University of Alberta

Synthetic Studies on Novel Facially Amphiphilic Sesquiterpenoid- and 11β-Steroid-Amino Acid Conjugates and Transition Metal Mediated Steroid-Amino Acid Derived Biometallosurfactants

by

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Dedication

I offer this dissertation unto the Lotus feet of the Supreme Personality of Godhead:
Lord Shri Krishna and Srimati Radharani
as well as
my visionary father late Manharbhai D. Pandya, loving mother Induben M.
Pandya and family

Abstract

Sesquiterpenoid- and steroid-amino acid conjugates demonstrate a broad array of interesting biological properties, as the different segments of the conjugate function collectively to regulate conformation, recognition, transport and solubility. The current project involved developing a facile methodology to synthesize metabolically stable facially amphiphilic conjugates by appending either amino acids (chapter 2) or cationic metal ligand-amino acid complexes (chapter 3) as hydrophilic segments on steroid progesterone and sesquiterpene amorpha-4,11-diene scaffolds.

Aminosteroids and C-11 substituted steroids have attracted long lasting interest due to their diverse pharmacological properties. As the stereoselective C-11β functionalization of a steroid imposes severe steric hindrance due to the C-18 and C-19 angular methyl groups, access to 11β-aminoprogesterone is a challenge. Chapter 2 describes stereoselective syntheses of a new family of aminosteroids: 11β-aminoprogesterone (11β-NH_2-Pro) (**76**) and its derivatives, including its glycine **77** and L-/D- alanine- **78**/**79** based conjugates, by nucleophilic substitution or reductive amination. Additionally, a synthesis of the 12-amorpha-4,11-dienyl-(*S*)-glycine (**80**) conjugate is also discussed.

Biological testing of the aminoprogesterone derivatives revealed that some of them selectively inhibit 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), similar to that of their structural analogue 11β-hydroxyprogesterone. Moreover, two compounds, 11β-azidoprogesterone (**81**) and 11β-*N*-(*o*-nosyl)-aminoprogesterone (**93**), which did not significantly inhibit 11β-HSDs, had

antagonist properties on the mineralocorticoid receptor (MR). The 11β-aminoprogesterone derivatives form the basis for the further development of improved modulators of corticosteroid action for treatment of electrolyte disturbances and chronic inflammatory disorders.

Chapter 3 discusses the efforts towards developing a new class of amidoglutarate-tethered cationic cobalt(III) based biometallosurfactant complexes Λ-α-Co[(*S,S*-picbipyrro-amidoglutarate)(11β-NH-Pro-Gly)]$^{2+}$ (**138**) and Λ-α-Co[(picenMe$_2$-amidoglutarate)(11β-NH-Pro-Gly)]$^{2+}$ (**139**) bearing chiral rigid or achiral non-rigid N_4-tetradentate and N,O-bidentate ligand derived from the steroid-based *N*-substituted amino acid *N*-(11β-NH-Pro)-Gly (**77**). However, no desired complexation was realized. Subsequently, model studies identifying the critical impeding factors of complexation suggested that unfavorable steric interactions between *N*-substituted alkyl group of amino acids and the pyridyl rings of N_4-tetradentate ligands caused the complexation to fail. The synthesized *cis*-α-Co[N_4Cl_2]$^+$ (**161**, **171-174**) and Λ-α-Co[N_4(AA)]$^{2+}$ (**185, 186**, **190-192**) complexes were characterized by comparing their ^{1}H-NMR and CD spectral features to their structural analogues for which X-ray crystallographic studies have been reported.

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Table of Contents

List of Figures

List of Schemes

List of Tables

List of Abbreviations

$[\alpha]_D$	specific rotation
Å	angstrom
AA	amino acid
Ac	acetyl
Ac_2O	acetic anhydride
AcOH	acetic acid
ACS	American Chemical Society
AIBN	2,2′-azobis(2-methylpropionitrile)
Ala	alanine
Alloc	allyloxycarbonyl
Am	amafalone
AMD	age-related macular degeneration
Aq.	aqueous
AR	androgen receptor
Arg	arginine
Asn	asparagine
Asp	aspartic acid
BAIB	bis-acetoxyiodobenzene
Bn	benzyl
Bu_4NI	tetrabutylammonium iodide
c	concentration
CAM	ceric ammonium molybdate

CBX	carbenoxolone
Cbz	carbobenzyloxy
CCl_4	carbon tetrachloride
CD	circular dichroism
CIP	Cahn–Ingold–Prelog
CKD	chronic kidney disease
CMC	critical micelle concentration
CMIA	carbonyl metalloimmunoassays
Co_2B	cobalt boride
CoA	coenzyme A
Cohex	hexaaminecobalt(III) chloride
COS-7	African Green Monkey SV40-transfered kidney fibroblast cell line
CPC	centylpyridinium chloride
CrO3	chromium trioxide
CSA	cationic steroid antibiotics
δ	chemical shift
Δε	molar circular dichroism
d	doublet (in NMR)
Dap	2,3-diaminopropionic acid
DCE	dichloroethane
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone

DEAD	diethyl azodicarboxylate
DIAD	diisopropyl azodicarboxylate
DIBAL	diisobutylaluminum hydride
DIPEA	*N*, *N*-diisopropylethylamine
DMAP	4-(dimethylamino)pyridine
DMF	dimethylformamide
DMP	Dess-Martin periodinane
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DNs	dinitrobenzenesulfonyl
ε	molar extinction coefficient
E. coli	*Escherichia coli*
ED_{50}	median effective dose
Equiv	equivalent
ESI	electronespray ionization
Et	ethyl
Et_2O	diethyl ether
Et_3N	triethylamine
EtOAc	ethyl acetate
FPP	farnesyl pyrophosphate
FTIR	fourier transform infrared spectroscopy
GA	glycyrrhetinic acid
GABA	γ-aminobutyric acid

GC	glucocorticoid
GI_{50}	half maximal growth inhibition
Glu	glutamic acid
Gly	glycine
GPP	geranyl pyrophosphate
GR	glucocorticoid receptor
h	hour
HEK-293	human embryonic kidney 293 cells
HeLa	Henrietta Lacks (cervical cancer cell line)
HIV	human immunodeficiency virus
HMPA	hexamethylphosphoramide
hMR	human mineralocorticoid receptor
HN_3	hydrazoic acid
HPLC	high pressure liquid chromatography
HR-MS	high resolution mass spectrometry
HRT	hormone replacement therapy
HSD1	hydroxysteroid dehydrogenase type 1
HSD2	hydroxysteroid dehydrogenase type 2
Hz	hertz
IBX	2-iodoxybenzoic acid
IC_{50}	half maximal inhibitory concentration
IPA	isopropyl alcohol
IPP	isopentenyl pyrophosphate

