oxalyl chloride ethyl mono ester (10.3 g; 75.5 mmol) in DCM (100 mL) drop-wise with stirring at 0 °C under argon, ice bath was removed and stirred the mixture for 30 min at room temperature. The reaction mixture was washed with water (200 mL * 2x) followed by brine (200 mL) and dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure and the crude product was purified using column chromatography (pet-ether : EtOAc) to get 16.6 g (86%) of the title compound as a brownish solid with HPLC-purity 99%.

-

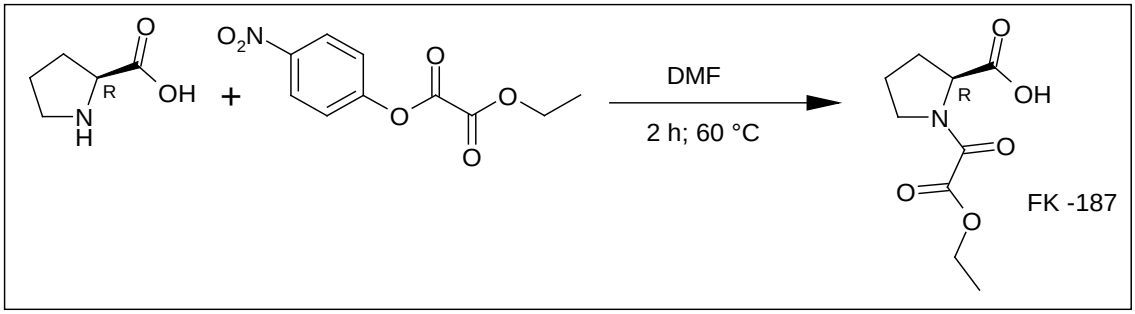
m.p. 82-84 °C
Lit. m.p. 80-81 °C

Ref: [2006JAFC1868]

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 8.18-8.31 (m, 2 H), 7.29-7-40 (m, 2 H), 4.38 (q, 2 H), 1.36 (t, 3 H).

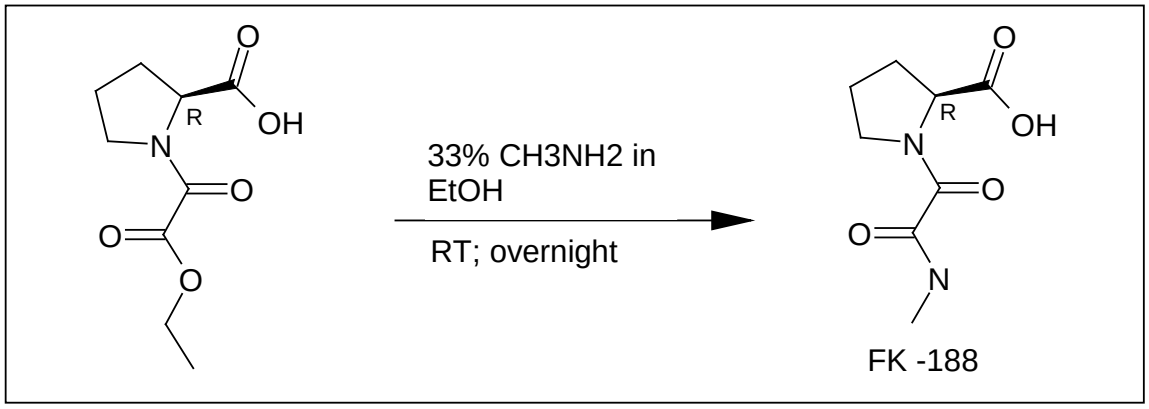
13C NMR (200 MHz, $CDCl_3$) δ (ppm) 156.5, 155.1, 154.3, 146.0, 125.4, 122.1, 64.1, 13.9.

1-(2-Ethoxy-2-oxo-acetyl)pyrrolidine-2-carboxylic acid (FK-187)



To a solution of D-Proline (2.0 g; 17.4 mmol) in DMF (50 mL) was added oxalic acid ethyl ester 4-nitophenyl ester (4.2 g; 17.4 mmol) and stirred for 2 hrs at 60 °C under argon. The solvent was removed under reduced pressure to get the crude compound and was used in the next step without further purification.

1-[2-(Methylamino)-2-oxo-acetyl]pyrrolidine-2-carboxylic acid (FK-188)



To a solution of crude compound FK-187 in ethanol (50 mL) was added a 33% methylamine solution in ethanol (50 mL) and stirred overnight at room temperature. Volatiles were removed under reduced pressure. The crude compound was purified by column chromatography (DCM : MeOH) to get 3.5 g (quant.) of the title compound as an off-white solid with HPLC-purity 99%.

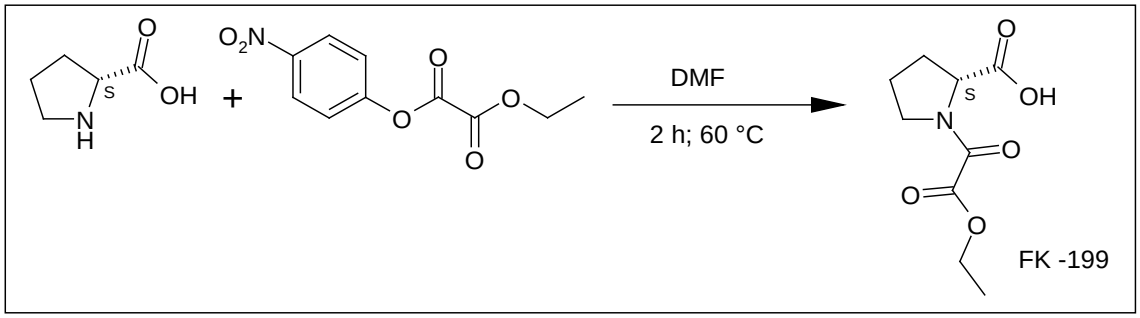
m.p. 52-54 °C

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 8.46 (br.s, 1 H), 7.87 (br.s, 1 H), 4.15-4.98 (m, 1 H), 3.46-4.13 (m, 2 H), 2.45 (s, 3 H), 1.71-2.31 (m, 4 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 178.4, 177.5, 162.3, 161.8, 160.5, 160.4, 63.4, 63.2, 49.1, 48.5, 32.2, 29.0, 25.9, 25.4, 24.8, 22.2.

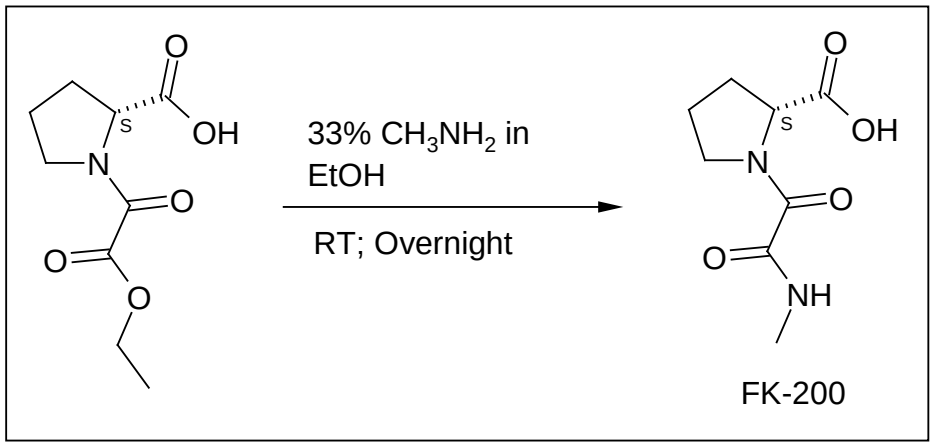
(All carbons signals appeared twice in this 13C NMR spectrum)

1-(2-Ethoxy-2-oxo-acetyl)pyrrolidine-2-carboxylic acid (FK-199)



To a solution of L-Proline (2.0 g; 17.4 mmol) in DMF (50 mL) was added oxalic acid ethyl ester 4-nitophenyl ester (4.2 g; 17.4 mmol) and stirred it at 60 °C for 2 hrs under argon. The solvent was removed under reduced pressure to get the crude compound which was used in the next step without purification.

1-[2-(Methylamino)-2-oxo-acetyl]pyrrolidine-2-carboxylic acid (FK-200)



To a solution of crude compound FK-199 in ethanol (50 mL) was added a 33% methylamine solution in ethanol (50 mL) and stirred overnight. The solvent was removed under reduced pressure. The crude compound was purified by column chromatography (DCM : MeOH) to get 3.5 g (quant.) of the title compound as an off-white solid with HPLC-purity 99%.

m.p. 53-54 °C

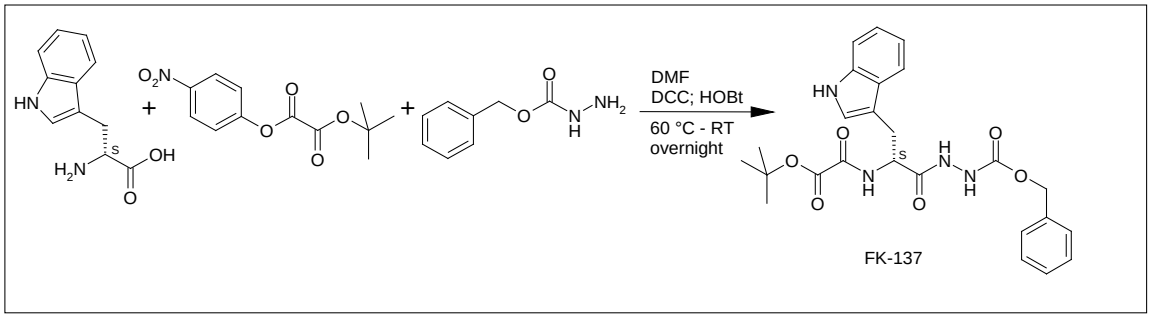
1H NMR (200 MHz, $CDCl_3$) δ (ppm) 8.46 (br.s, 1 H), 7.86 (br.s, 1 H), 4.15-4.97 (m, 1 H), 3.46-4.13 (m, 2 H), 2.45 (s, 3 H), 1.71-2.32 (m, 4 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 178.4, 177.5, 162.3, 161.8, 160.5, 160.4, 63.4, 63.2, 49.1, 48.5, 32.2, 29.0, 25.9, 25.4, 24.8, 22.2.

(All carbons signals appeared twice in this 13C NMR spectrum)

5. Synthesis of modified peptide (A)

tert-Butyl 2-[[(1S)-2-(2-benzyloxycarbonylhydrazino)-1-(1H-indol-3-ylmethyl)-2-oxo-ethyl]amino]-2-oxo-acetate (FK-137; Cbz-NTrpO-OBoc)

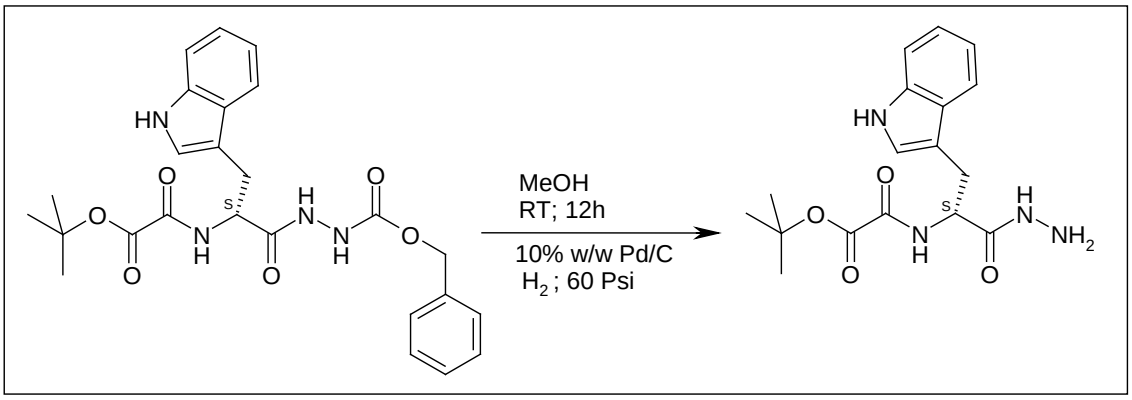


To a solution of L-Tryptophan (5.00 g; 24.5 mmol) in DMF (100 mL), oxalic acid *tert*-butylester 4-nitrophenyl ester (7.86 g; 29.4 mmol) was added and stirred at 60°C for 1.5 h under argon. The reaction mixture was cooled to room temperature. HOBt (3.47 g; 25.7 mmol), Cbz-NHNH2 (4.27 g; 25.7 mmol) and DCC (5.30 g; 25.7 mmol) were added to the reaction mixture and stirred overnight at room temperature under argon. DMF was removed under reduced pressure. The reaction mixture was diluted with EtOAc and cooled in freezer for 4 hrs to completely separate the DCHU formed. The DCHU was removed by filtration. The organic phase was washed with 1N HCl, 10% $NaHCO_3$ and finally with brine (3x) and dried over anhydrous sodium sulphate. The solvent was removed to get the crude product which was purified by column chromatography to get 10.30 g (87%) of the title compound with HPLC-purity 99%.
m.p. 68-70 °C.

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 8.60 (br.s, 1 H), 8.28 (br.s, 1 H), 7.71 (br.s, 1 H), 7.52 (br.s, 1 H), 6.78-7.24 (m, 10 H), 4.86-4.98 (m, 2 H), 4.56-4.71 (m, 1 H), 3.00-3.22 (m, 2 H), 1.34 (s, 9 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 170.6, 158.4, 157.6, 156.2, 136.1, 135.5, 128.5, 128.3, 128.1, 127.4, 124.2, 122.0, 119.6, 118.4, 111.5, 108.9, 85.0, 67.8, 53.0, 28.0, 27.6.

***tert*-Butyl 2-[[(1S)-2-hydrazino-1-(1H-indol-3-ylmethyl)-2-oxo-ethyl] amino]-2-oxo-acetate (FK-164; NTrpO-OBu^t)**



In a Parr apparatus a solution of FK-137 (20.00 g; 16.6 mmol) in MeOH (50 mL) was hydrogenated using 10% Pd/C (2.00 g, 10% w/w) at 60 psi for 12 hrs at room temperature. The catalyst was removed by filtration through sintered glass funnel and washed with MeOH (50 mL). The filtrate was evaporated to give 6.70 g (93%) of the product as an off-white solid with HPLC-purity 99%.

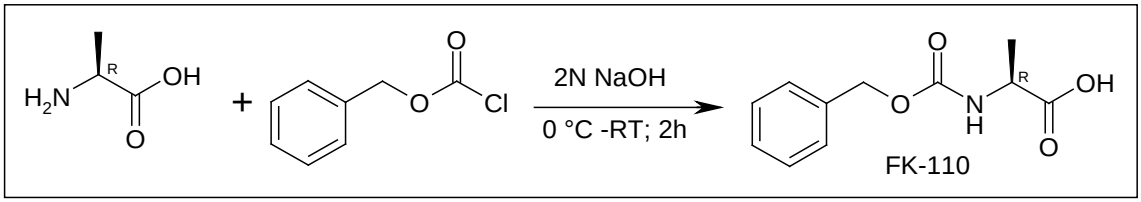
m.p. 83-85 °C.

Ref: [2003BMC2715]

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 8.82 (br.s, 1 H), 7.84-8.03 (m, 2 H), 7.46-7.63 (m, 1 H), 6.87-7.32 (m, 5 H), 4.56-4.70 (m, 1 H), 3.06-3.33 (m, 2 H), 1.48 (s, 9 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 170.7, 158.8, 157.5, 136.2, 127.2, 123.5, 122.1, 119.6, 118.4, 111.5, 109.5, 85.0, 53.4, 28.3, 27.6.

(2R)-2-(Benzyloxycarbonylamino)propanoic acid (FK-110)



A solution of 2 N NaOH (240 mL) and benzyloxycarbonyl chloride (70.51 mL; 493.9 mmol) were added simultaneously drop-wise from two separate syringes into D-alanine (40.00 g; 448.9 mmol) in 2 N NaOH (264 mL) with magnetic stirring and ice cooling. After approximately 1 h, the mixture was allowed to react at room temperature for another 1 h. Water (400 mL) was added to the mixture and it was extracted with ether. The aqueous layer was acidified with 2 N HCl (240 mL) to adjust pH 2-3. The product was extracted using ether (250 mL * 5x). The combined organic phase was dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure to get 25.00 g (90%) of the title compound as a white solid with HPLC-purity 95%.

m.p. 78-80°C.

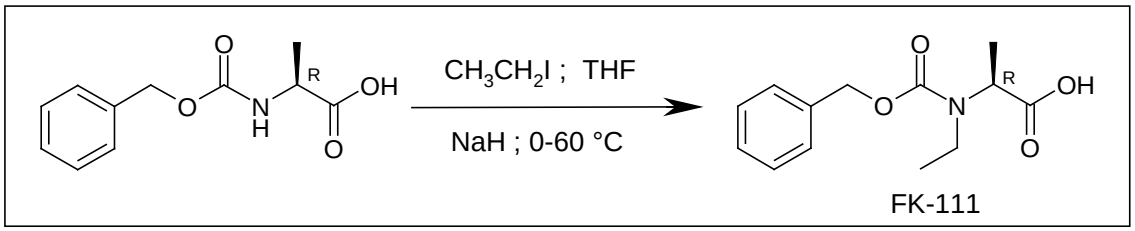
$[\alpha]_D^{20}$ = +19.601° (c 1.3, MeOH)

Ref: [2008JMC6371] on page 6378

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 10.74 (br.s, 1 H), 7.18-7.32 (m, 5 H), 5.35 (br.s, 1 H), 4.94-5.13 (m, 2 H), 4.15-4.45 (m, 1 H), 1.36 (d, *J*=7.2 Hz, 3 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 177.8, 155.9, 136.1, 128.6, 128.3, 128.2, 67.2, 49.5, 18.4.

(2S)-2-[Benzyloxycarbonyl(ethyl)amino]propanoic acid (FK-111)



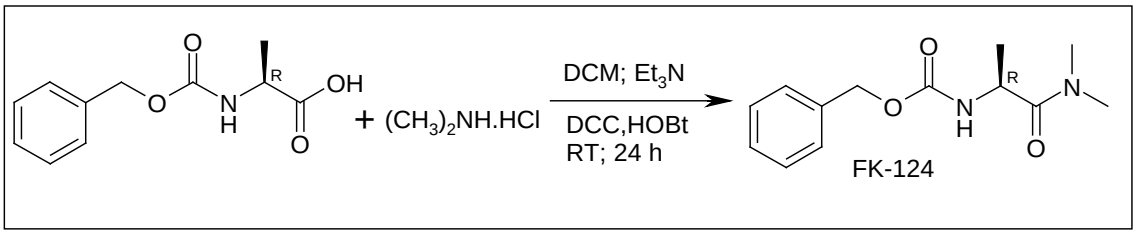
To a solution of *N*-Carbobenzoxy amino acid (10.00 g; 44.8 mmol) in THF (200 mL) was added ethyl iodide (83.85 mL; 537 mmol) this mixture was cooled to 0°C. Sodium hydride (5.37 g, 60% suspension in mineral oil; 224 mmol) was added in portions. The cooling bath was removed and the reaction mixture was heated to 60°C. The temperature was maintained for one week. Excess of sodium hydride was quenched with water and the resulting suspension was extracted with EtOAc (100 mL * 3x). The aqueous fraction was then acidified with citric acid solution to pH 4 and extracted with EtOAc (100 mL * 3x). The ethyl acetate solution was washed with brine, dried over anhydrous sodium sulphate, and evaporated to give 4.0 g (36%) of the title compound as a brown liquid with HPLC-purity 96%.

Ref: [2008TA970]

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 9.64 (br.s, 1 H), 7.27-7.46 (m, 5 H), 5.15 (s, 2 H), 4.07-4.67 (m, 1 H), 3.06-3.62 (m, 2 H), 1.49 (d, *J*=7.2 Hz, 3 H), 1.18 (t, 3 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 177.3, 156.4, 136.4, 128.5, 128.0, 127.8, 67.4, 55.2, 40.9, 15.0, 14.2.