J	coupling constant in Hertz
L-statin	4-amino-3-hydroxy-6-methylheptanoic acid
λ	wavelength
LC-MS	liquid chromatography coupled with mass spectrometry
LDA	lithium diisopropylamide
$LiAlH_4$	lithium aluminium hydride
m	multiplet
M	molar
M.P.	melting point
m/z	mass to charge ratio
MBz	4-methoxybenzoyl
MC	mineralocorticoid
MCF-7	Michigan cancer foundation-7 (cancer cell line)
MDR	multidrug resistance
Me	methyl
MHz	megahertz
MMP	matrix metalloproteinase
MR	mineralocorticoid receptor
MRA	mineralocorticoid receptor antagonism
Ms	methanesulfonyl
MS	molecular sieve
MsCl	methanesulfonyl chloride
MW	microwave

υ	wavenumber
NAD^+	β-nicotinamide adenine dinucleotide
$NADP^+$	β-nicotinamide adenine dinucleotide phosphate
NaOCl	sodium hypochlorite
nM	nanomolar
NMR	nuclear magnetic resonance
o-Ns/*o*-nosyl	ortho-nitrobenzenesulfonyl
PBu_3	tributyl phosphine
Pd/C	palladium on carbon
PDC	pyridinium dichromate
Phe	phenylalanine
$PhOPPh_2$	phenyl diphenylphosphinite
Picbipyrro	*N*,*N*′-di(2-picolyl)-2,2’-bispyrrolidine
Piccyhxn	*N*,*N*′-di(2-picolyl)-1,2-cyclohexanediamine
$PiccyhxnMe_2$	*N*,*N*′-dimethyl-[*N*,*N*′-di(2-picolyl)]-1,2-cyclohexanediamine
Picen	*N*,*N*′-di(2-picolyl)ethane-1,2-diamine
$PicenMe_2$	*N*,*N*′-dimethyl-[*N*,*N*′-di(2-picolyl)]ethane-1,2-diamine
Picpn	3-methyl-1,6-di(2-pyridyl)-2,5-diazahexane
$PicpnMe_2$	*N*,*N*′-dimethyl-3-methyl-1,6-di(2-pyridyl)-2,5-diazahexane
PicpyrrMe	*N*,*N*′-dimethyl-*N*′-mehyl-aminomethylpyrrolidine
PMe_3	trimethylphosphine
pNZ	para-nitrobenzyloxycarbonyl
PPh_3	triphenyl phosphine

ppm	parts per million
Pro	proline
Py	pyridine
q	quartet
R_f	retardation factor (thin layer chromatography)
rt	room temperature
s	singlet (in NMR)
SAR	structure activity relationship
SDR	short-chain alcohol dehydrogenase
Sec-	secondary
Spp.	species
t	Triplet (in NMR)
TABCO	2,4,4,6-tetrabromo-2,5-cyclohexadienone
t-Bu	*tertiary*-butyl
TEMPO	2,2,6,6-tetramethylpiperidinyloxy
tert-	tertiary
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
TFE	trifluoroethanol
THF	tetrahydrofuran
TLC	thin layer chromatography
$TMSCH_2N_3$	trimethylsilylmethyl azide
$TMSN_3$	trimethylsilyl azide

TMSOTf	trimethylsilyl trifluoromethanesulfonate
t_R	retention time
Trt	trityl
UV-vis	ultraviolet-visible spectroscopy
Val	valine
Z-DNA	left handed double helical DNA structure

Chapter 1 : Terpenoid-Amino Acid Conjugates

1.1 Introduction

Historically, nature has acted as the ultimate pharmacy in providing a plethora of natural products (secondary metabolites) to combat many human diseases. Recently, improved analytical techniques have simplified the isolation and identification of active natural products. This is typically followed by mode of action studies. Synthetic derivatives with superior activity may be prepared through structure-activity relationship studies. Many of these natural products have gone on to become current drug candidates. By 2008, almost 50% of drugs were either natural products or synthetic derivatives thereof.[1]

Less than 10% of the natural biodiversity has been evaluated for potential biological activity.[2] As such, there has been increased attention in finding new natural product-derived drug candidates by combining and expanding the biological functions of natural products through the formation of natural product hybrids. Theoretically, many hybrids consisting of combinations of different natural products could be prepared. This strategy is not confined to natural products themselves, as synthetic compounds can also be hybridized to generate conjugates with novel functions and biological activities.

1.1.1 Natural Product Conjugates

A covalently linked union of two or more natural product fragments gives rise to natural product hybrid molecules, also known as conjugates or chimeras.

This approach seems to be beneficial as it offers a unique platform to create an unlimited variety of hybrid structures from known natural products. These hybrids possess the potential to act as therapeutic leads, possessing novel properties with unprecedented synergetic biological activities arising from the combination of two or more active compounds. From a general standpoint, this concept of conjugates is not new, and is inspired by nature itself.

Nature derives a plethora of natural products from biosynthetic pathways, such as the shikimate and mevalonate pathways. However, an increasing number of natural product conjugates derived from mixed biosynthetic pathways have been discovered lately.[3-5] For example, as shown in Figure 1.1, the antimicrobial compound thiomarinol (**1**) was isolated from marine bacterium *Alteromonas rava* sp. nov. SANK 73390. Thiomarinol is a conjugate of pseudomonic acid C (**2**) and holothin (**3**).[6] Interestingly, unlike parent compounds **2** and **3**, conjugate **1** displays broad and potent antimicrobial activity against Gram-positive and Gram-negative bacteria.

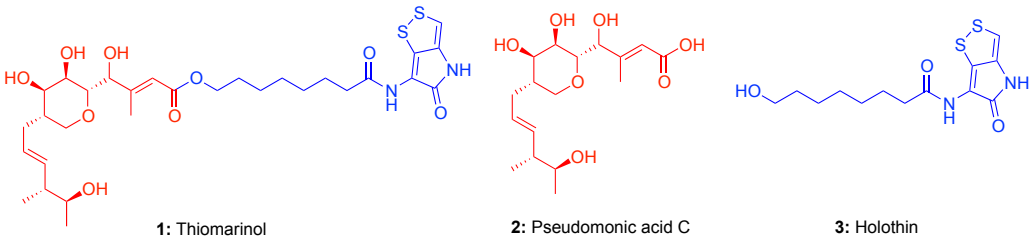


Figure 1.1 Naturally occurring antimicrobial hybrid thiomarinol.

Inspired by nature, chemists have increasingly paid attention to synthesizing conjugates/hybrids based on scaffolds such as C_{60}-fullerene[4, 7], terpenoids (mainly sesquiterpenoids[3, 8] and triterpenoids/steroids[9]), taxoids,[10] anthracyclines,[11] and β-lactams.[12] Several different functional entities such as

amines/polyamines,[13-14] amino acids/peptides,[4-5, 9] enediynes,[15] nucleic acids,[16] carbohydrates,[9, 17] porphyrins[18] and active pharmacophores have also been used.[3, 19] Among these available scaffolds, terpenoids have been particularly attractive due to their broad biological profile and natural abundance.

1.1.2 Conjugates Based on Terpenoid Scaffolds

Terpenoids, also called isoprenoids, are one of the largest groups of natural products found in nature. They are synthesized from two or more isoprene units (five-carbon building blocks). These compounds are commonly used as raw materials for their flavors and fragrances. However, they are also used for their biologically active properties against microorganisms, plants, insects and animals.[20]

1.1.2.1 Biosynthetic pathways for sesquiterpenoids and triterpenoids (steroids)

Based on the number of isoprene building blocks, terpenoids are classified as monoterpenoids (C_{10}), sesquiterpenoids (C_{15}), diterpenoids (C_{20}), sesterterpenoids (C_{25}), triterpenoids/steroids (C_{30}), tetraterpenoids (C_{40}) and polyterpenoids $(C_5)_n$. Of these classes, sesqui- and triterpenoids are of special interest, as they display a wide spectrum of biological activities. A brief overview of the biosynthetic pathway for these two terpenoids is depicted in Figure 1.2.[21]

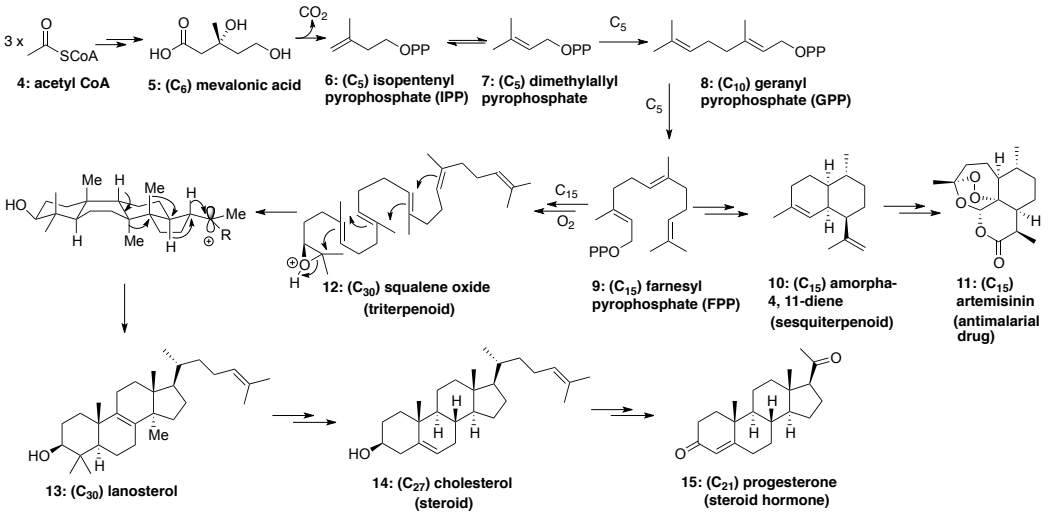
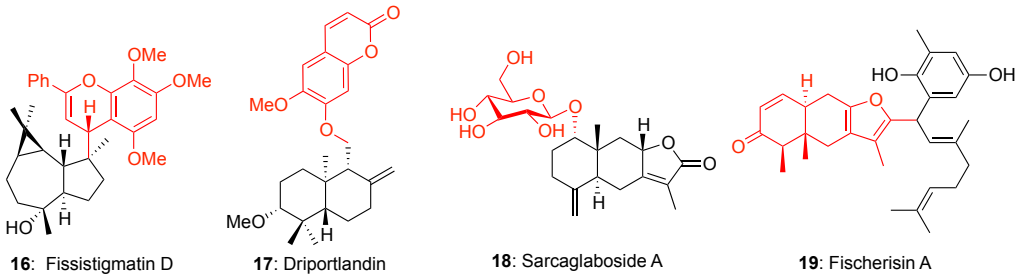


Figure 1.2 Biosynthetic pathways for sesquiterpenoids and triterpenoids (steroids).

The key intermediate of terpenoid biosynthesis is mevalonic acid (**5**), which is formed from three molecules of acetyl-CoA (**4**). After losing a carbon atom as CO_2, mevalonic acid yields the C_5 precursor isopentenyl pyrophosphate (IPP) (**6**). IPP is connected to dimethylallyl pyrophosphate (**7**) by a simple allylic proton transfer. Condensation of **6** with **7** offers geranyl pyrophosphate (GPP) (**8**), the starting point for all of the monoterpenes. Repeating the alkylation with another molecule of IPP gives farnesyl pyrophosphate (FPP) (**9**), the starting point for the C_{15} sesquiterpenes. In the sesquiterpene series, diverse cyclizations lead to an amazing variety of products such as amorpha-4,11-diene (**10**) and artemisinin (**11**), a well-known antimalarial drug. Dimerization of FPP gives a triterpenoid squalene oxide (**12**), which subsequently yields cholesterol (**14**) through a lanosterol (**13**) intermediate. Other steroids, such as progesterone (**15**), are derived from cholesterol.

1.1.2.2 Conjugates based on sesquiterpenoids frameworks

Sesquiterpenoids are widespread in higher plants, fungi, algae, marine invertebrates and microorganisms, and show a wide range of biological and pharmacological properties.[22] Fissistigmatin D (**16**) (Figure 1.3) was isolated from a creeper, *Fissistigma bracteolatum* Chatt, growing in the north of Vietnam.[23] It is an example of a group of natural product conjugates consisting of both flavonoid and sesquiterpene moieties in a single scaffold. It has been proposed that the biosynthesis of this interesting hybrid involves a mixed pathway combining a sesquiterpene-type unit with a chalcone/flavonoid unit. In South-East Asia, the extract of these creepers is used in traditional medicine to stop bleeding, cure infections and improve blood circulation.[23]



16: Fissistigmatin D **17**: Driportlandin **18**: Sarcaglaboside A **19**: Fischerisin A

Figure 1.3 Naturally occurring sesquiterpenoid-based conjugates.

Driportlandin (**17**), a sesquiterpene-coumarin conjugate isolated from the whole dried plant of *Euphorbia,* showed a significant effect in inhibiting the efflux-pump activity mediated by P-glycoprotein.[24] It proved to be effective in reversing multidrug resistance (MDR) in mouse lymphoma cells.