Benzyl *N*-[(1S)-2-(dimethylamino)-1-methyl-2-oxo-ethyl]carbamate (FK-124)

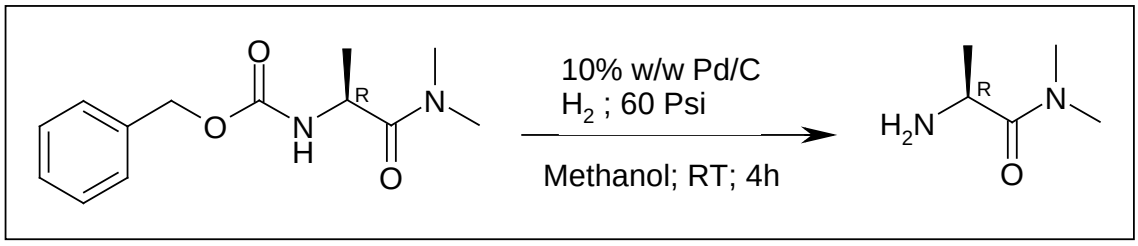


To a solution of Z- D-Alanine (25.00 g; 111.9 mmol) in DCM (600 mL), HOBt (15.89 g; 117.6 mmol) and DCC (24.26 g; 117.6 mmol) were added. To this a solution of dimethylamine hydrochloride (9.59 g; 117.6 mmol) neutralized with triethylamine (16.46 mL; 117.6 mmol) in DCM (50 mL) was added and the mixture was stirred at room temperature for 24 hrs. The precipitated DCHU was removed by filtration and the filtrate was concentrated under reduced pressure. The additional DCHU was removed by subsequent triturating with cold EtOAc and filtration. The ethyl acetate solution was washed with 1N HCl (400 mL * 2x), 10 % $NaHCO_3$ (400 mL * 2x), brine (300 mL), dried over anhydrous sodium sulphate, and concentrated to give 25.81g (92%) of the product as a thick liquid.

$[\alpha]_D^{20}$ = +21.601° (c 1.4, MeOH)

Ref: [2003 OBC 965]

(2S)-2-Amino-*N*,*N*-dimethyl-propanamide (FK-135)



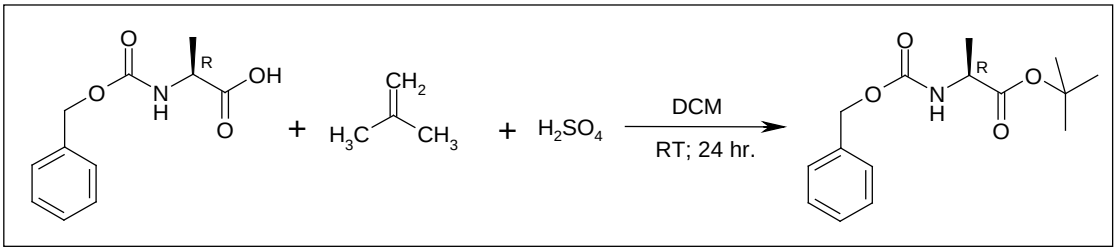
In a Parr apparatus a solution of FK-124 (25.8 g; 115.57 mmol) in MeOH (150 mL) was hydrogenated using 10% Pd/C (246 mg, 10% w/w) at 60 Psi for 4 hrs at room temperature. The catalyst was filtered using sintered glass funnel, washed with MeOH (50 mL) and the filtrate was evaporated to give 8.69 g (65%) of the product as a colourless thick liquid.

Ref: [2003BMCL2715]

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 5.08-5.50 (br.s, 2 H), 3.71-4.08 (m, 1 H), 2.81-3.08 (m, 6 H), 1.23-1.33 (m, 3 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 173.67, 46.54, 36.68, 35.79, 19.27.

***tert*-Butyl (2S)-2-(benzyloxycarbonylamino)propanoate (FK-161)**



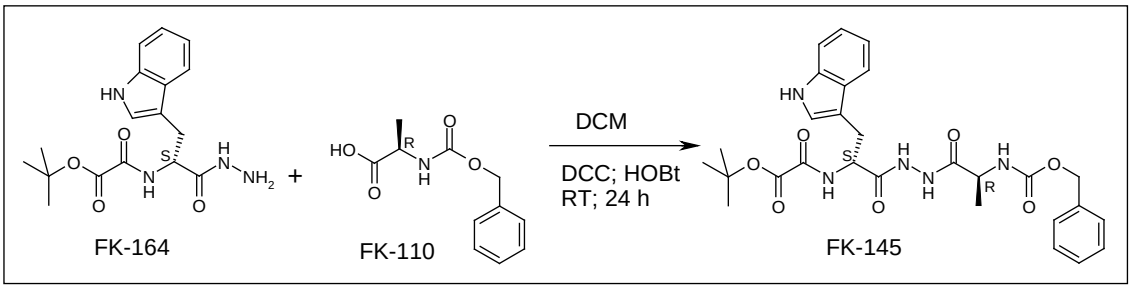
Cbz-D-Ala-OH (10.0 g; 44.8 mmol) was suspended in DCM (300 mL) and sulphuric acid (1 mL) was added. Isobutylene was bubbled in reaction mixture for 2 hrs with stirring. The resulting solution was stored at room temperature for 66 hrs. A solution of 5% potassium hydroxide solution (200 mL) was added to the reaction mixture. The organic layer was separated, washed with 100 mL of water, dried over sodium sulphate and finally filtered through Celite. The solvent was removed under reduced pressure. The resulting thick oil was dissolved in diisopropyl ether (20 mL), filtered through Celite and diluted with n-heptane (100 mL). The product, which rapidly crystallized, was separated after chilling an hour in ice-water and washed with diisopropyl ether to get 8.6 g (69%) of the title compound as colourless liquid with HPLC-purity 95%.

Ref: [1960JACS3359] on page 3361.

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 7.28-7.40 (m, 5 H), 5.31-5.45 (m, 1 H), 5.07-5.15 (m, 2 H), 4.17-4.35 (m, 1 H), 1.46 (s, 9 H), 1.38 (d, *J*=7.04 Hz, 3 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 172.2, 155.6, 136.4, 128.5, 128.1, 81.9, 66.8, 50.2, 27.9, 18.9.

***tert*-Butyl 2-[[(1S)-2-[2-[(2S)-2(benzyloxycarbonylamino)propanoyl] hydrazino]-1-(1H-indol-3-ylmethyl)-2-oxo-ethyl]amino]-2-oxo-acetate (FK-145; Cbz-Ala-NTrpO-OBu^t)**



To a solution of FK-164 (5.10 g; 14.6 mmol) in DCM (50 mL), HOBt (2.07 g; 15.3 mmol) was added, followed by DCC (3.16 g; 15.3 mmol). To this a solution of Cbz-D-Ala-OH (3.27 g; 14.6 mmol) in DCM (50 mL) was added and mixture was stirred for 24 hrs at room temperature under argon. Precipitated DCHU was removed by filtration and the filtrate was evaporated. The additional DCHU was removed by subsequent triturating with cold EtOAc and filtration. The EtOAc solution was washed with 1N HCl (100 mL * 2x), 10% $NaHCO_3$ (100 mL), brine (100 mL), dried over anhydrous sodium sulphate and concentrated. Crude compound was purified by column chromatography (MeOH : DCM) to obtain 2.0 g (25%) of the title compound as an off-white solid with HPLC-purity 95%.

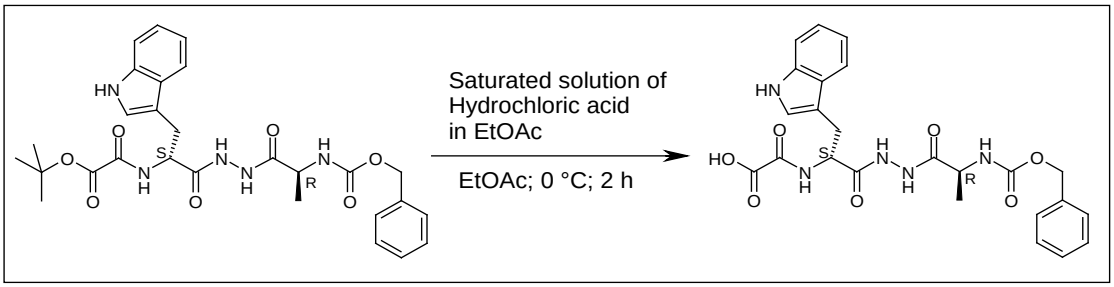
m.p. 110-112 °C

1H NMR (400 MHz, DMSO-d6) δ (ppm) 10.88 (br.s, 1 H), 10.33 (br.s, 1 H), 10.05 (br.s, 1 H), 8.57 (br.s, 1 H), 7.64-7.68 (m, 1 H), 7.53-7.58 (m, 1 H), 7.30-7.40 (m, 6 H), 7.22-7.26 (m, 1 H), 7.04-7.11 (m, 1 H), 6.95-7.02 (m, 1 H), 5.00-5.08 (m, 2 H), 4.58-4.67 (m, 1 H), 4.13-4.22 (m, 1 H), 3.12-3.29 (m, 2 H), 1.41-1.47 (m, 9 H), 1.29 (d, *J*=7.2 Hz, 3 H).

13C NMR (400 MHz, DMSO-d6) δ (ppm) 172.0, 169.8, 159.8, 158.0, 156.1, 137.5, 136.5, 128.8, 128.3, 128.2, 127.7, 124.5, 121.4, 118.9, 118.8, 111.8, 109.8, 83.7, 65.9, 53.1, 49.1, 27.9, 27.8, 18.8.

2-[[(1S)-2-[2-[(2S)-2-(Benzyloxycarbonylamino)propanoyl]hydrazino]-1-(1H-indol-3-ylmethyl)-2-oxo-ethyl]amino]-2-oxo-acetic acid

(FK-147; Cbz-Ala-NTrpO-OH)

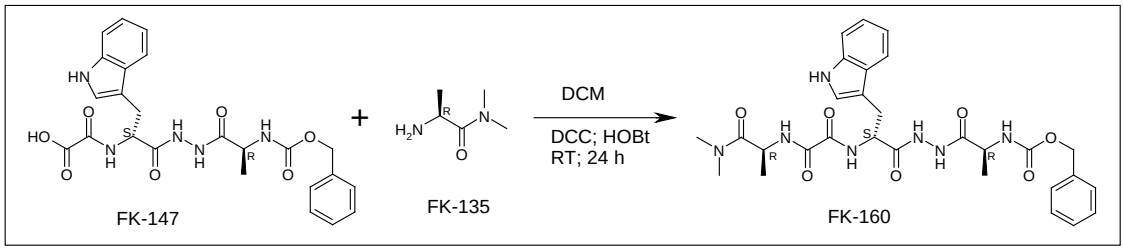


To a solution of FK-145 (200 mg; 360 mmol) in EtOAc (10 mL) was added a hydrochloric acid solution saturated in EtOAc (10 mL) drop-wise at 0°C, the reaction mixture was further stirred at room temperature for 2 hrs. The solvent was evaporated under reduced pressure and the solid was dried under vacuum to get 180 mg (quant.) of the title compound as a white solid with HPLC-purity 96%.

1H NMR (400 MHz, DMSO-d6) δ (ppm) 10.85 (br.s, 1 H), 10.32 (br.s, 1 H), 10.04 (br.s, 1 H), 8.55-8.67 (d, *J*=8.5 Hz, 1 H), 7.61-7.71 (d, *J*=7.8 Hz, 1 H), 7.49-7.59 (d, *J*=7.5 Hz, 1 H), 7.27-7.42 (m, 6 H), 7.19-7.24 (m, 1 H), 6.92-7.12 (m, 2 H), 4.97-5.08 (m, 2 H), 4.61-4.71 (m, 1 H), 4.12-4.21 (m, 1 H), 3.11-3.30 (m, 2 H), 1.27 (d, *J*=7.2 Hz, 3 H).

13C NMR (400 MHz, DMSO-d6) δ (ppm) 172.0, 169.8, 162.0, 158.4, 156.1, 137.4, 136.5, 128.8, 128.3, 128.2, 127.7, 124.4, 121.4, 118.9, 118.8, 111.8, 109.9, 65.9, 53.0, 49.1, 27.9, 18.8.

Benzyl *N*-[(1S)-2-[2-[(2S)-2-[[2-[[(1S)-2-(dimethylamino)-1-methyl-2-oxo-ethyl]amino]-2-oxo-acetyl]amino]-3-(1H-indol-3-yl)propanoyl] hydrazino]-1-methyl-2-oxo-ethyl]carbamate (FK-160)



FK-147 FK-135 FK-160

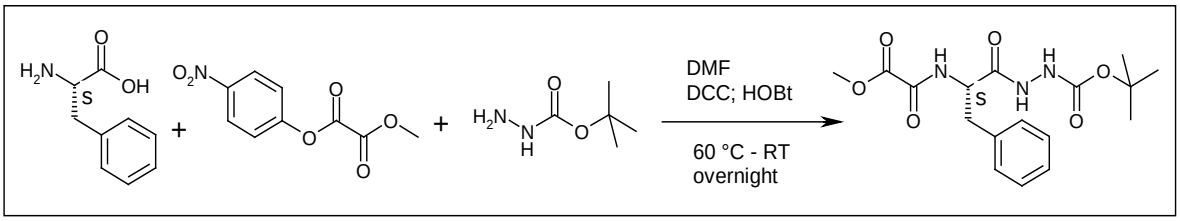
To a solution of FK-147 (130 mg; 0.26 mmol) in DCM (10 mL) was added HOBt (36 mg; 0.27 mmol) and DCC (56 mg; 0.27 mmol). A solution of FK-135 (30 mg; 0.26 mmol) in DCM (10 mL) was added to the reaction mixture and stirred overnight at room temperature under argon. Precipitated DCHU was removed by filtration and DCM was removed under reduced pressure. The additional DCHU was removed by subsequent triturating with cold EtOAc and filtration. The EtOAc solution was washed with 1N HCl (100 mL * 2x), 10% $NaHCO_3$ (100 mL), brine (100 mL), dried over anhydrous sodium sulphate and concentrated to give the crude compound which was crystallised from EtOAc (10 mL) to give an off-white solid.

Yield 64%.

Compound was insoluble in common NMR solvents (e.g. CDCl3, MeOD, DMSO, CF3COOD) and due to this solubility issue could not be identified.

6. Rearranged Product

<u>Methyl 2-[[(1S)-1-benzyl-2-(2-tert-butoxycarbonylhydrazino)-2-oxo-ethyl]amino]-2-oxo-acetate</u> (FK-19; Boc-NPheO-OMe)



To a solution of L-phenylalanine (2.00 g; 12.0 mmol) in DMF (50 mL) was added oxalic acid methyl ester 4-nitrophenyl ester (3.24 g; 14.0 mmol) and stirred at 60°C for 1.5 h under argon. The reaction mixture was cooled to room temperature. HOBt (1.70 g; 13.0 mmol), *tert*-butyl carbazate (1.90 g; 14.0 mmol) and DCC (2.59 g; 13.0 mmol) were added to the reaction mixture and stirred overnight at room temperature under argon. The solvent was removed under reduced pressure and the residue was re-dissolved in EtOAc, cooled in freezer for 4 hrs to completely separate the DCHU formed. The DCHU was removed by filtration. The organic phase was washed with 1N HCl, 10% $NaHCO_3$ and finally with brine (3x) and dried over anhydrous sodium sulphate. The solvent was evaporated to get the crude product which was purified by column chromatography (pet-ether : EtOAc) to get 3.2 g (74%) of the title compound as an off-white solid with HPLC-purity 97%.

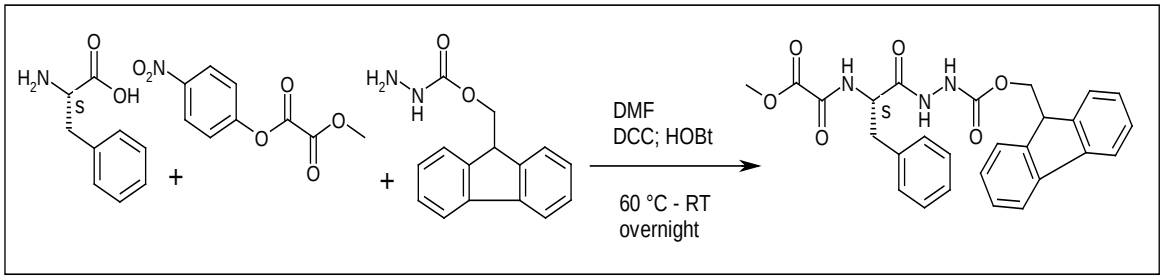
m.p. 62-64 °C

Ref: [2007DISS1 on p102]

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 8.65 (br.s, 1 H), 7.90 (br.s, 1 H), 7.11-7.19 (m, 5 H), 6.77 (br.s, 1 H), 4.72-4.86 (m, 1 H), 3.72 (s, 3 H), 2.95-3.24 (m, 2 H), 1.35 (s, 9 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 169.7, 160.2, 156.4, 155.4, 135.9, 129.3, 128.6, 127.1, 82.0, 53.7, 53.2, 37.6, 28.1.

Methyl 2-[[(1S)-1-benzyl-2-[2-(9H-fluoren-9-ylmethoxycarbonyl) hydrazino]-2-oxo-ethyl]amino]-2-oxo-acetate
(FK-44; Fmoc-NPheO-OMe)



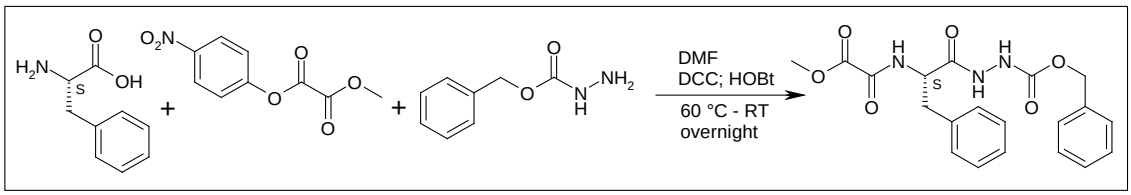
To a solution of L-phenylalanine (2.00 g; 12.0 mmol) in DMF (50 mL) was added oxalic acid methyl ester 4-nitrophenyl ester (3.51 g; 15.6 mmol) and stirred at 60°C for 1.5 h under argon. The reaction mixture was cooled to room temperature. HOBt (1.70 g; 13.0 mmol), Fmoc-hydrazide (3.56 g; 14.0 mmol) and DCC (2.59 g; 13.0 mmol) were added to the reaction mixture and stirred overnight at room temperature under argon. The solvent was removed under reduced pressure and the residue was re-dissolved in EtOAc. The mixture was cooled in freezer for 4 hrs to completely separate the DCHU formed. The DCHU was removed by filtration. The organic phase was washed with 1N HCl, 10% $NaHCO_3$ and finally with brine (3x) and dried over anhydrous sodium sulphate. The solvent was evaporated and the crude product was purified by column chromatography (pet-ether : EtOAc) to get 3.9 g (66%) of the target compound as a white solid with HPLC-purity 94%.

m.p. 85-87 °C.

Ref: [2007DISS1 on p102]

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 8.75 (br.s, 1 H), 7.87 (br.s, 1 H), 7.61 (d, *J*=7.4 Hz, 2 H), 7.42 (d, *J*=7.4 Hz, 2 H), 7.18-7.32 (m, 3 H), 6.95-7.15 (m, 7 H), 4.60-4.85 (m, 1 H), 4.18-4.38 (m, 2 H), 4.03-4.12 (m, 1 H), 3.62 (m, 3 H), 2.81-3.33 (m, 2 H).
13C NMR (200 MHz, $CDCl_3$) δ (ppm) 170.0, 160.06, 156.57, 156.19, 143.45, 143.42, 141.22, 135.68, 129.26, 128.71, 127.80, 127.14, 125.12, 119.98, 68.07, 53.68, 53.34, 46.77, 37.65.

Methyl 2-[[(1S)-1-benzyl-2-(2-benzyloxycarbonylhydrazino)-2-oxo-ethyl]amino]-2-oxo-acetate (FK-55; Cbz-NPheO-OMe)



To a solution of L-phenylalanine (2.00 g; 12.0 mmol) in DMF (50 mL) was added oxalic acid methyl ester 4-nitrophenyl ester (3.51 g; 14.0 mmol) and stirred at 60°C for 1.5 h under argon. The reaction mixture was cooled to room temperature. HOBt (1.70 g; 13.0 mmol), Cbz-$NHNH_2$ (1.99 g; 12.0 mmol) and DCC (2.59 g; 13.0 mmol) was added to the reaction mixture and stirred overnight at room temperature under argon. The solvent was removed under reduced pressure and the residue was re-dissolved in EtOAc, cooled in freezer for 4 hrs to completely separate the DCHU formed. The DCHU was removed by filtration. The organic phase was washed with 1N HCl, 10% $NaHCO_3$ and finally with brine (3x) and dried over anhydrous sodium sulphate. The solvent was evaporated to get the crude product which was purified by column chromatography (pet-ether : EtOAc) to get 3.3 g (69%) as an off-white solid with HPLC-purity 97%.