Sarcaglaboside A (**18**), a sesquiterpene glycoside isolated from *Sarcandra glabra*, showed pronounced hepatoprotective activities (at 10^{-4} M *in vitro*) against D-galactosamine-induced toxicity in WB-F344 rat hepatic epithelial stem-like

cells.[25] Interestingly, it appears to be more potent than bicyclol, an anti-hepatitis drug. In 2011, Xie et al.[26] isolated novel sesquiterpenoid-geranylhydroquinone based conjugates fischerisin A and B from the roots of *Ligularia fischeri*. Fischerisin A (**19**) exhibited *in vitro* cytotoxicity against cultured human oral epidermoid carcinoma (KB) and human breast cancer (MCF-7) cell lines with IC_{50} values of 9.7 and 10.2 mM respectively.

Besides these naturally occurring conjugates, synthetically derived sesquiterpenoid-based conjugates have also been explored. Some examples are shown in Figure 1.4.

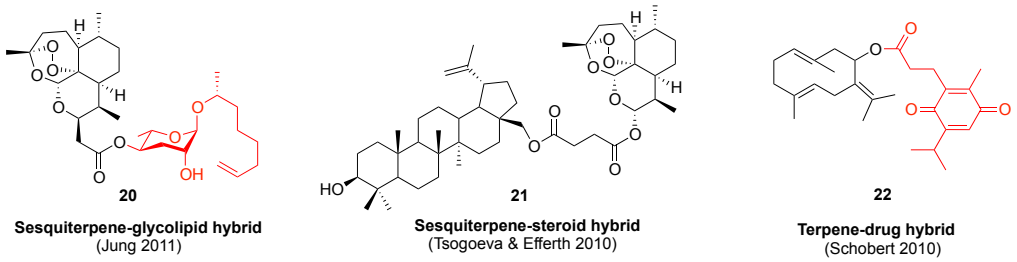


Figure 1.4 Synthetically derived sesquiterpenoid-based conjugates.

Jung and co-workers[27] synthesized novel artemisinin-glycolipid hybrid **20** that showed exceptional *in vitro* activity against oral cancer, higher than either artemisinin or the glycolipid alone. Compound **20** also exhibited five-fold more activity than common anti-cancer drugs cisplatin and paclitaxel. The groups of Efferth and Tsogoeva[28] have collaboratively constructed novel hybrid **21**, consisting of artesunate and a betulin-based steroid cytotoxic natural product. This conjugate inhibited leukemia and MDR cell line growth in a more pronounced manner (IC_{50} values ~ 10 μM) than betulin itself (IC_{50} values ~ 60 μM).

Schobert and co-workers[29] have prepared terpene conjugates of the weak anticancer drug thymoquinone, a constituent of black seed oil. Amongst all of the

conjugates reported, germacrone conjugate **22** achieved the best result against MDR MCF-7 breast carcinoma cells.

1.1.2.3 Conjugates based on steroid (triterpenoid) frameworks

Steroids are a valuable class of natural products, widely occurring in mammalian tissues. These compounds possess a rigid framework with varying levels of functionalization, and an ability to penetrate cell membranes and bind to specific hormonal receptors. Steroids have been widely explored as a scaffold for conjugation with other chemically or biologically relevant molecules. Many natural and synthetic steroid-based conjugates are known to have interesting new physicochemical and biological profiles. For example, as shown in Figure 1.5, cephalostatin 1 (**23**) is a steroid-based dimeric natural product hybrid having a higher biological activity than the monomer. It contains a pyrazine unit, conjugated to highly oxygenated steroid moieties on each side. Cephalostatin 1 was isolated from the marine worm *Cephalodiscus gilchristi* and exhibited a GI_{50} value of 2.2 nM, when screened against human leukemia and lymphoma cell lines.[30] Yang et al. isolated the naturally occurring saponin (amphipathic glycoside) extensumside A (**24**) from *Myriopteron extensum*, which exhibited significant cytotoxic/anti-proliferation activity against eight cancer cell lines.[31]

Pachystermine A (**25**) is a naturally occurring β-lactam-steroid found in the buxaceous plant *Pachysandra terminalis*.[32] This hybrid molecule is composed of a steroid and a four membered β-lactam ring (a pharmacophore of common potent antibacterial agents).

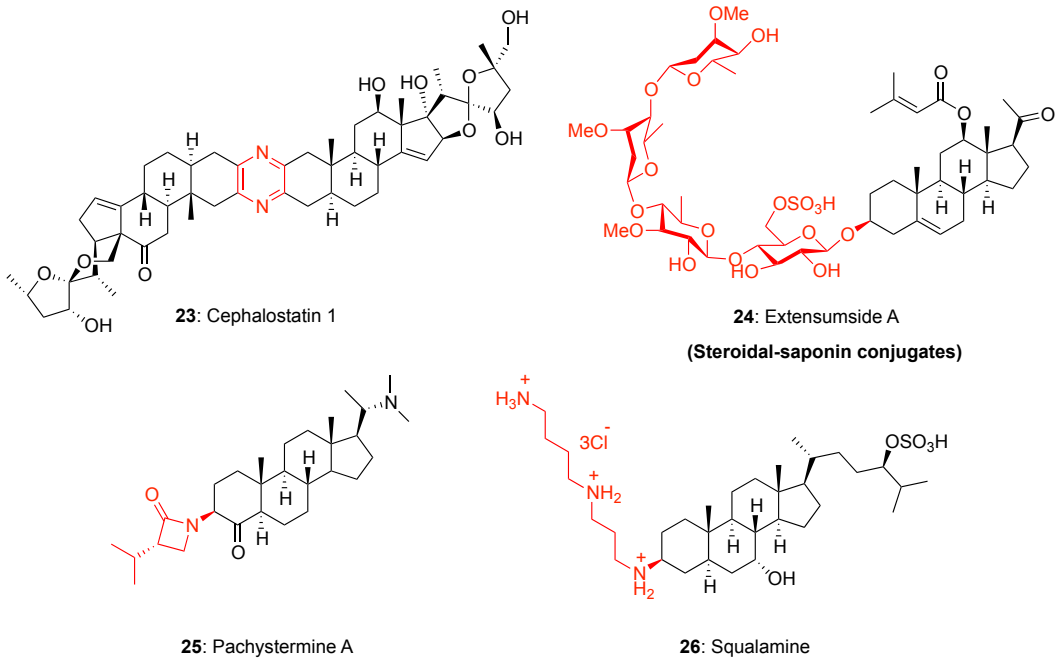


Figure 1.5 Naturally occurring steroid-based conjugates.

Squalamine (**26**) is a sterol-spermidine/polyamine conjugate that was isolated from *Squalus acanthias,* the dogfish shark.[33] Recently, this unusual natural product has attracted significant attention due to its high potency and broad biological profile as an antibacterial, antifungal, antimalarial, antiprotozoal and antiangiogenic agent.[34] Squalamine (**26**) is also useful for the treatment of various cancers (including lung, ovarian, and brain cancers), age-related macular degeneration (AMD) and the control of body weight in men.[34]

In addition to the naturally occurring steroidal hybrids, a variety of synthetically derived steroid-based hybrids are being explored for their biological activities.[3-5, 9] A few representative examples are shown in Figure 1.6, such as steroid-polyamine **27**,[34] steroid-carbohydrate **28**,[35] steroid-drug **29**,[36-37] steroid-C_{60}-porphyrin **30**,[38] steroid-enediyne **31**[38] and steroid-amino acid **32**.[39]

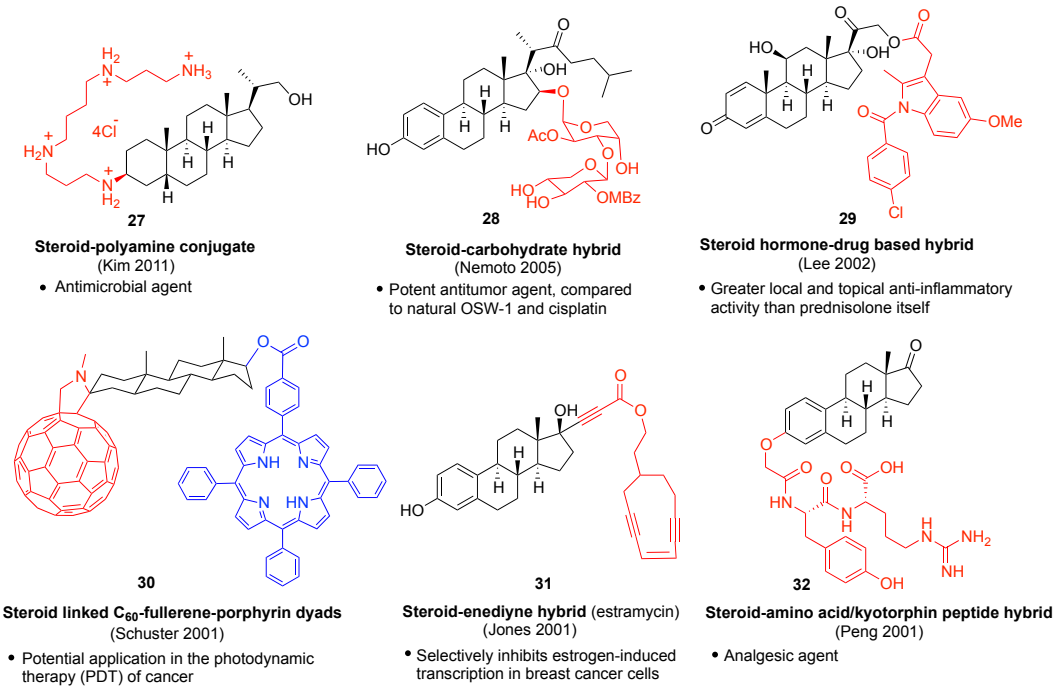


Figure 1.6 Synthetically derived steroid-based conjugates.

It is worth mentioning that in most of these cases, the observed biological profile of the hybrid is better than that of the individual parts. Among all of the functional entities bearing terpenoid-based frameworks (specifically sesquiterpenoids and steroids), introducing amino acid functionalities to the terpenoid-based scaffolds is a particularly attractive goal. Such terpenoid-amino acid conjugates are amphipathic in nature and can possess a novel biological profile with the ability to act as an efficient vector in site-specific drug delivery. The following section is a brief introductory survey of the existing natural and synthetic terpenoid-amino acid-based conjugates.

1.1.3 Terpenoid-Amino Acid Conjugates

Installation of an amino acid or peptide on a terpenoid scaffold offers the combination of a hydrophilic functionality and a hydrophobic carrier in the same

molecule. This represents an important class of molecules (i.e. lipopeptide-based biosurfactants)[40-43] for drug development, as sesquiterpenoids and steroids tend to be poorly soluble in aqueous systems. Lately, much effort has been devoted to improving drug solubility by attaching amino acid functionalities. This can improve their pharmacokinetic profile, and maintain or even increase the biological activity of the parent terpenoids.

1.1.3.1 Sesquiterpenoid-amino acid conjugates

Sesquiterpenoid based amino acid conjugates are not widely found in nature. A few naturally-occurring conjugates based on sesquiterpene lactones are shown in Figure 1.7. Coustunolide (**33**), a sesquiterpene lactone with anticancer activity, was isolated from the dried roots of the Chinese herbal medicine *Saussurea lappa*. In an attempt to modulate its cytotoxicity, Yoshikawa and co-workers[44] isolated other coustunolide-amino acid conjugates, namely saussureamines A-E, from the same plant. Saussureamines A (**34**) and B (**35**) exhibited greater water-solubility and lower gastroprotective effects than did coustunolide (**33**) on gastric mucosal lesions in rats. However, their gastroprotective effects were greater than that of reference drug, certraxate. Inspired by nature, the same group synthesized several analogues with a variety of amino acids to determine its structure-activity profile.

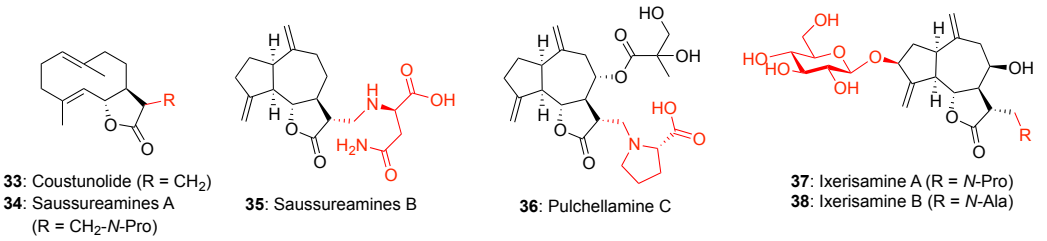
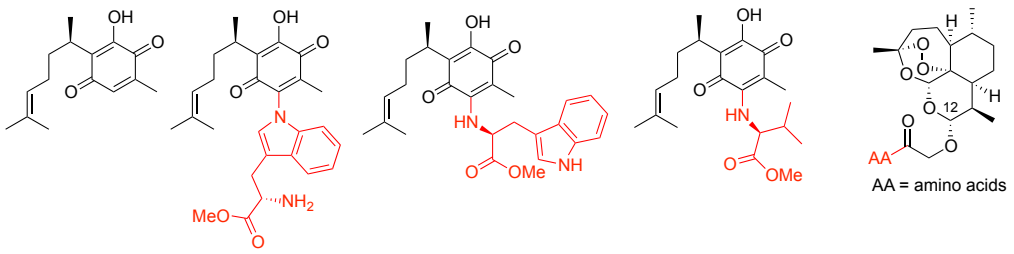


Figure 1.7 Naturally occurring sesquiterpenoid-amino acid-based conjugates.