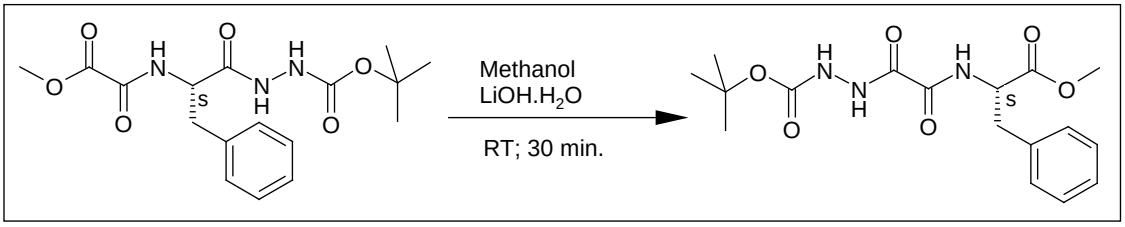
m.p. 60-62 °C

Ref: [2007DISS1 on p105]

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 8.56 (br.s, 1 H), 7.82 (br.s, 1 H), 7.06-7.29 (m, 10 H), 6.98 (br.s, 1 H), 4.98-5.09 (m, 2 H), 4.67-4.82 (m, 1 H), 3.66 (s, 3 H), 2.91-3.22 (m, 2 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 169.9, 160.1, 156.5, 156.1, 135.7, 135.4, 129.3, 128.7, 128.5, 128.4, 128.2, 127.2, 67.9, 53.2, 37.6.

<u>Methyl (2S)-2-[[2-(2-tert-butoxycarbonylhydrazino)-2-oxo-acetyl] amino]-3-phenyl-propanoate</u> (FK-20)



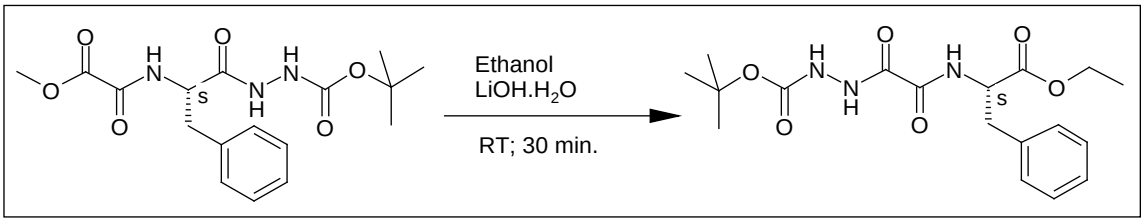
To a solution of FK-19 (1000 mg; 2.7 mmol) in MeOH (20 mL) was added $LiOH.H_2O$ (115 mg; 2.7 mmol) along with 2 drops of water. The solution was stirred at room temperature for 15 min. The solvent was removed on rotovap and the solid was dissolved in water and the compound was extracted with EtOAc (50 mL * 3x). The organic phase was dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure to get 200 mg (57%) of the title compound as a white solid with HPLC-purity 98%.

m.p. 50-53 °C.

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 9.00 (br.s, 1 H), 7.75 (br.s, 1 H), 7.01-7.27 (m, 5 H), 6.69 (br.s, 1 H), 4.76 (m, 1 H), 3.64 (s, 3 H), 3.04-3.12 (m, 2 H), 1.39 (s, 9 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 170.6, 158.1, 157.7, 154.2, 135.2, 129.1, 128.7, 127.3, 82.3, 53.6, 52.5, 37.8, 28.1.

Ethyl (2S)-2-[[2-(2-tert-butoxycarbonylhydrazino)-2-oxo-acetyl]amino]-3-phenyl-propanoate (FK-20-11)



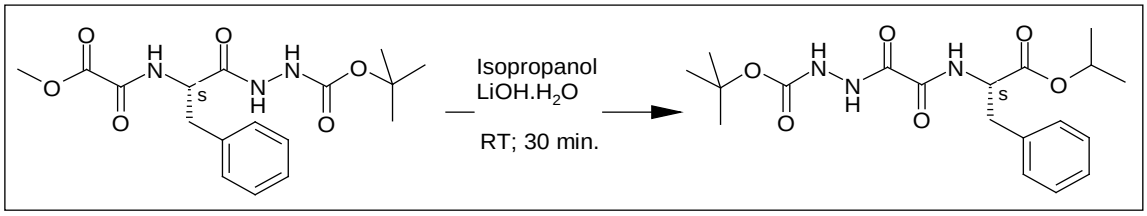
To a solution of FK-19 (1000 mg; 2.7 mmol) in EtOH (20 mL) was added LiOH.H2O (115 mg; 2.7 mmol) along with 2 drops of water. The solution was stirred at room temperature for 15 minutes. The solvent was removed using a rotovap and the solid was dissolved in water and the compound was extracted with EtOAc (50mL * 3x). The organic phase was dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure to get 483 mg (51%) of the title compound as a white solid with HPLC-purity 96%.

m.p. 61-63 °C.

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 9.14 (br.s, 1 H), 8.76 (br.s, 4 H), 8.25 (br.s, 1 H), 7.00-7.22 (m, 5 H), 4.66-4.84 (m, 1 H), 2.87-3.28 (m, 2 H), 1.92-2.01 (m, 2 H), 1.11-1.42 (m, 12 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 170.1, 160.4, 157.9, 155.9, 135.8, 129.3, 128.7, 127.1, 82.5, 60.5, 53.8, 37.5, 28.0, 14.1.

Isopropyl (2S)-2-[[2-(2-tert-butoxycarbonylhydrazino)-2-oxo-acetyl] amino]-3-phenyl-propanoate (FK-179)



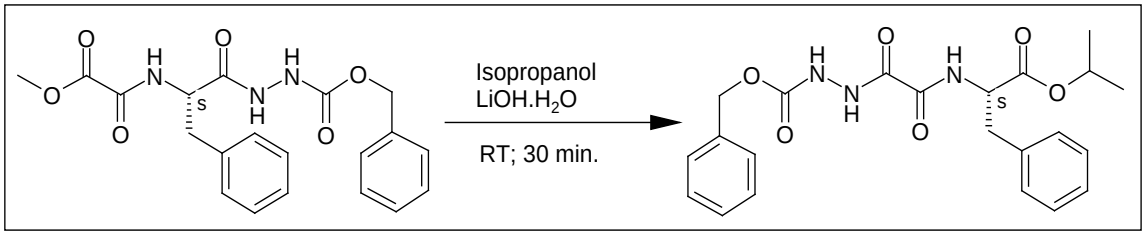
To a solution of FK-19 (1000 mg; 2.7 mmol) in isopropanol (20 mL) was added $LiOH.H_2O$ (115 mg; 2.7 mmol) along with 2 drops of water. The solution was stirred at room temperature for 15 min. The solvent was removed under reduced pressure and the solid was dissolved in water and the compound was extracted with EtOAc (50 mL * 3x). The combined organic phase was dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure to get 400 mg (42%) of the title compound as a white solid with HPLC-purity 99%.

m.p. 62-64 °C

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 8.37 (br.s, 1 H), 7.74 (br.s, 1 H), 7.10-7.27 (m, 5 H), 6.58 (br.s, 1 H), 4.94-5.09 (m, 1 H), 4.66-4.81 (m, 1 H), 2.96-3.26 (m, 2 H), 1.36 (s, 9 H), 1.24 (d, *J*=6.5 Hz, 6 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 169.6, 159.2, 156.9, 155.2, 135.8, 129.3, 128.7, 127.1, 82.0, 71.8, 53.2, 37.5, 28.1, 21.4.

Isopropyl 2-[[2-(N'-benzyloxycarbonylhydrazino)-2-oxo-acetyl]amino]-3-phenyl-propanoate (FK-193-1-1)



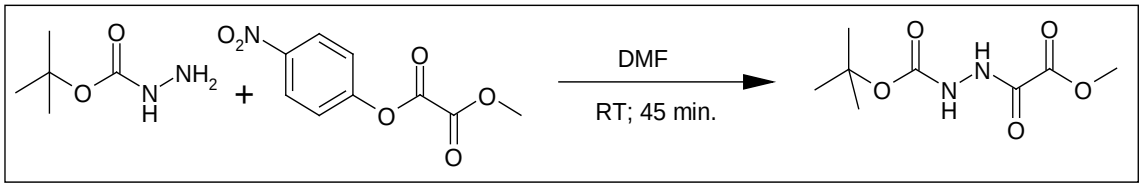
To a solution of FK-55 (1000 mg; 2.5 mmol) in isopropanol (20 mL) was added $LiOH.H_2O$ (105 mg; 2.5 mmol) along with 2 drops of water. The solution was stirred at room temperature for 15 minutes. The solvent was removed under reduced pressure and residue was re-dissolved in water and the compound was extracted with EtOAc (50 mL * 3x). The combined organic phase was dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure to get 450 mg (42%) of the title compound as an off-white solid with HPLC-purity 96%.

m.p. 53-54 °C

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 8.53 (br.s, 1 H), 7.75 (br.s, 1 H), 7.11-7.31 (m, 10 H), 6.93 (br.s, 1 H), 4.90-5.05 (m, 3 H), 2.89-3.20 (m, 2 H), 1.21 (d, *J*=1.6 Hz, 3 H), 1.17 (d, *J*=1.8 Hz, 3 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 164.7, 153.8, 151.8, 150.8, 130.4, 130.2, 124.1, 123.5, 123.3, 123.1, 122.9, 121.9, 66.6, 62.6, 48.0, 32.3, 16.2.

***tert*-Butyl 2-[methoxy(oxo)acetyl]hydrazinecarboxylate (FK-37)**



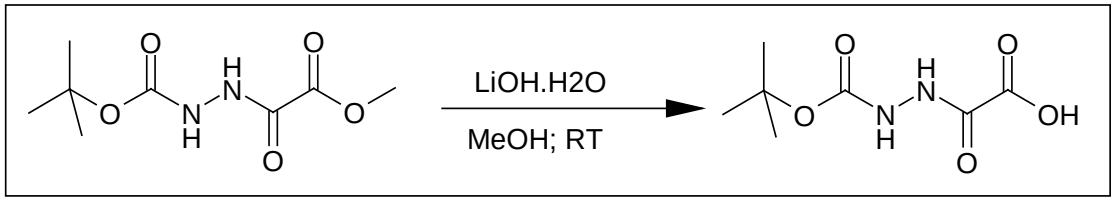
To a solution of *tert*-butyl carbazate (2.0 g; 15.0 mmol) in DMF (30 mL) was added methyl 4-nitrophenyl oxalate (3.8 g; 17.0 mmol) and stirred at room temperature for 45 min under argon. The reaction mixture was washed with 10% $NaHCO_3$ solution and extracted with EtOAc (3x). The combined organic phase was dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure. The crude compound obtained was further purified by column chromatography (pet-ether : EtOAc) to yield 830 mg (25%) of the title compound with HPLC-purity 94%.

m.p. 93-94 °C

1H NMR (200MHz, $CDCl_3$) δ (ppm) 9.27 (br.s, 1 H), 7.27 (br.s, 1 H), 3.83 (s, 3 H), 1.39 (s, 9 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 159.56, 155.40, 154.69, 82.31, 53.60, 27.96.

[2-(*tert*-Butoxycarbonyl)hydrazino](oxo)acetic acid (FK-38)



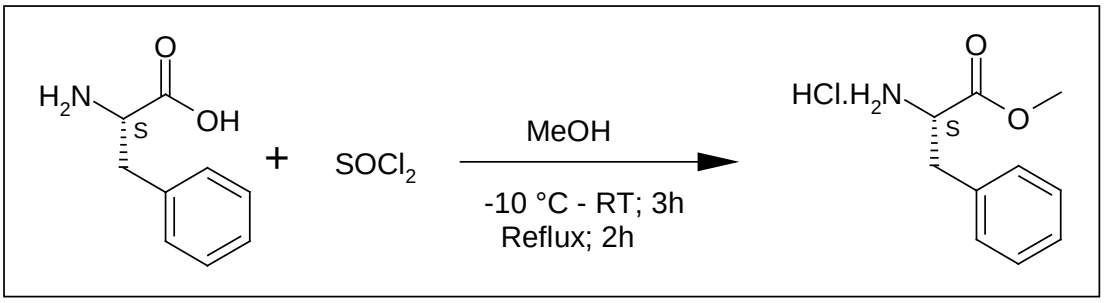
To a solution of FK-37 (830 mg; 38.0 mmol) in MeOH (15 mL) was added $LiOH.H_2O$ (160 mg; 38.0 mmol) along-with two drops of water. The reaction mixture was stirred at room temperature for 30 min. The solvent was removed under reduced pressure and residue was re-dissolved in water and was extracted with EtOAc (50 mL * 3x). Aqueous phase was cooled to ice bath temperature and then added 5N HCl drop-wise till the pH 3. The resulting mixture was extracted with EtOAc (3x) and the combined organic phase was washed with brine and dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure and the crude compound was purified by column chromatography to give 200 mg (25%) of the title compound with HPLC-purity 99%.

m.p. 121-122 °C

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 1.4 (s, 9 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 161.32, 158.17, 154.60, 79.45, 27.93.

Phenylalanine methyl ester hydrochloride (FK-35)



To a pre-cooled MeOH (40 mL) at -10 °C, thionyl chloride (3.96 g; 33.0 mmol) was added drop-wise in a round bottomed flask. After stirring for 15 min, L-phenylalanine (5.00 g; 30.0 mmol) was added to this mixture and let it warm to room temperature with constant stirring for 3 hrs. The reaction mixture was refluxed for 2 hrs. Volatiles were removed under reduced pressure and the residue was washed with ethanol to yield 3.2 g (58%) of the title compound as a white solid with HPLC-purity 97%.

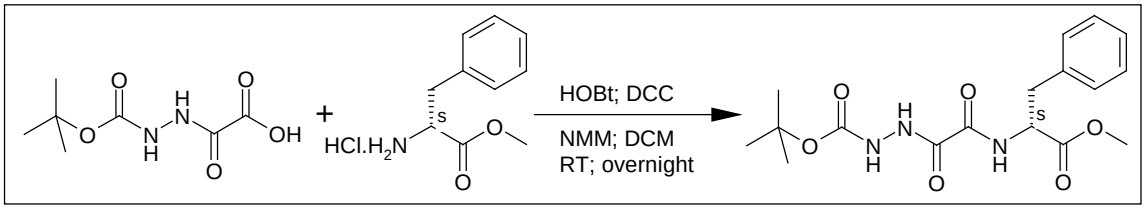
m.p. 162-163 °C

Lit. m.p. 158-160 °C

Ref: [2008SC684]

13C NMR (200 MHz, DMSO-d_6) δ ppm 169.24, 134.75, 129.32, 128.50, 127.16, 53.23, 52.42, 35.73.

Methyl *N*-[[2-(*tert*-butoxycarbonyl)hydrazino](oxo) acetylphenylalaninate (FK-43)



To a solution of compound FK-38 (120 mg; 0.80 mmol) in DCM (20 mL) was added HOBt (114 mg; 0.84 mmol) and DCC (173mg; 0.84 mmol) and stirred. A solution of phenylalanine methylester hydrochloride (173 mg; 0.80 mmol) dissolved in DCM (2 mL) and NMM (89 mg; 0.88 mmol) was added to the pre-stirred reaction mixture. The reaction mixture was stirred overnight at room temperature under argon. DCM was removed under reduced pressure and the residue was re-dissolved in EtOAc. The ethyl acetate mixture was cooled in freezer for 4 hrs to completely separate the DCHU formed and was removed by filtration. The organic phase was washed with 1N HCl, 10% $NaHCO_3$ and finally with brine, dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure and the crude compound was further purified by column chromatography (pet-ether : EtOAc) to get 160 mg (36%) of the title compound as a white solid with HPLC-purity 98%.

m.p. 50-52 °C

1H NMR (200 MHz, $CDCl_3$) δ (ppm) 9.06 (br.s, 1 H), 7.79 (br.s, 1 H), 7.02-7.24 (m, 5 H), 6.79 (br.s, 1 H), 4.70-4.82 (m, 1 H), 3.63 (s, 3 H), 3.04-3.12 (m, 2 H), 1.39 (s, 9 H).

13C NMR (200 MHz, $CDCl_3$) δ (ppm) 170.64, 158.17, 157.77, 154.28, 135.28, 129.14, 128.72, 127.30, 82.25, 53.55, 52.54, 37.74, 28.06.

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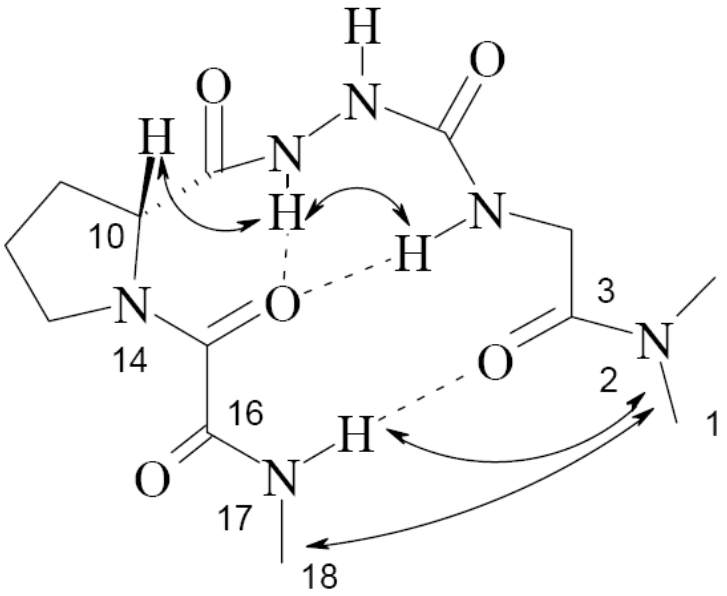
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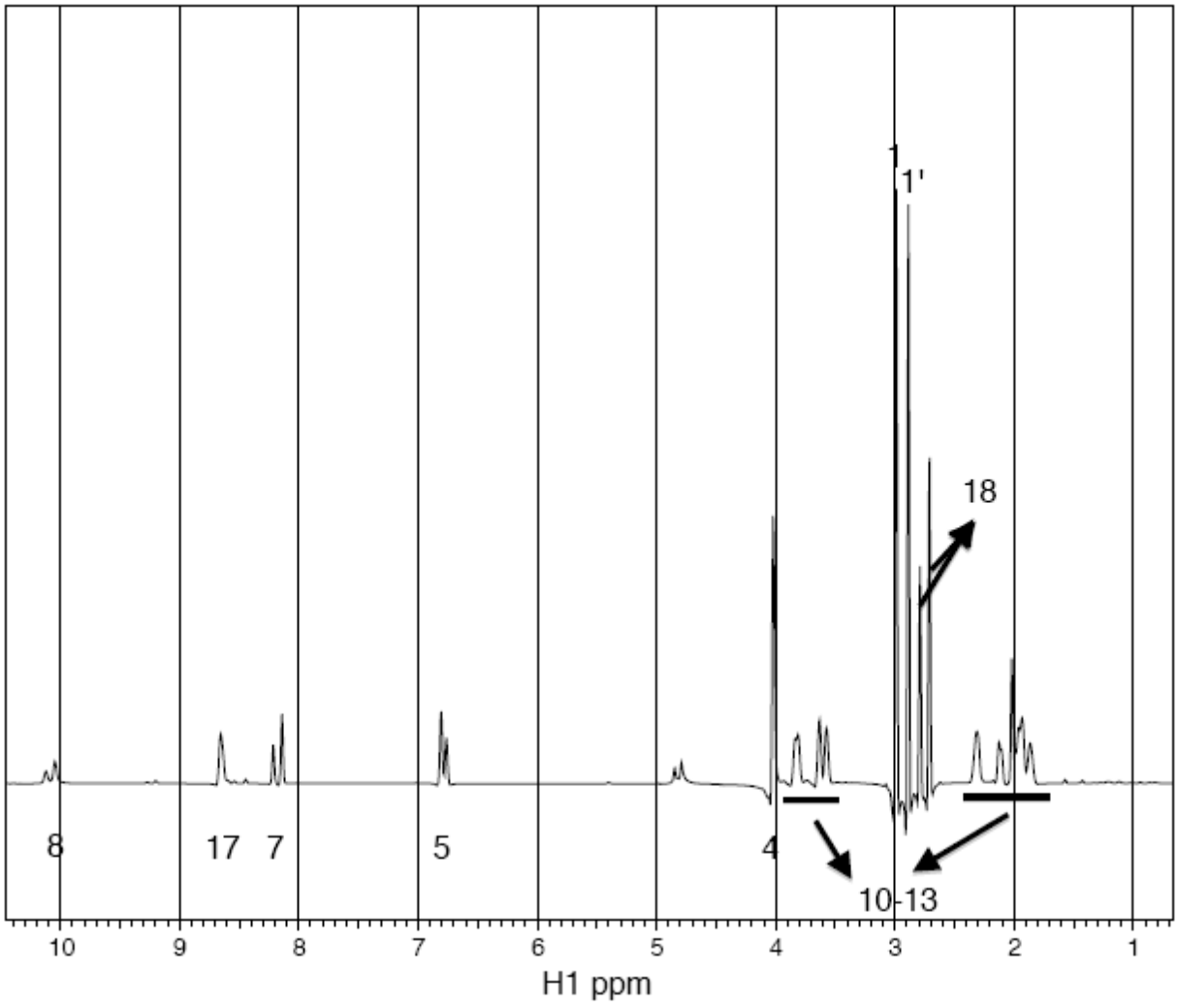
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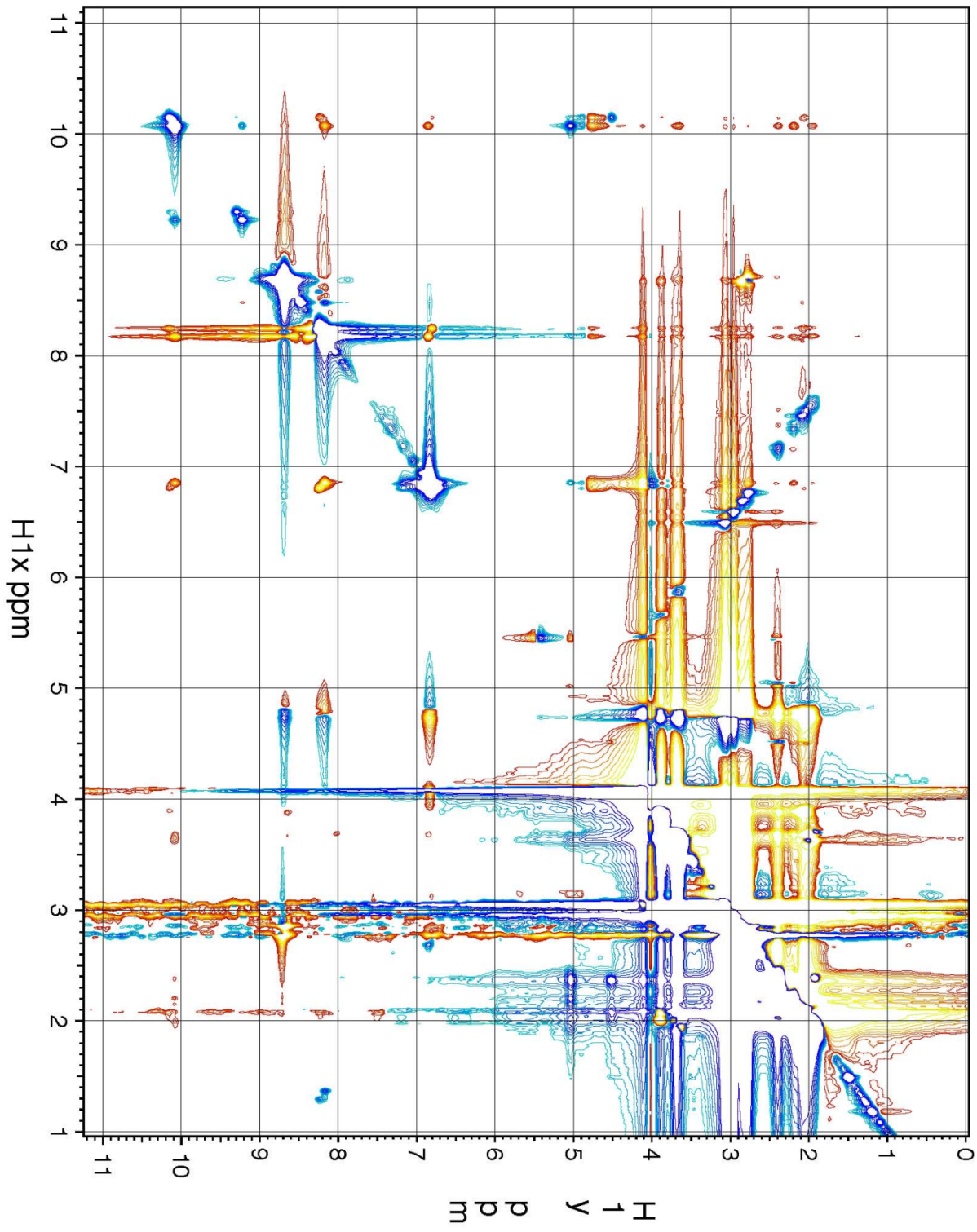