The pulchellamines are another example of naturally occurring lactone-based conjugates. Seven of these conjugates, bearing amino acids such as L-asparagine, L-phenylalanine, L-proline, L-valine, L-tryptophan, L-isoleucine and γ-aminobutanoic acid (GABA), were isolated from aerial parts of the Korean medicinal plant *Saussurea pulchella*.[45] Pulchellamine C (**36**) exhibited promising cytotoxic activity *in vitro* in the micromolar range against human skin, ovary, colon and lung cancer cell lines. Very recently, Cha et al.[46] isolated novel glucosides of sesquiterpene-amino acid conjugates, ixerisamine A (**37**) and ixerisamine B (**38**), from traditional Chinese perennial herb *Ixeris dentate*. These sesquiterpene lactone-amino acid conjugates exhibited relatively poor inhibitory activity (ED_{50} > 30 μM) on proliferation of tumor cells compared to the sesquiterpene lactone itself (ED_{50} ~ 2 μM). Generally, it is believed that the loss of the exo methylene group on the γ-lactone via the Michael-type attack of an endogenous amino acid is responsible for the decreased cytotoxicity of these sesquiterpene-amino acid based conjugates.[46]

Perezone (**39**) (Figure 1.8) is a sesquiterpenoid benzoquinone isolated from the roots of Mexican traditional medicinal plants from the genus *Perezia*.[47] It plays an important role in inhibiting the aggregation of platelets due to a

hypoglycemic effect. It is also considered to be a cardioprotective agent, showing cytotoxicity against several cancer cell lines.



39: Perezone **40** (Lozada 2012) **41** (Lozada 2012) **42** (Lozada 2012) **43** (Adam 1997)

Figure 1.8 Synthetically-derived sesquiterpenoid-amino acid-based conjugates.

Recently, Lozada and co-workers[47] synthesized several aromatic (**40, 41**) and aliphatic (**42**) amino acid conjugates of this sesquiterpene to study their cytotoxic and antioxidant activities. The addition of aliphatic amino acid esters lowered this activity in comparison to the parent aromatic compound. Interestingly, **40** was selective against the K-562 leukemia cancer cell line (IC_{50} ~ 4.5 μM) while **41** was a potent antioxidant (IC_{50} ~ 5.5 μM) possessing better activity in comparison to reference drug α-tocopherol and perezone (**39**).

Artemisinin (**11**) is a well-known antimalarial drug biosynthesized from amorpha-4,11-diene (**10**).[48] Compound **11** falls into the class of amorphadiene-based sesquiterpenoids. In search for possible new biological activities offered by artemisinin-based amino acid conjugates, Adam and co-workers synthesized such conjugates such as **43** by attaching a variety of cyclic and linear amino acid ethyl esters at the 12β and 12α position of dihydroartemisinin.[48] Synthesizing new amino acid conjugates based on the relatively unexplored yet easily accessible amorpha-4,11-diene (**10**) scaffold is of further interest.

1.1.3.2 Steroid/triterpenoid-amino acid conjugates

Steroid-amino acid conjugates have been widely studied, and have been reported to play important roles in the cellular processes of living systems. They display diverse activities, including enhancing antiarrhythmic activity, delivering prodrugs to specific target tissues, and acting as synthetic receptors for oligopeptides.[9] In addition, some of these conjugates act as protease-like catalysts, mimic natural cationic peptide antibiotics, or stimulate permissive action (i.e. enhancing the effect of peptides by increasing the number of peptide receptors).[49-50]

Marine sponges are a rich source of steroids with highly functionalized scaffolds and highly variable side chains. As shown in Figure 1.9, polymastiamide A (**44**) is a tyrosine-conjugated steroid isolated from the Norwegian marine sponge *Polymastia boletiformis*.[51] Compound **44** is the first example of this new class of marine natural products, and exhibits *in vitro* antimicrobial activity against various human and plant infectious agents. Subsequent investigations led to the isolation of several other analogues of polymastiamide A, namely polymastiamides B-F.[52] This class of conjugates covers a number of important natural products, such as bufotoxin (**45**), a 3-arginyl-derived steroid isolated from the venom of the Chinese hoptoad.[53]

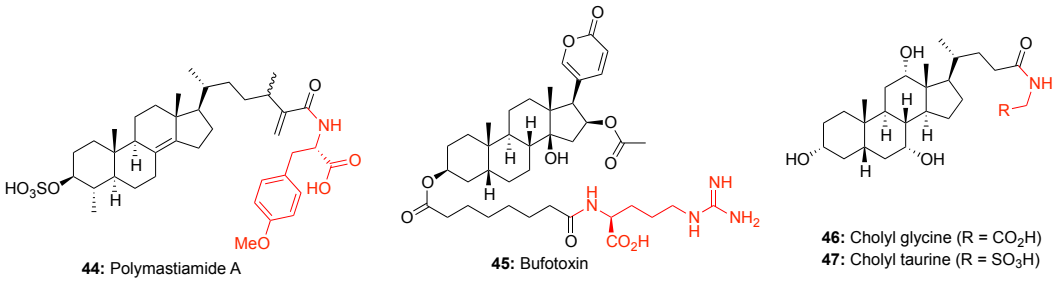
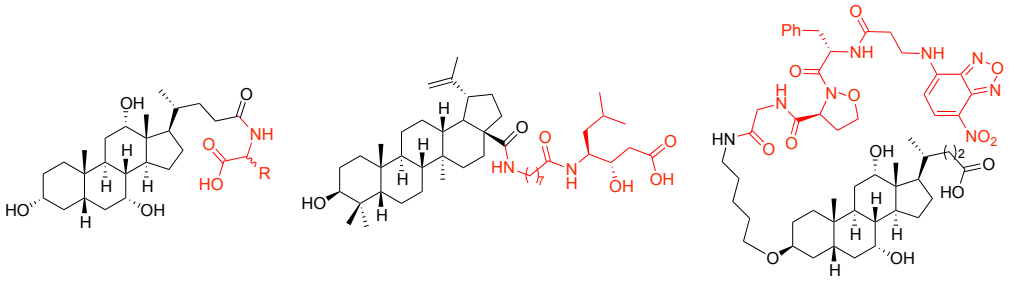


Figure 1.9 Naturally occurring steroid-amino acid-based conjugates.