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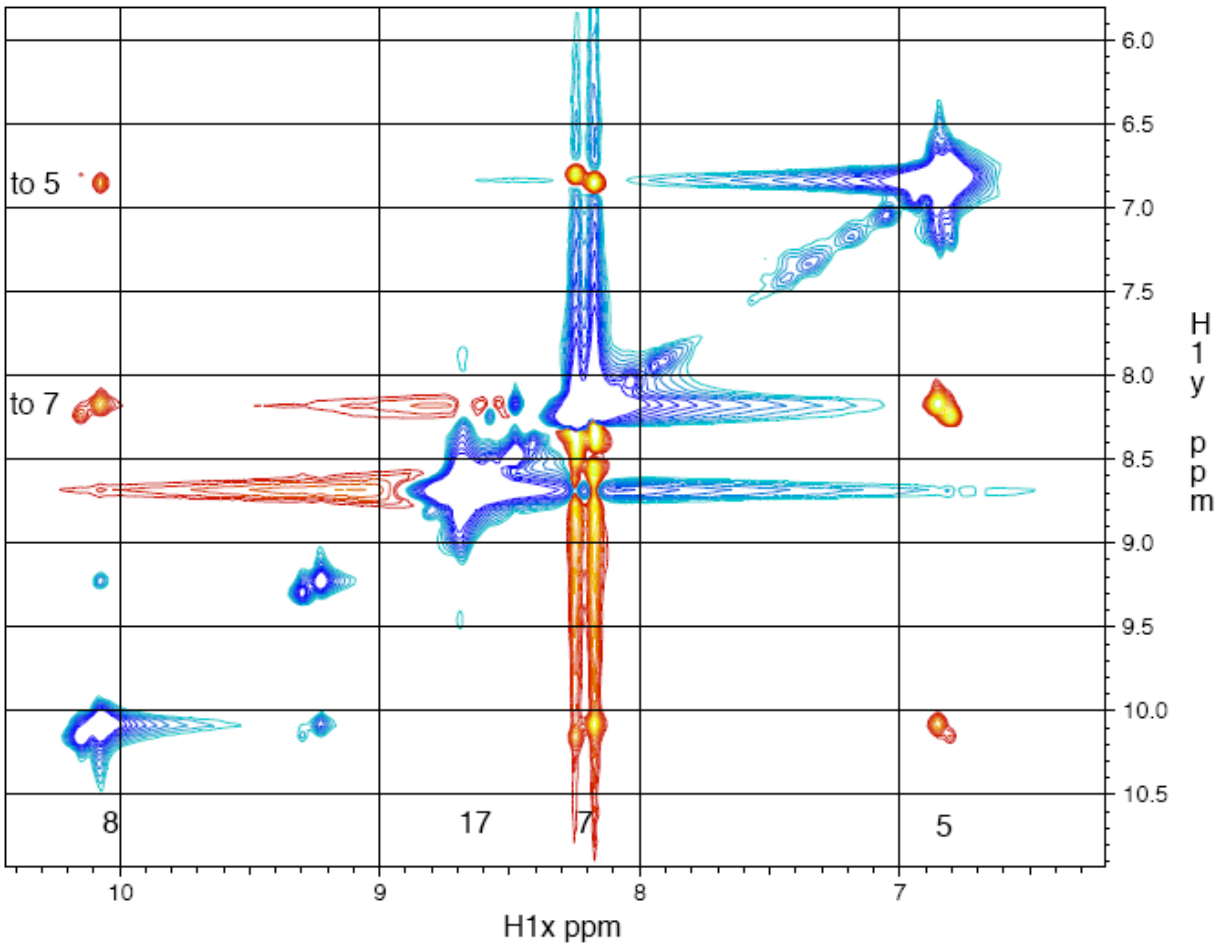

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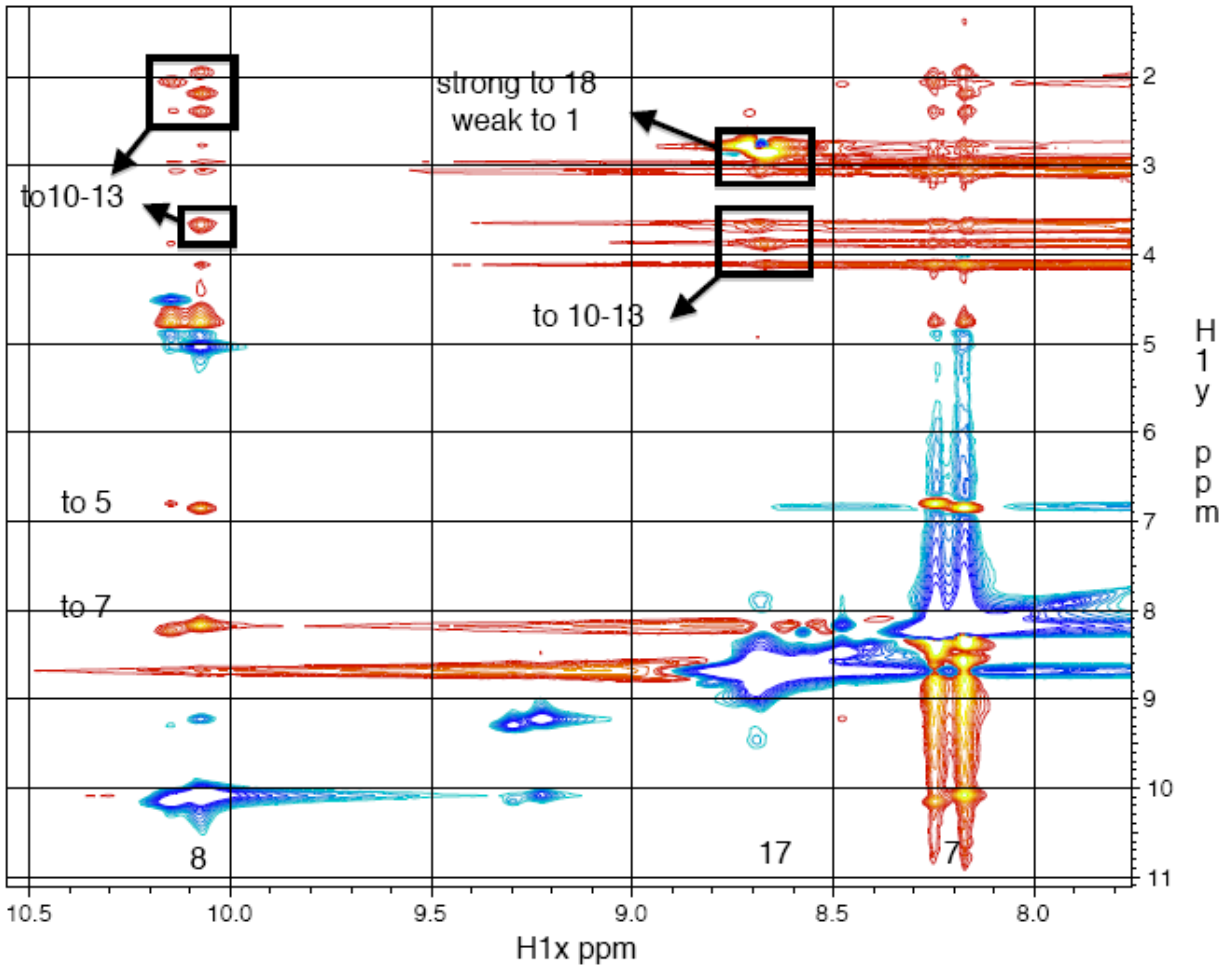

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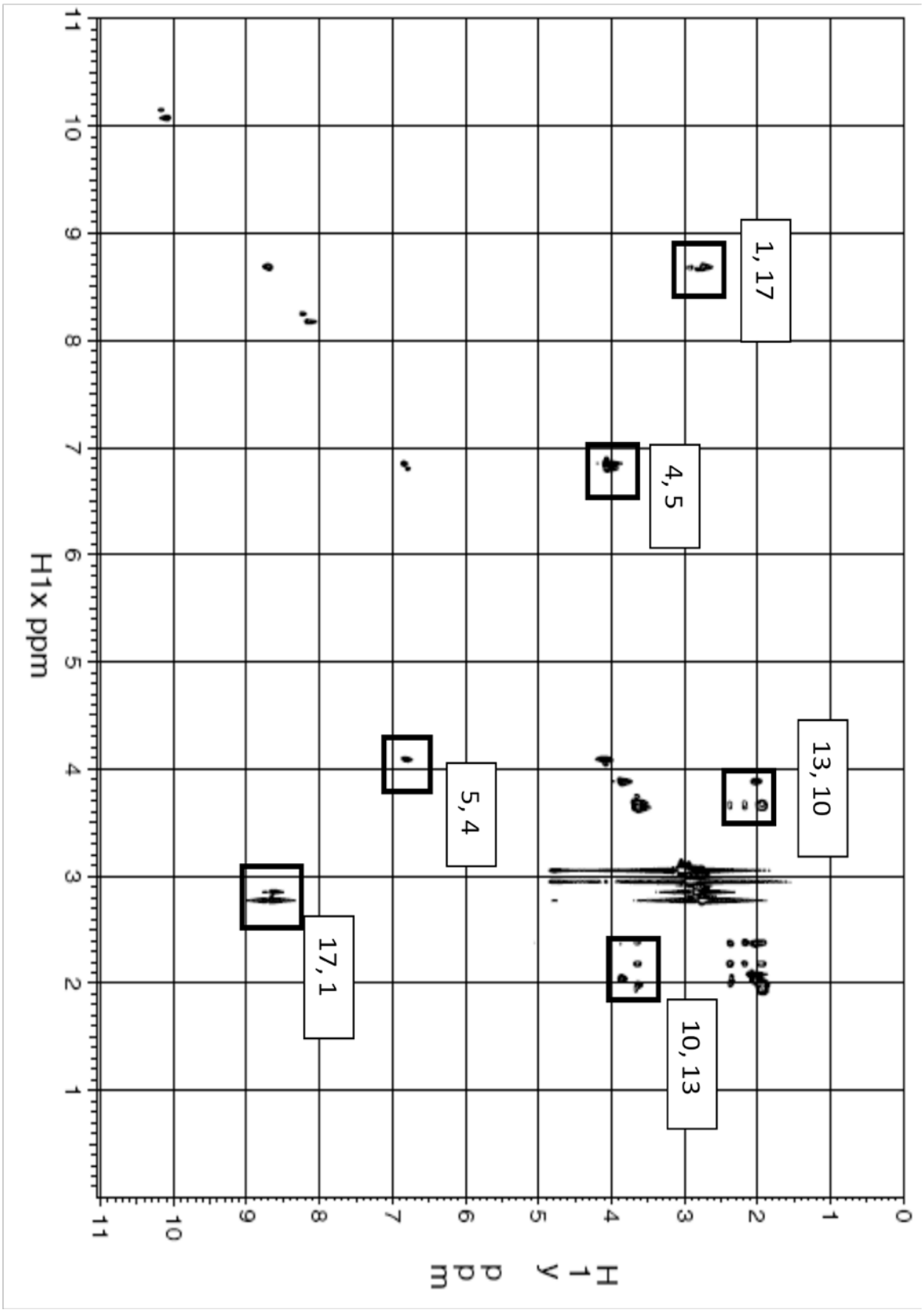

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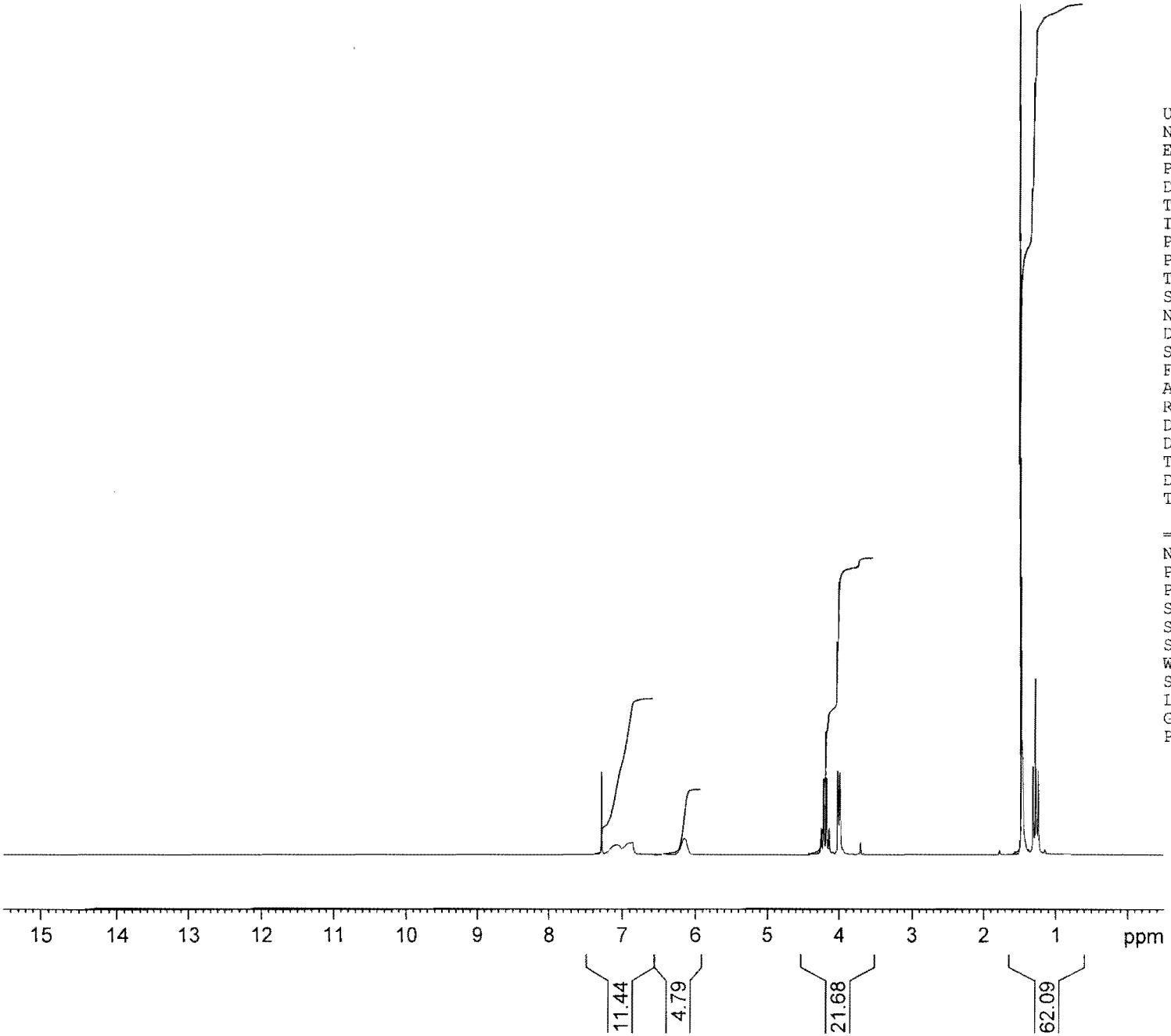

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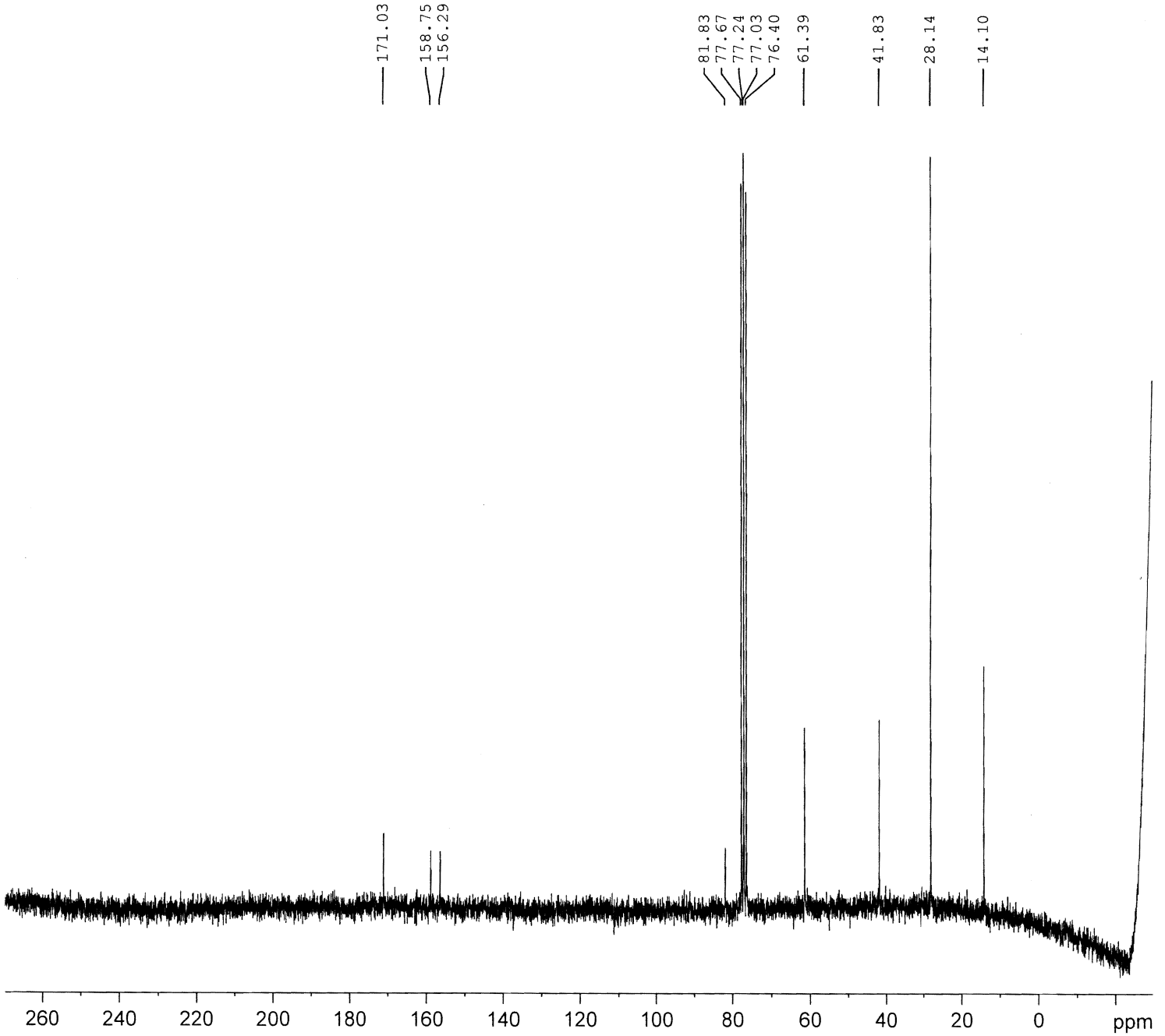

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9 Dec 2009

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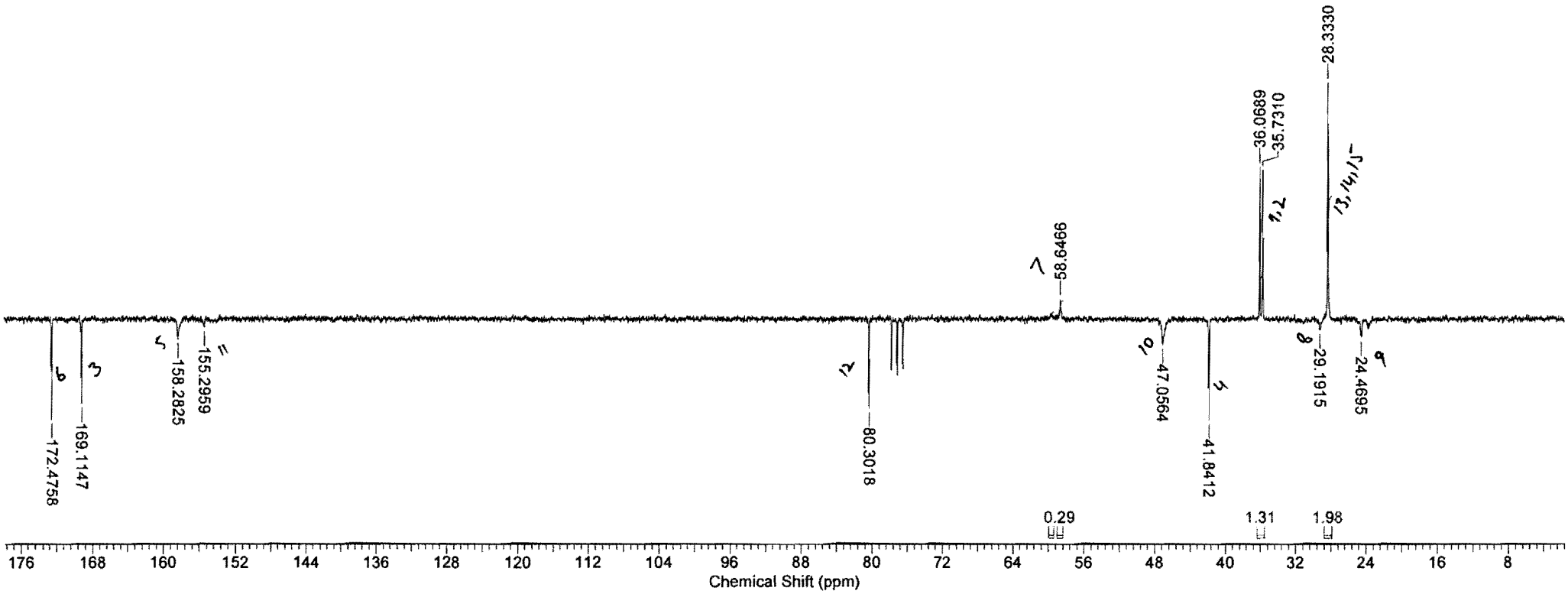

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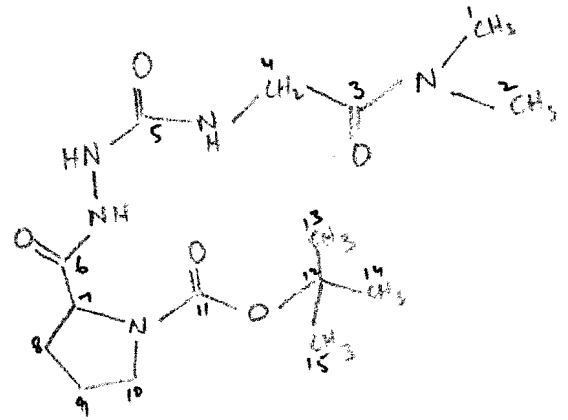

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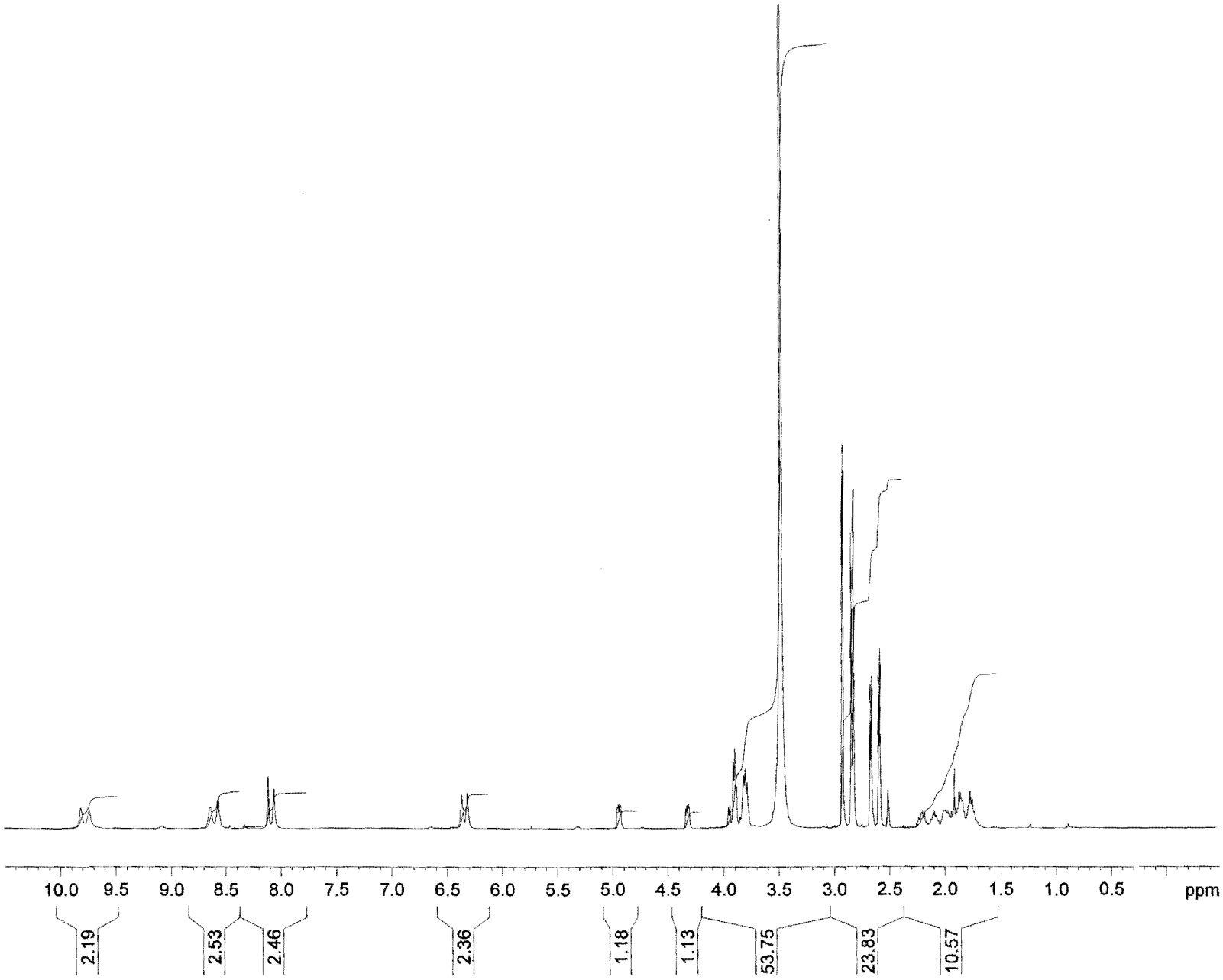


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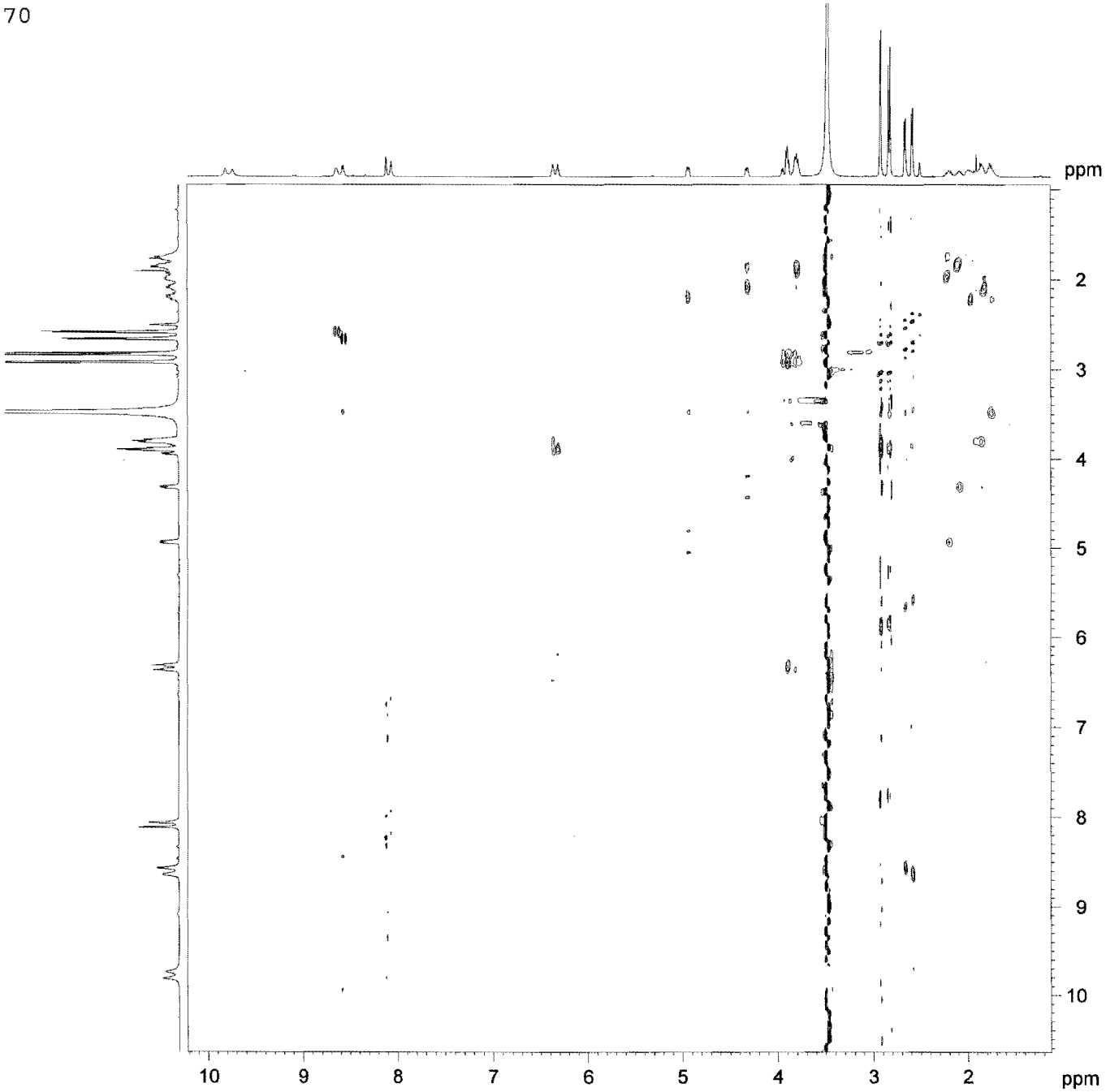


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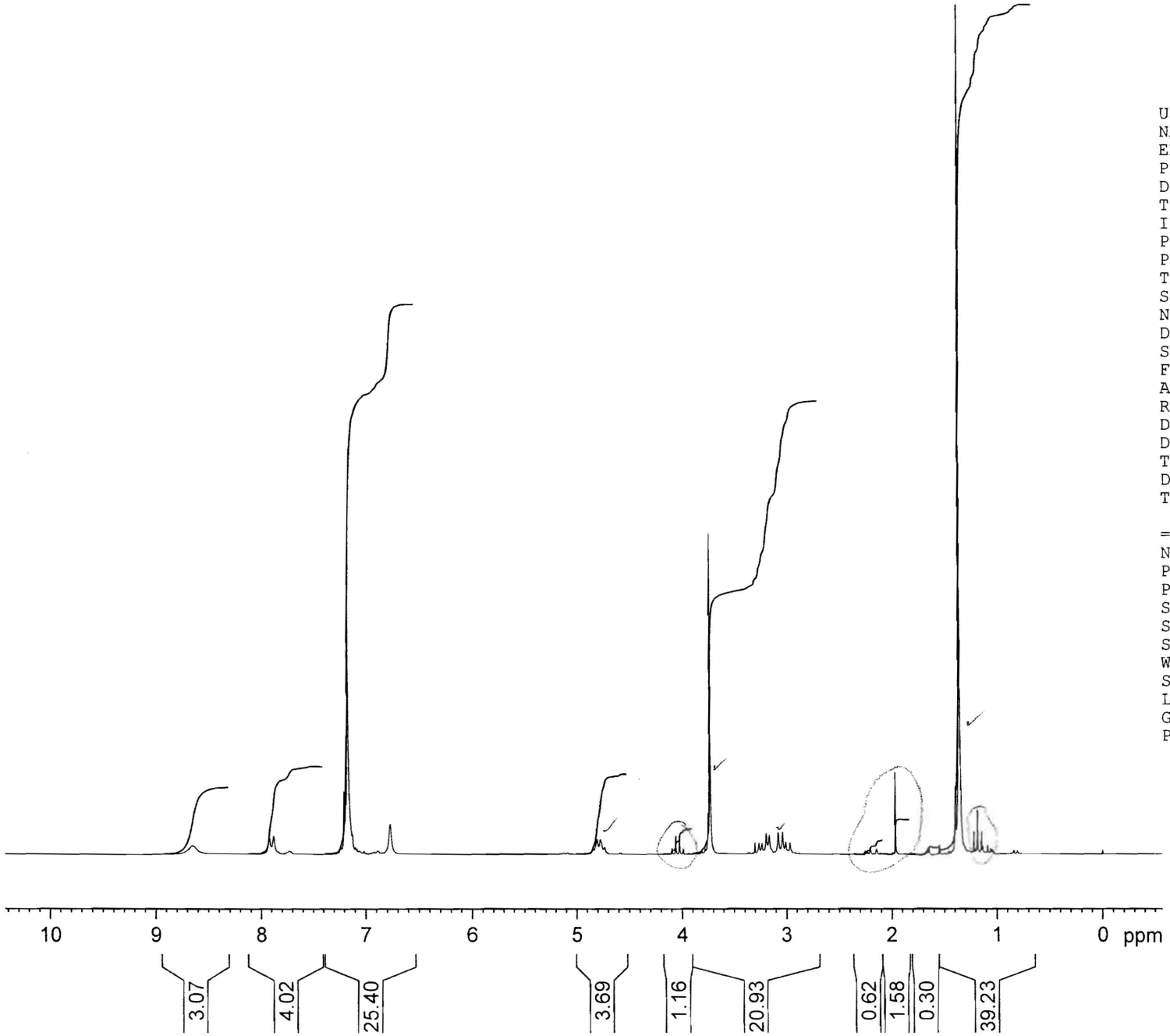

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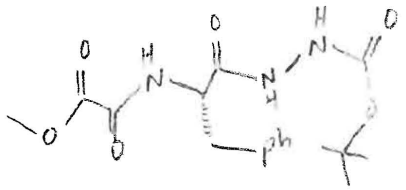
FK-19-5



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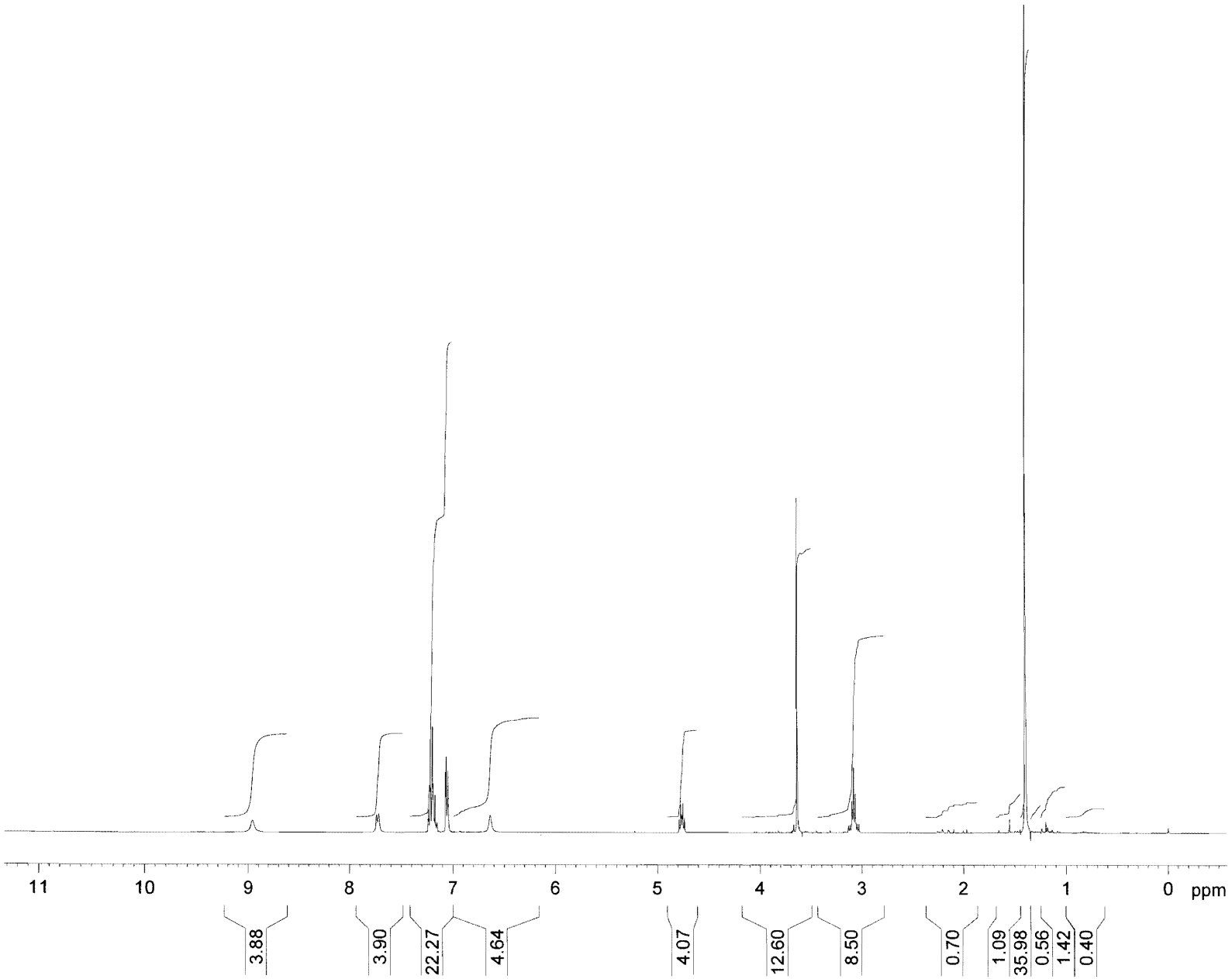
FK-20-8-1