Commonly known conjugates such as cholyl glycine (**46**) and cholyl taurine (**47**) contain a glycyl or a taurinyl group at the 17- position of the steroid, respectively. This has led to the development of analogues of steroid-amino acid conjugates for estrogen-dependent biological activity,[54] ^{14}C-cholyl-glycine breath test,[55] and treating depression.[56] For example, Marcelis and co-workers[57] synthesized a series of cholic acid derivatives (**48**) with a variety of α-amino acids coupled via an amide bond. These conjugates (Figure 1.10) form small micelles in aqueous solutions and were found to behave as novel organogelators, forming stable, transparent and thermoreversible gels in aromatic solvents. The authors showed that varying the amino acid side group had a modest influence on micellization behavior, namely a small decrease in critical micelle concentration (CMC) upon increasing the amino acid hydrophobicity.

Mayaux et al.[58] conjugated the unusual amino acid L-statin (4-amino-3-hydroxy-6-methylheptanoic acid)[59] with amide derivatives of betulinic acid. This novel conjugate **49** has been claimed to interfere specifically with virus-cell fusion and inhibited HIV replication at a concentration as low as 0.02 μg/mL.



48
(Marcelis 2003)

49
(Lepecq 1994)

50
(Petzinger 1992)

Figure 1.10 Synthetically derived steroid-amino acid-based conjugates.

Kramer et al.[60] were interested in investigating the potential of bile acids as "Trojan horses" to deliver drugs specifically to the liver, and to improve the membrane permeability/intestinal absorption of peptide drugs. Their structure-activity relationship studies revealed that 25% of peptide-bile acid conjugate **50** was secreted intact from portal blood into bile within 40 min. Less than 4% of the parent oxaprolylpeptide, (4-nitrobenzo-2-oxa-1,3-diazol-β-Ala-Phe-5-oxaproline-Gly), a fluorescent peptide inhibitor of prolyl-4-hydroxylase was secreted intact. This opens up important pharmacological options for the development of liver-specific drugs based on steroid-drug conjugates.

Hydrocortisone, prednisolone, and urotoxins (Glu-Asp-Gly-OH, His-Gly-Glu-OH, His-Gly-Lys-OH, and His-Gly-Lys-$NHNH_2$) isolated from uremic fluid, are known to have immunosuppressive activities.[61] To enhance their permissive action, Peng and co-workers[62] synthesized steroid–urotoxin conjugates (**51**, **52**) by introducing the urotoxins into convenient sites (C-3, C-21) on prednisolone and hydrocortisone via amidation or condensation reactions (Figure 1.11). These conjugates exhibited higher immunosuppressive activities than that of hydrocortisone, prednisolone, and the urotoxins alone, as well as when tested in combination.

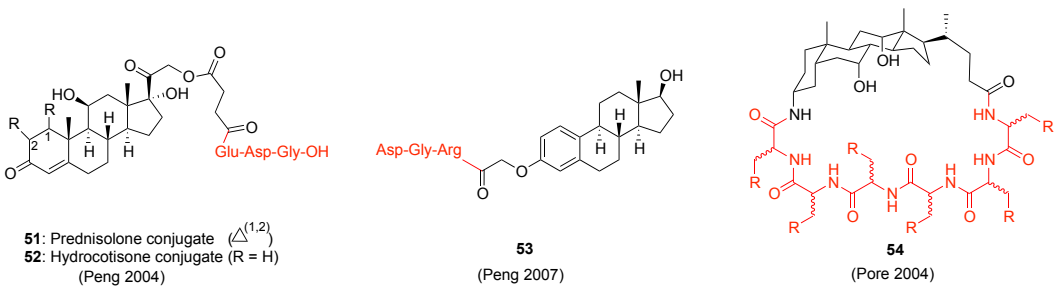


Figure 1.11 Synthetic steroid-amino acid-based conjugates.

The same group also conjugated tripeptide RGD (Arg-Gly-Asp), a common motif used by integrins for cell adhesion, at C-3 and C-17 of estrogenic sex hormones such as estradiol and estrone.[63] The orally active estradiol-RGD peptide conjugate **53** enhanced anti-osteoporosis potency, reduced the adverse effects of hormone replacement therapy (HRT), and could be used for cell adhesion studies.[64] Recently, Pore and coworkers[65] synthesized analogues of conjugate **54,** featuring a novel amphiphilic topology using bile acids and a variety of amino acids for membrane disruption.

1.1.3.2.1 Aminosteroid-amino acid conjugates

Steroids containing a nitrogen functionality (aminosteroids) offer a wide spectrum of biological activities. Antibacterial, antimalarial, antiviral and neurosteroid effects have all been observed. In addition to the steroid-amino acid conjugates mentioned above, aminosteroid-amino acid conjugates have also been synthesized and studied. Campbell et al. synthesized 3α-aminosteroid Org 6001 [amafalone (Am)] (**55**), which possessed an interesting antiarrhythmic activity in animal models.[66] Subsequently, Mokotoff and co-workers reported[67] the synthesis of peptidyl aminosteroids **56**. These conjugates exhibited similar antiarrhythmic effects to amafalone when administered intravenously to rats.

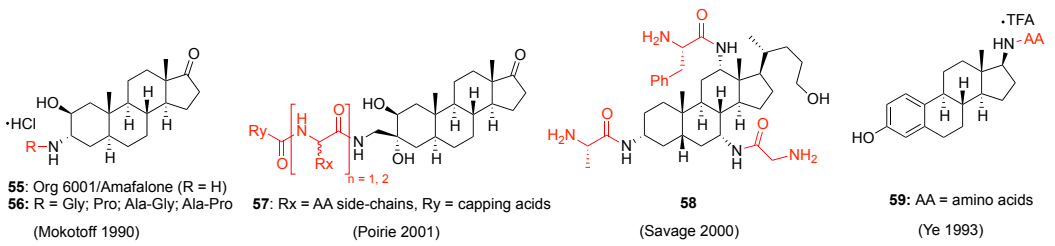


Figure 1.12 Synthetic aminosteroid-amino acid-based conjugates.

In order to design potential inhibitors of 17β-hydroxysteroid dehydrogenase (17β-HSD), Poirie and co-workers[68] used solid-phase synthesis to prepare libraries of 3β-peptido-aminomethyl-3α-hydroxy-5α-androstan-17-ones (**57**). Among them, 3β-(*N*-heptanoyl-L-phenylalanine-L-leucine)-aminomethyl-3α-hydroxy-5α-androstan-17-one (not shown) inhibited the enzyme with an IC_{50} value of 227 nM, twice as potent as the natural substrate Δ^4-dione.