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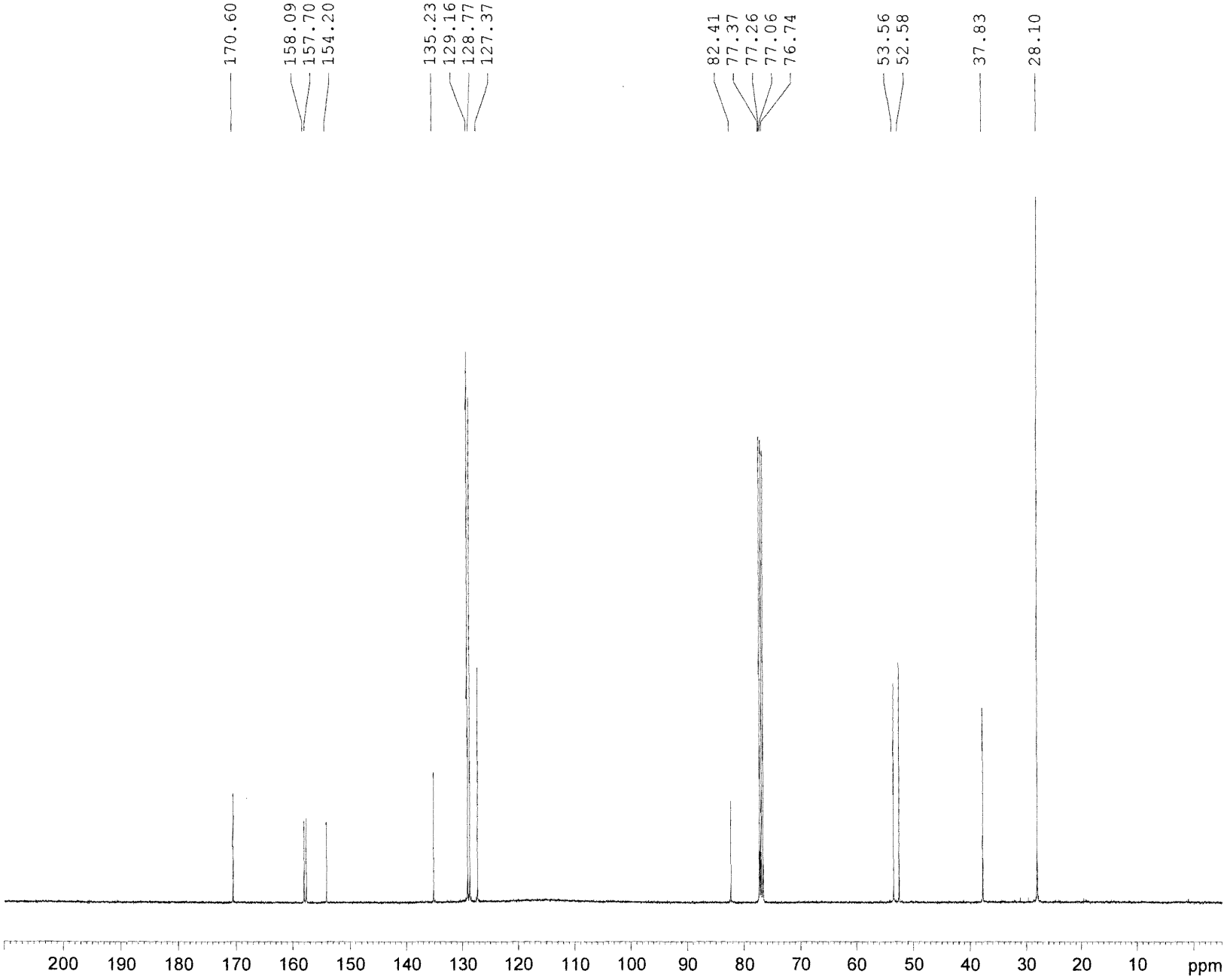

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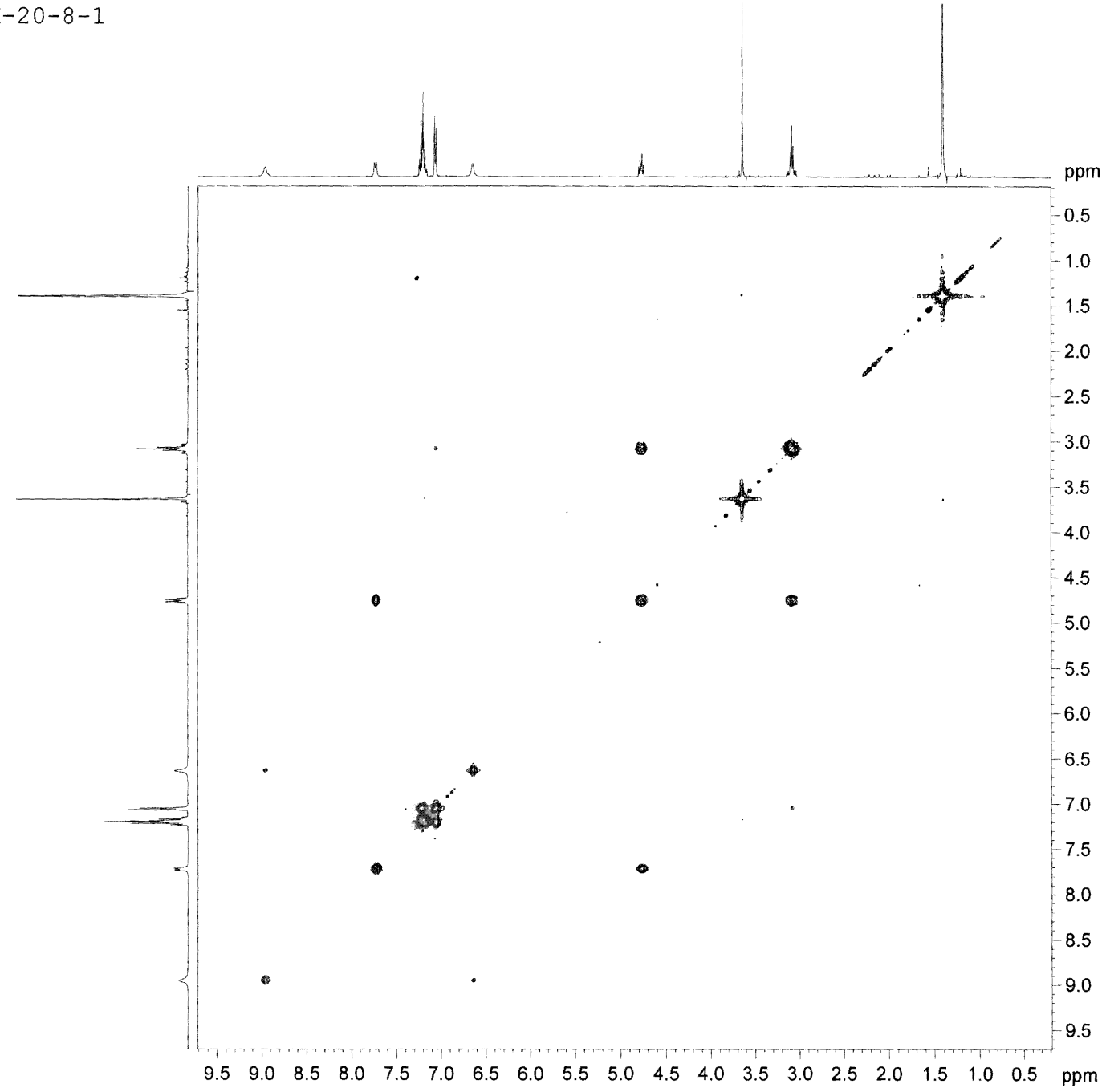


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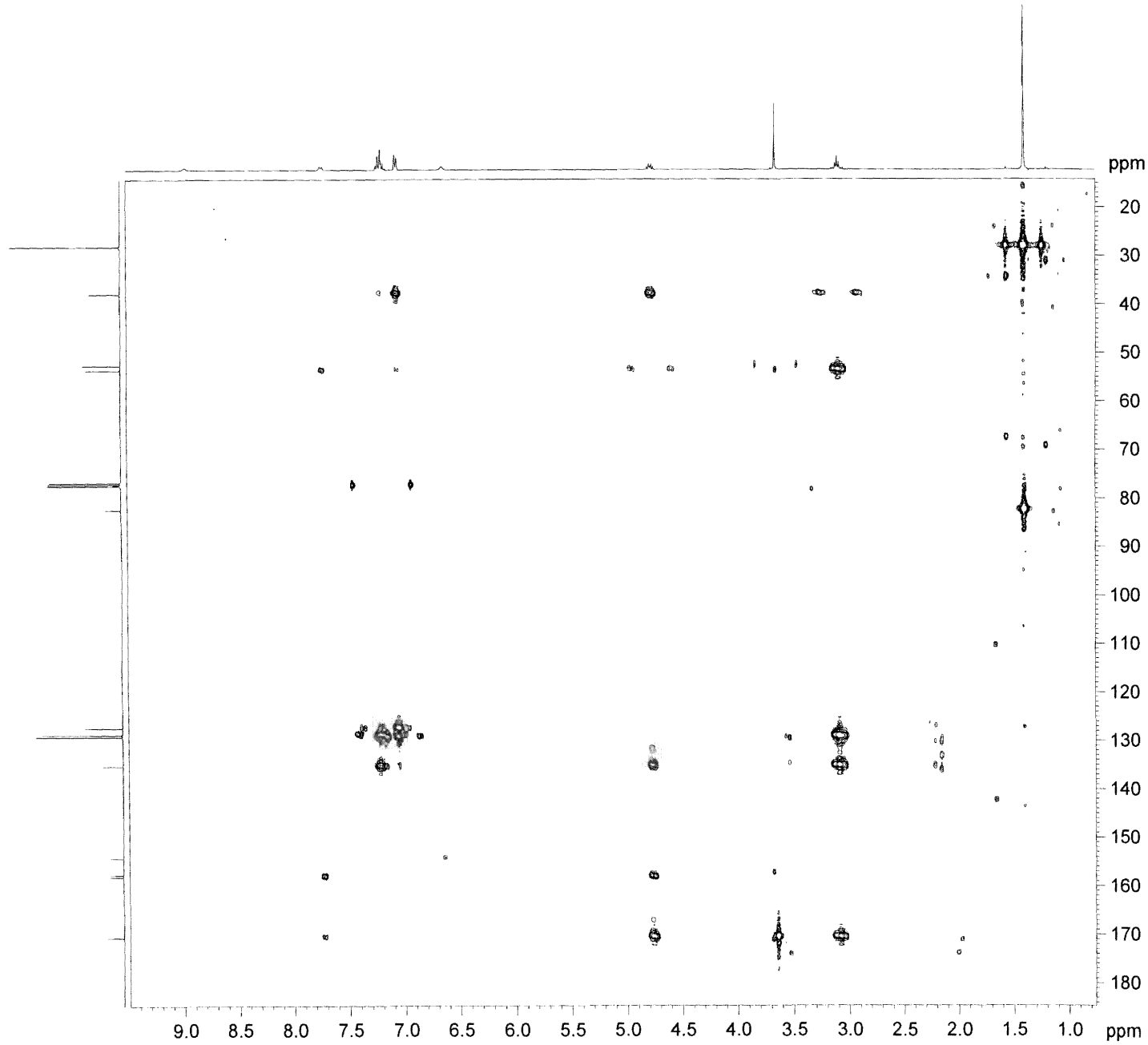


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GB                     0
PC                  1.40

F1 - Processing parameters
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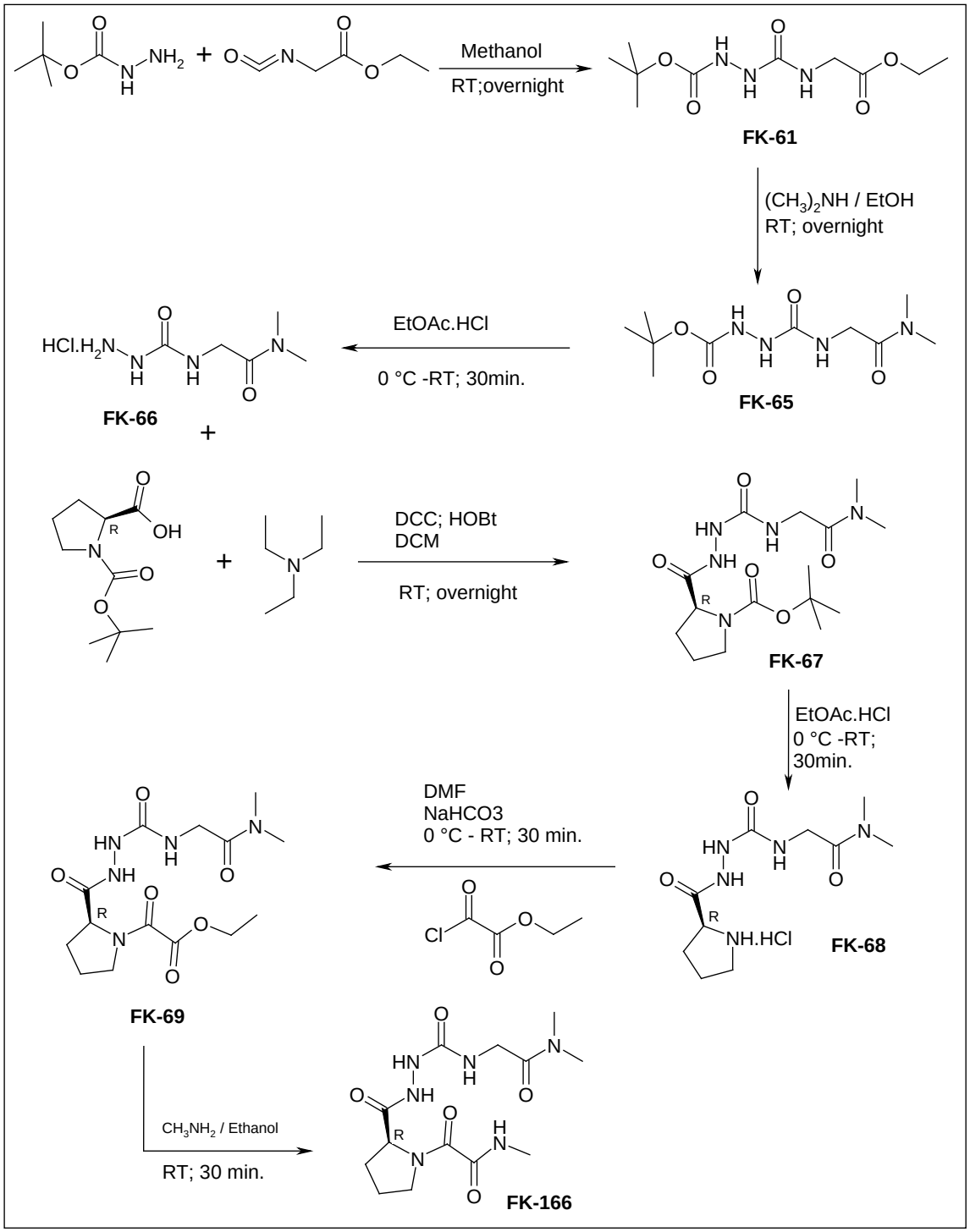
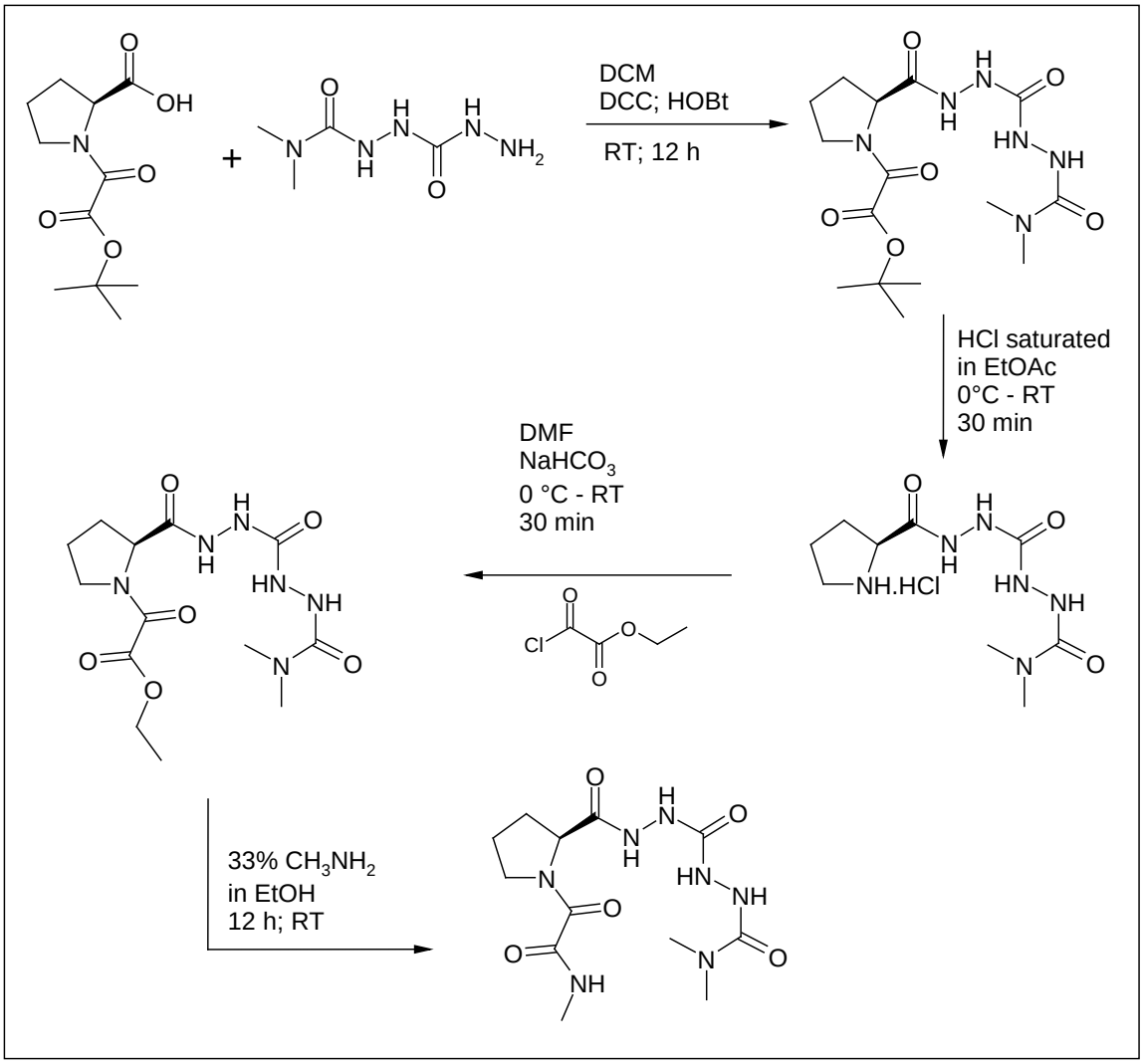
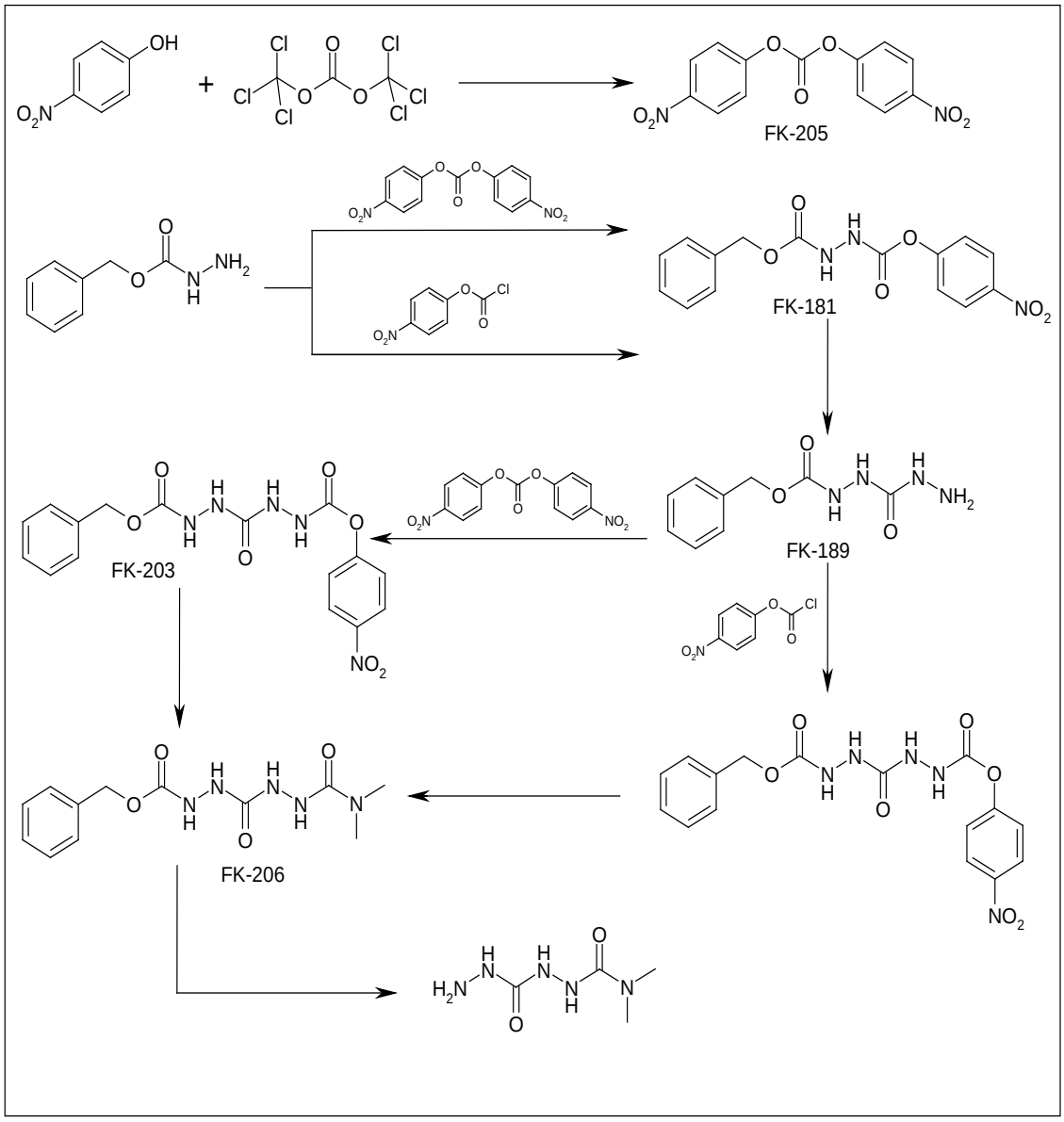
SCHEMES



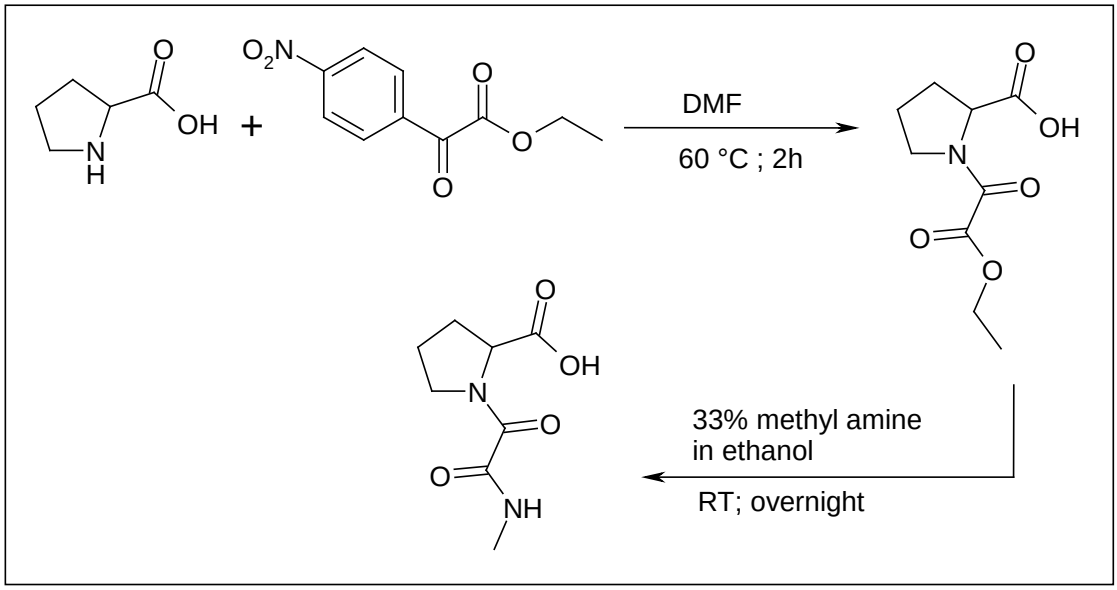
Scheme 1: Synthetic route for the synthesis of a β-turn mimic.



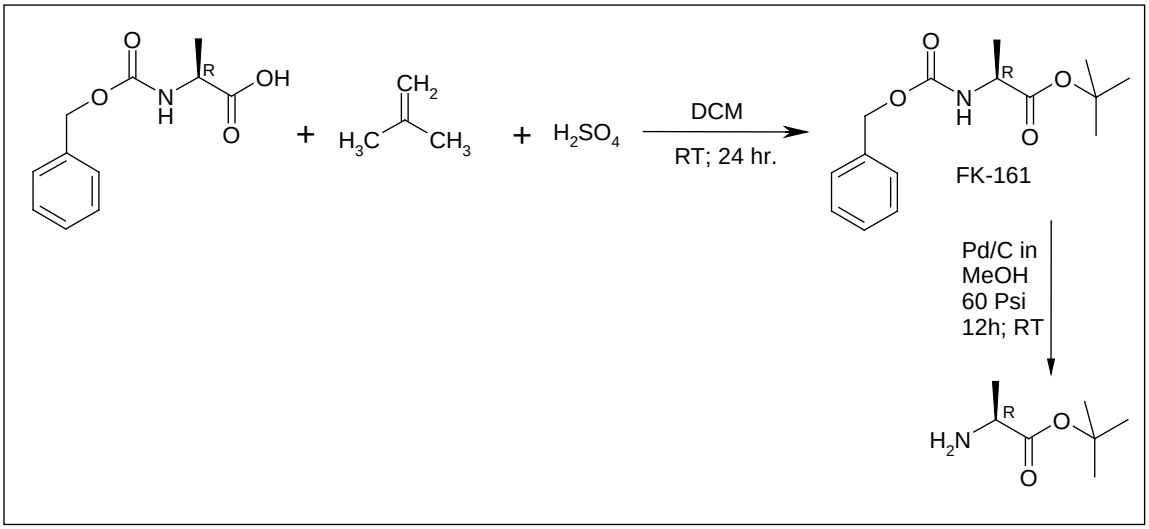
Scheme 2: Synthetic route for a NXO β-turn mimic with carbohydrazide substructure.



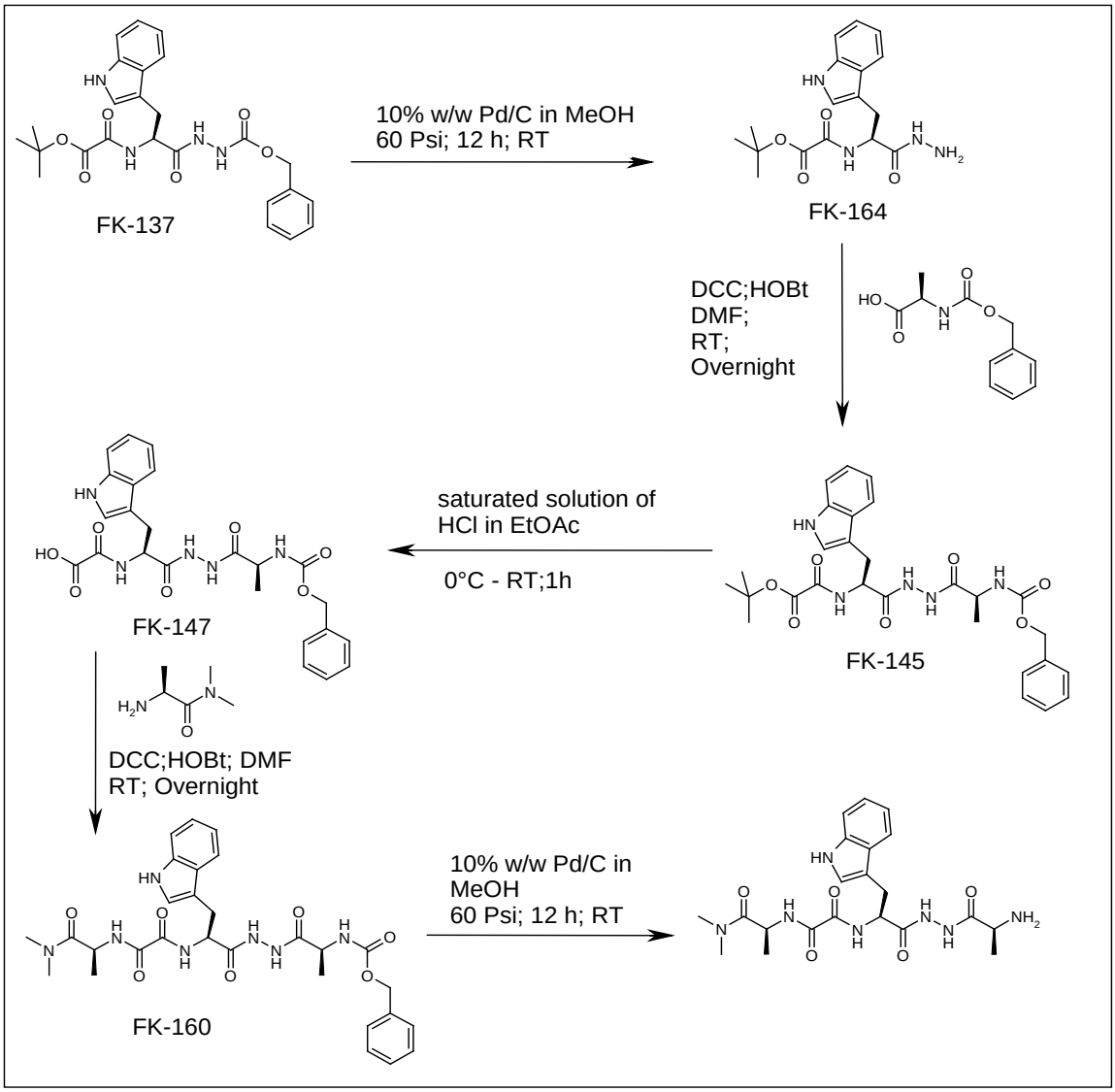
Scheme 3: Synthetic route for carbohydrazide intermediate for NXO β-turn mimic.



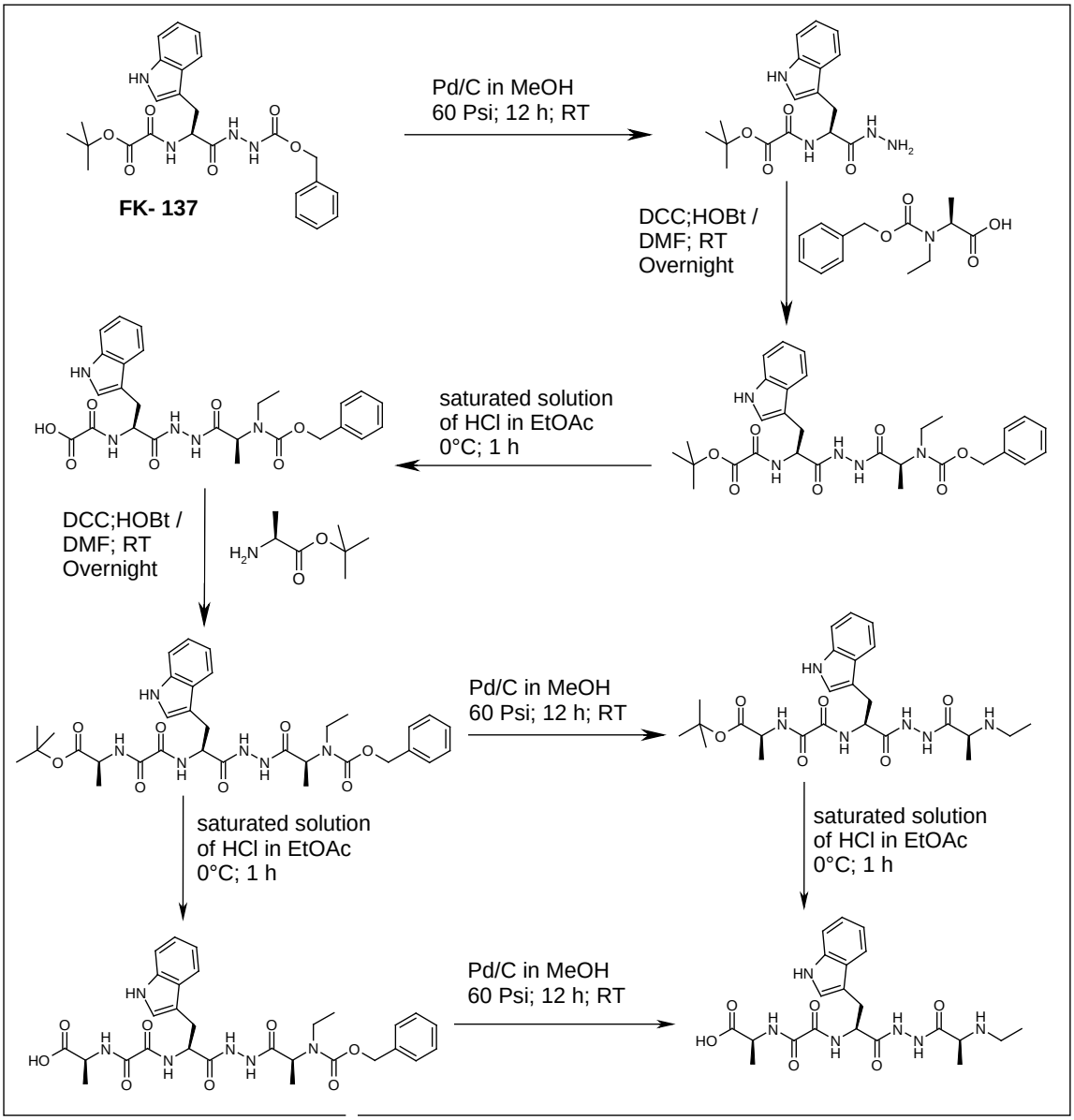
Scheme 4: Synthetic route for FK-188 & FK-200 using D and L-proline.



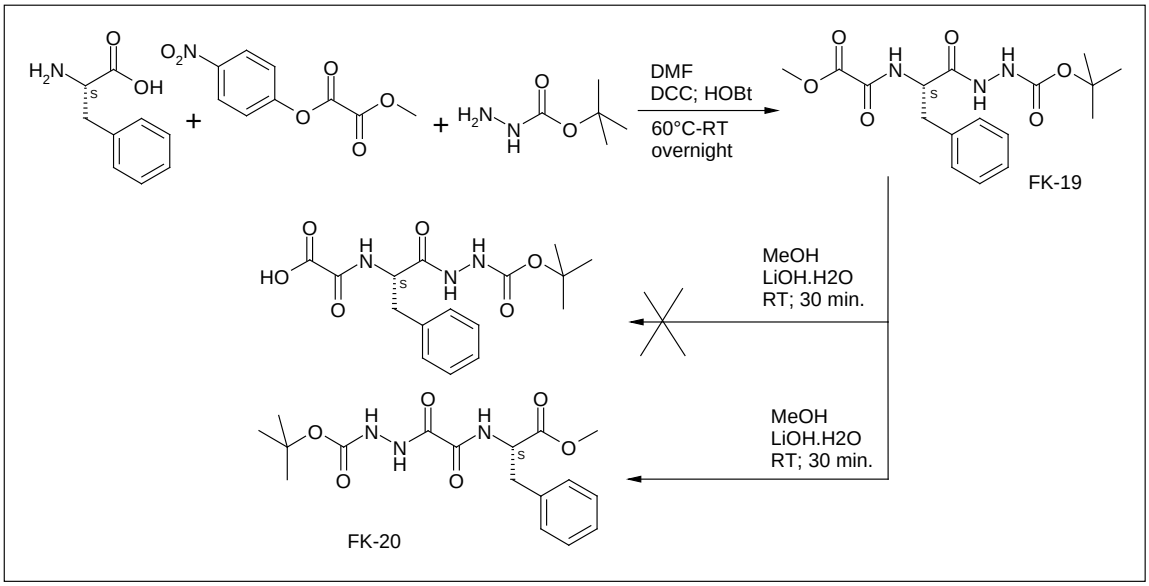
Scheme 5: Synthetic route for *tert*-butyl alaninate



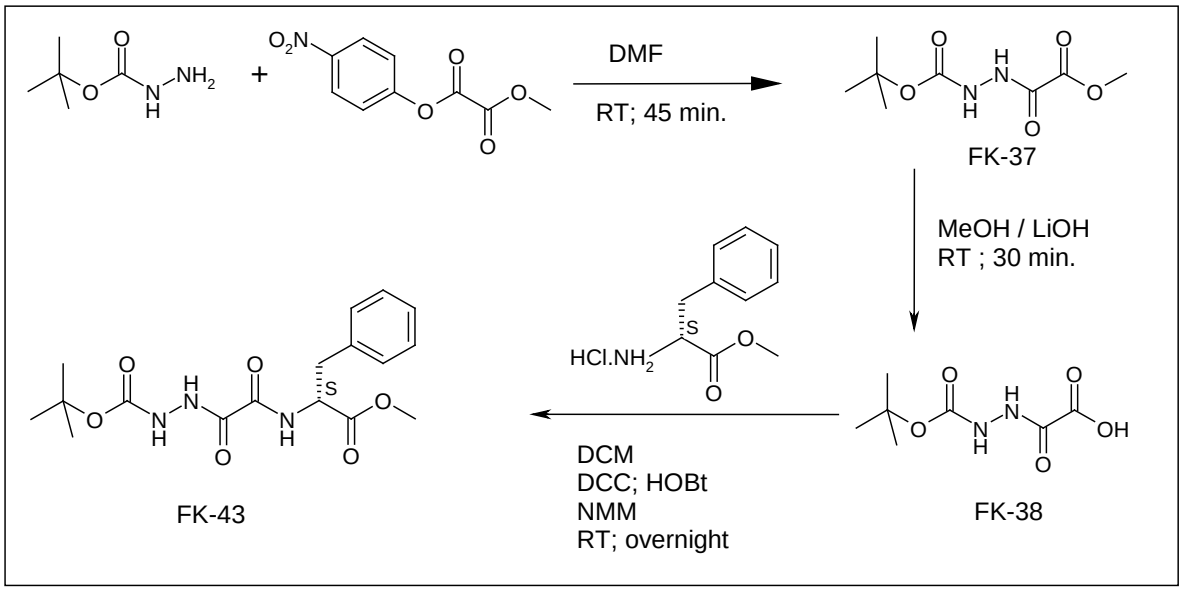
Scheme 6: Synthetic route for modified tripeptide (A) of β-sheet mimic.



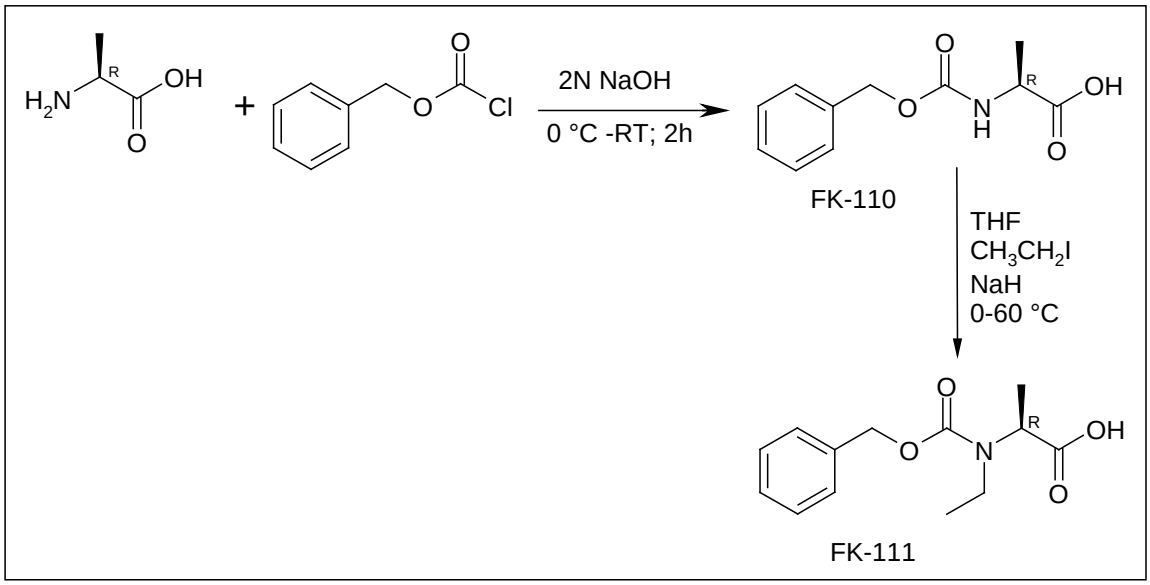
Scheme 7: Synthetic route for modified tripeptide (B) of β-sheet mimic.



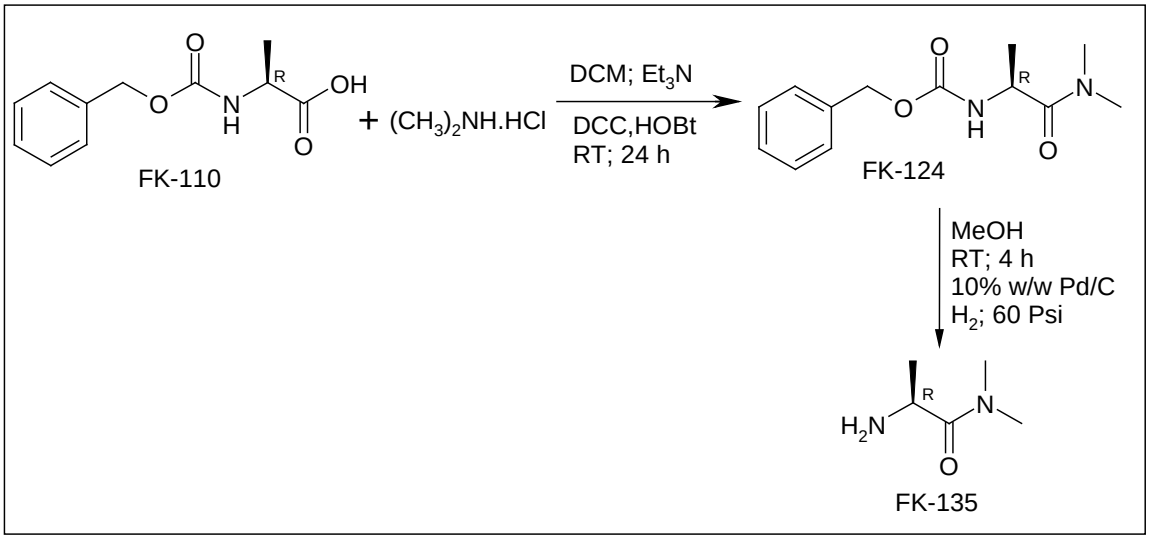
Scheme 8: Synthesis of rearranged product.



Scheme 9: Synthetic route for NXO-rearranged product.



Scheme 10: Synthesis of (2S)-2-[Benzyloxycarbonyl(ethyl)amino]propanoic acid.



Scheme 11: Synthesis of (2S)-2-Amino-*N*,*N*-dimethyl-propanamide.

13rd International Electronic Conference on Synthetic Organic Chemistry (ECSOC-13), 1-30 November 2009.
http://www.mdpi.org/ecsoc-13/ & http://www.usc.es/congresos/ecsoc/13/

[c013]

13th Electronic Conference on Synthetic Organic Chemistry
Institute of Applied Synthetic Chemistry
Vienna University of Technology

Surprise in the Lithium Hydroxide Hydrolysis of a *NXO*-Compound.

Khan Farhan A. ; Jaywant .Phopase and Ulrich Jordis*

Institute of Applied Synthetic Chemistry, Vienna University of Technology,

Getreidemarkt 9/163 1060 Vienna

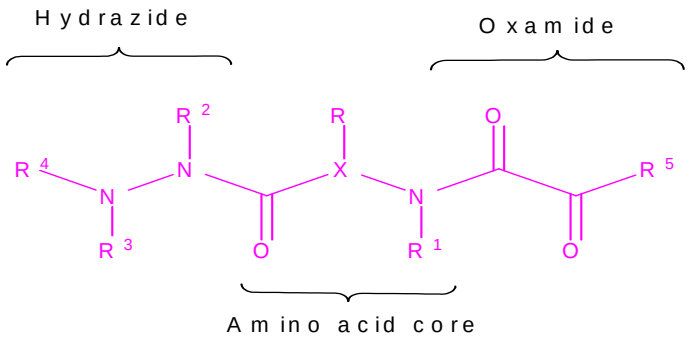
(*) Corresponding author. Email: ujordis@ioc.tuwien.ac.at

Abstract. This paper describes the unexpected outcome of the lithium hydroxide hydrolysis of the *NXO*-compound Boc-*NPheO*-OMe (**1**) and an independent synthesis of the product thus obtained, as well as the synthesis of 1,2,5 triazine-3,4,7- trione from **1**.

We submit these results for discussion to ECSOC 13 and hope to receive constructive comments and suggestions.

Introduction. Modified peptides are designed to mimic the biological function of natural peptides with modified stability, degradation or reactivity properties. Peptide mimics are prepared using modified amino acids or amino acid analogs. Earlier we have introduced modified amino acids[(1)] and have named them *NXO* compounds of the general formula **(A),** wherein X connects an acid hydrazide with an oxalic amide and additionally contains any residue of a natural or unnatural amino acid. These *NXO* compounds represent amino acid derivatives, where the basic amino group functionalized into an acidic oxamide group and the carboxylic acid is turned into an intrinsically basic hydrazide.

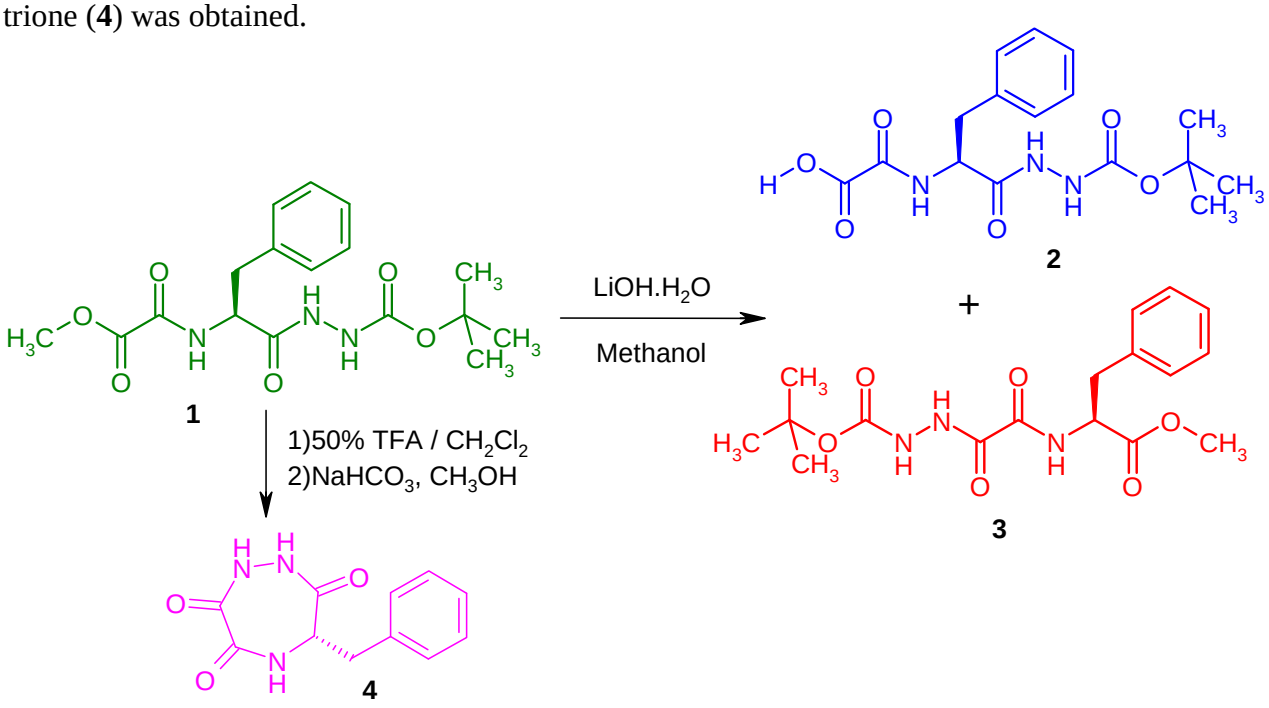
NXO-compounds (**A**)



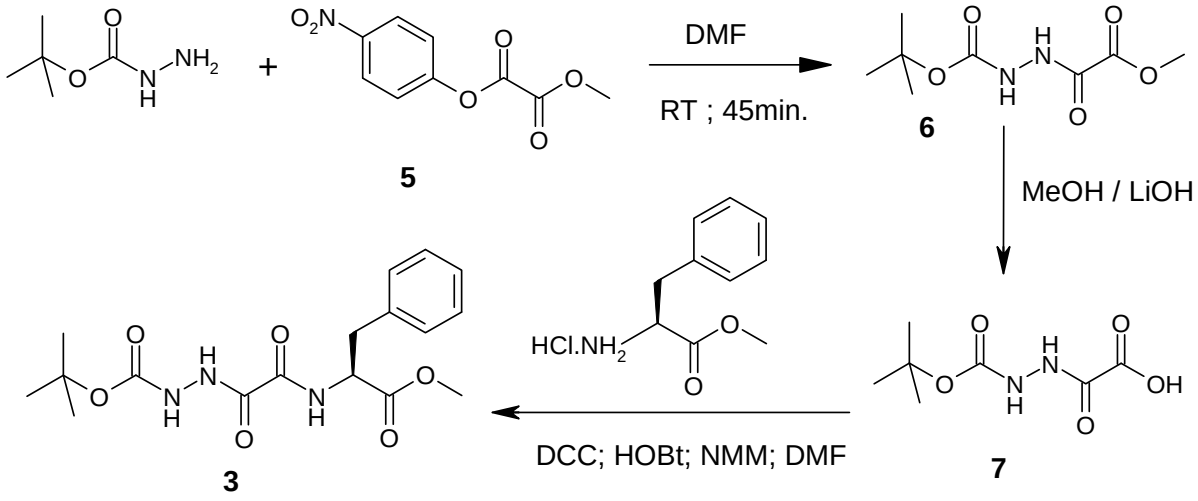
Methyl esters serve as protecting groups, which retain enough reactivity to be converted into amides or peptides. Their use for the protection of the carboxyl group is, however, rather limited as their removal, especially with a growing peptide chain usually is not efficient. The simple and most frequent used method for the hydrolysis of methyl ester is saponofication with aqueous alkali, mostly in the presence of varying amounts of organic solvents such as dioxane, methanol, ethanol, acetone, dimethylformamide etc.[2,3] . More specifically lithium hydroxide in methanol is used frequently for the hydrolysis of methyl esters in peptide chemistry.

Results and Discussion

While studying the chemical behaviour of the NXO-Compound derived from phenylalanine Boc-*NPheO*–OMe (**1**) we observed, in addition to the expected product **2,** another compound **3,** that was isolated and showed to have the same number of carbon peaks in 13C spectra to the starting material **1.** 2D NMR studies established the structure **3** for the isolated compound. The structure was further confirmed by an independent synthesis (*Scheme 1*). On treatment of **1** with TFA the novel dihydro-1,2,5-triazine-3,4,7-trione (**4**) was obtained.



The independent synthesis of **3** started from Boc-hydrazine. The intermediated **6** and **7** have not been reported before. DCC coupling of **7** with the Phe-OMe gave **3** while was identical in all respects with the compound obtained from **1.**



Scheme 1: Preparation of methyl*N*-[[2-(*tert*-butoxycarbonyl)hydrazino](oxo)acetyl]phenylalaninate**(3)**

Conclusion: In summary, we identified the structure of an unexpected product obtained from Boc-NPheO-OMe and developed an independent synthesis. We invite the scientific community to comment or explain this behaviour or suggest further experiments.

Experimental:

Reaction of Boc-NPheO-OMe with LiOH

To a solution of Boc-NPheO-OMe **1** (345mg; 94mmol) in 10 mL of methanol $LiOH.H_2O$ (39mg; 94mmol) was added and stirred at room temperature for 15min. The solvent was removed under vacuum; the remaining solid was dissolved in water and extracted with ethyl acetate. The organic phase was dried (Na_2SO_4) to give compound **3** Yield 58%. HPLC 98% 13C NMR 200MHz ($CDCl_3$) 28.07, 37.79, 52.55, 53.55, 82.34, 127.33, 128.74, 129.14, 135.24, 154.22, 157.71, 158.11, 170.60. The aqueous phase was adjusted to pH3 by dropwise addition of 5N HCl at 0°C. Extraction with ethyl acetate, washing of the organic phase with brine, drying using Na_2SO_4 and evaporation gave 130mg of **2**

(S)-6-Benzyl-dihydro-1,2,5 triazepane-3,4,7-trione (4)

[1]To the solution of **1** (1.0g, 2.74mmol.) in dichloromethane (15mL) TFA (15mL) was added drop-wise and the reaction mixture stirred at RT for 30 min. under argon. The reaction mixture was then evaporated and dried. The residue obtained was dissolved in dichloromethane (15mL) and washed with 10% $NaHCO_3$. The aqueous solution was extracted with dichloromethane (3 x 15mL), the combined organic layers were dried over Na_2SO_4 and evaporated under vacuum. The residue obtained was refluxed overnight in MeOH (15mL) under nitrogen. The solvent was evaporated and the crude compound obtained was purified by column chromatography to give 350mg (54%) of desired compound as a white solid. 1H NMR (200MHz, $CDCl_3$) (ppm) 8.3 (br s,1H), 7.1-7.4 (m, 5H), 6.7 (br s, 1H),4.7-4.9 (m, 1H), 3.04-3.36(m,2H). 13C NMR (200MHz, $CDCl_3$) (ppm) 171.83, 159.2, 158.6, 135.7, 129.23, 128.67, 127.1, 53.1, 37.5.

***tert*-Butyl 2-[methoxy(oxo)acetyl]hydrazinecarboxylate (6)**

To a solution of t-butylcarbazate (2.00g; 15mmol) in 20mL of DMF *tert*-butyl 4-nitrophenyl oxalate (3.75g; 17mmole was added and stirred under argon at room temperature for 45 min. 50mL of 10% $NaHCO_3$ was added and the reaction mixture was extracted with ethyl acetate and dried over Na_2SO_4. Ethyl acetate was removed in vacuum, to get the target compound **6.** Yield 25%; HPLC >93%; 13C NMR 200MHz ($CDCl_3$) 27.96, 53.60, 82.31, 154.69, 155.40, 159.56.

[2-(*tert*-Butoxycarbonyl)hydrazino](oxo)acetic acid (7)

A solution of **6** (830mg; 38mmol) and $LiOH.H_2O$ (160mg; 38mmol) in 15 mL of methanol and 2 drops of water were stirred at room temperature for 30min. Volatiles were removed under vacuum, the solid was dissolved in water and extracted three times with ethyl acetate. The aqueous phase was adjusted to pH3 by drop-wise addition of 5N HCl at 0°C. Extraction with ethyl acetate, washing of the organic phase with brine, drying using Na_2SO_4 and evaporation gave desired product in that was used as such in the next step. HPLC >99%; 13C NMR 200MHz ($CDCl_3$) 27.93, 79.45, 154.60, 158.17, 161.32.

Methyl-*N*-[[2-(*tert*-butoxycarbonyl)hydrazino](oxo)acetyl]phenylalaninate (3)

To a solution of **7** (120mg; 0.8mmol.) in 20mL of dichloromethane, HOBt (114mg; 0.84mmol) and DCC (173mg; 0.84mmol) were added and stirred under argon. Phenylalanine methyl ester hydrochloride (173mg; 0.8mmol) in 5mL of dichloromethane was treated with NMM (0.08mL), and the mixture was added to above solution followed by stirring under argon. Volatiles were removed under vacuum, the reaction mixture re-dissolved in ethyl acetate and cooled for 4 hours. This was filtered and the precipitated DCCU formed was washed with ethyl acetate. The combined organic phases were extracted with 1N HCl, 10% $NaHCO_3$ and brine, dried over Na_2SO_4 and evaporated to give **3** as colorless solid. Yield 26% ; HPLC >98% ; 13C NMR 200MHz ($CDCl_3$) 28.06, 37.74, 52.54, 53.55, 82.25, 127.30, 128.72, 129.14, 135.28, 154.28, 157.77, 158.17, 170.64.

Acknowledgements. KHAN Farhan Ahmed is thankful to HEC (Higher Education Commission of Pakistan) for financial support.

References.

1. Phopase, J.; Jordis, U. PCT Int. (2007), WO 2007095980.

2. Schröder, E.; Lübke, K., in: The Peptides Academic; New York, (1995); Vol.1, p55.

3. Houben-Weyl "Methods of Organic Chemistry", Vol. E22a Synthesis of peptides and Peptidomimetics. P195-197.

Farhan Ahmed Khan

Institute of Applied Synthetic Chemistry
Technical University of Vienna
Getreidemarkt 9/163, A-1060 Vienna, AUSTRIA
Tel: +43 1 58801 163 473
Mobile: +43 650 9111708
Email: fkhan@ioc.tuwien.ac.at

ACADEMIC CREDENTIALS

PhD.: (2008-2012)

Thesis: Synthesis and optimization of NXO building blocks and their Application in the synthesis of peptidomimetics.

Institute of Applied Synthetic Chemistry, Vienna University of Technology, Austria.
Advisor: Prof. Dipl.-Ing. Dr. Techn. Ulrich Jordis

MSc.: *Organic Chemistry*: (2002-2005)

Thesis: Furan Ring Transformations

Islamia University Bahawalpur, Bahawalpur, Pakistan
Advisor: Prof. Dr. Misbah-ul Ain Khan (Prof. Emeritus)

AREAS OF RESEARCH:

- Synthesis of heterocyclic compounds
- Bio-organic chemistry
- Peptide Chemistry

AWARDS:

- Four year scholarship was awarded under ***Overseas Scholarship Program for PhD in Selected Fields*** *by* ***Higher Education Commission of Pakistan*** to support my PhD. at TU Wien (Vienna University of Technology).

- Scholarship was awarded under ***Indigenous Scholarship Program for PhD*** *by* ***Higher Education Commission of Pakistan*** to support my PhD. at Islamia University Bahawalpur, Pakistan. (It was returned back to avail the Overseas scholarship).

RESEARCH EXPERIENCE

- 2003-2005: worked on a research topic for partial fulfillment of requirement of my Master Degree.

Nature of work: Synthesis of heterocyclic compounds.

- 2008-till date: working on my PhD project in TU Wien.

Nature of work: Synthesis of some artificial building blocks named as NXO-compounds. My work is mostly related to peptide synthesis. During this research work a good hands on experience was gained on 1H NMR, 13C NMR, FT-IR, MS, LC/MS, HPLC, GC-MS, X-ray, Microwave reactions and Kugelrohr-distillations.

PUBLICATION:

- Khan, Farhan A.; Phopase, Jaywant; Jordis, Ulrich. **Surprise in the lithium hydroxide hydrolysis of a NXO-compound.** International Electronic Conference on Synthetic Organic Chemistry, 13th, Nov. 1-30, 2009 (2009).

CONFERENCES ATTENDED:

- 6th International & 16th National Chemistry Conference held in (2007) at Department of Chemistry, Bahauddin Zakariya University, Multan, PAKISTAN.

- XXIVth European Colloquium on Heterocyclic Chemistry held in (2010) at Technical University of Vienna, Vienna, AUSTRIA.

REFERENCES:

Prof. Dipl.-Ing. Dr.techn. Ulrich Jordis Institute of Applied Synthetic Chemistry Vienna University of Technology Getreidemarkt 9-163 OC 1060 Wien ATU 37675002 Email: ujordis@pop.tuwien.ac.at Tel: +43-1-58801 x 15460	*Prof. Dr. Abdul Jabbar* Department of Chemistry The Islamia University of Bahawalpur. Bahawalpur, Pakistan. Email: abdul_jabbar06chem@yahoo.com Tel: +92 300 9684586
Dipl.-Ing. Dr. Laszlo Czollner Senior Scientist Institute of Applied Synthetic Chemistry Vienna University of Technology Getreidemarkt 9-163 OC 1060 Wien ATU 37675002 Email:laszlo.czollner@tuwien.ac.at Tel: +43-1-58801 x 15432	*Prof. Dr. Misbahul Ain Khan* Institute of Chemistry, University of The Punjab, Lahore, Pakistan. & Prof. Emeritus & Ex-Vice Chancelor The Islamia University of Bahawalpur. Bahawalpur, Pakistan. Email: misbahakhan@gmail.com