Based on the observation that peptides such as polymyxin B sensitize Gram-negative bacteria to hydrophobic antibiotics by increasing the permeability of their outer membranes, Savage and co-workers[69] developed an efficient means of conjugating cholic acid derivatives with three amino acids (**58**, Figure 1.12), which sensitized Gram-negative bacteria to erythromycin and novobiocin. Similarly, cationic steroid antibiotics (CSA) were synthesized by conjugating tripeptides to a triaminoanalogue of cholic acid.[70] Recently Ye et al. synthesized a series of *N*-protected amino acid-estradiol derivative conjugates **59** which exhibited cytotoxic effects against both MCF-7 and HeLa cell lines.[71] Moreover, the biological studies also showed that their interaction with estrogen receptors could be modulated by the properties of the conjugated amino acids.[54]

1.2 Outline of Research Described in the Thesis

As described above, terpenoid-amino acid conjugates, including aminosteroid-amino acid conjugates demonstrate a broad array of interesting biological properties. Therefore, we became interested in developing a methodology to synthesize a new class of such amphiphilic amino acid conjugates based on the relatively unexplored and biologically important steroid 11β-aminoprogesterone and sesquiterpene amorpha-4,11-diene scaffolds (Figure 1.13).

In spite of being crucial for the biological activities of steroid hormones, modification of the 11β site of steroids has not received much attention in the development of conjugates. This is likely due to the synthetic challenges associated with modifying this position. It was reasoned that functionalizing the sterically encumbered 11β position of progesterone with hydrophilic moieties such as amino acids (Chapter 2) and cationic metal ligand–amino acid complexes (Chapter 3) would be of especial interest as it offers facially amphiphilic structures with both polar and nonpolar faces, similar to those observed in steroid-based biosurfactant bile salts (**60**). Such facially amphiphilic steroidal biosurfactants are also capable of forming micellar aggregates and solubilizing biologically important hydrophobic materials.[72]

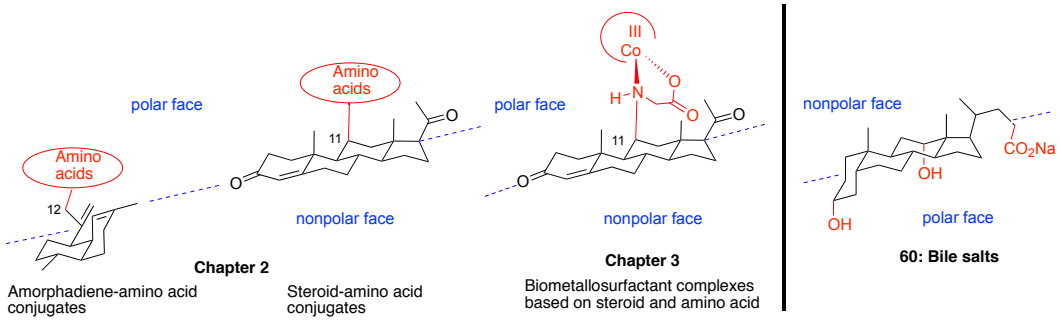


Figure 1.13 Thesis objectives: constructing novel facially amphiphilic sesquiterpenoid- and steroid-amino acid conjugates and steroid-amino acid based biometallosurfactants.

Besides the syntheses of 12-amorpha-4,11-dienyl-amino acid conjugate and 11β-substituted progesteronyl-amino acid conjugates, Chapter 2 also discusses initial evaluations of the synthesized 11β-substituted progesterone derivatives as modulators of 11β-hydroxysteroid dehydrogenases (11β-HSDs) and mineralocorticoid receptors (MR). These collaborative biological evaluations were performed by Professor Odermatt and co-workers at the University of Basel.

Chapter 3 discusses an alternate approach for accessing facially amphiphilic steroid-based structures by installing Co(III)-mediated tetradentate N_4-amino acid N,O based hexadentate complex at the 11β position of progesterone. Initial synthetic efforts towards the synthesis of such facially amphiphilic biometallosurfactant complexes and complexation model studies are discussed.

Finally, experimental data for the studies in Chapters 2 and 3 are listed in Chapter 4. Bibliographic citations are available in Chapter 5 and the crystal structure of 11β-amino progesterone is included in the appendix.

Chapter 2 : Stereoselective Synthesis & Evaluation of 11β-Aminoprogesterone-Amino Acid Conjugates* and Synthesis of 12-Amorpha-4,11-diene-Amino Acid Conjugate

2.1 Introduction

Terpenoid-amino acid conjugates constitute a class of amino acid-based biosurfactants or lipopeptides, where amino acids or peptides are linked to steroids or sesquiterpenoids. Some of these conjugates are natural products, such as sesquiterpenoid-amino acid-based saussureamine A[44] and pulchellamine C[45] as well as steroidal-amino acid-based polymastiamide A[51] and cholyl glycine.[9] These conjugates possess unique physicochemical and biological features due to their amphiphilic nature and ability to display the synergistic effects of two molecular entities. In addition to these natural conjugates, various synthetic terpenoid-amino acid conjugates are known to help deliver prodrugs to specific target tissues, to achieve permission action as well as to act as antibiotics, antiviral and antitumour agents, immunomodulators, steroid receptor modulators and enzyme inhibitors.[9, 40]

Inspired by such naturally occurring hybrids which combine a hydrophilic moiety (amino acids or peptides) with a lipophilic carrier (steroidal or sesquiterpenoidal derivatives), many researchers have been interested in

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* A version of this chapter has been published.

1. Pandya, K.; Dietrich, D.; Seibert, J.; Vederas, J. C.; Odermatt, A., Synthesis of sterically encumbered 11β-aminoprogesterone derivatives and evaluation as 11β-hydroxysteroid dehydrogenase inhibitors and mineralocorticoid receptor antagonists. *Bioorg. Med. Chem.* **2013**, *21*, 6274-6281.

developing methodology to synthesize a new class of terpenoid-amino acid conjugates. Among the terpenoids, the unexplored C-11β functionalized aminoprogesterone-based steroid conjugates and amorpha-4,11-diene (a biosynthetic precursor of antimalarial drug artemisinin) based sesquiterpene conjugates represent a new class of facially amphiphilic terpenoid-amino acid conjugates. A rationale for the selected targets is discussed in the remaining part of this introduction.

2.1.1 C-11 Functionalized Steroids: Modulators of 11β-Hydroxysteroid Dehydrogenases (11β-HSDs) and Mineralocorticoid Receptors (MRs)

Steroids are widely studied and are ideal building blocks for developing diverse conjugates due to their rigid architecture, broad biological activity profile, potential for varying levels of functionalization, and ability to penetrate cell membranes and bind to specific hormonal receptors.[5] Previous studies on steroidal derivatives have indicated that functionalization of steroids at C-11 often provides higher affinity binding with their respective receptors.[73] C-11 functionalization also appears to be crucial for the broad biological activity of numerous naturally occurring steroidal hormones[74] (e.g., **61**- **64**) and steroid-based drug molecules[74-75] (e.g., **65** and **66**) as shown in Figure 2.1.

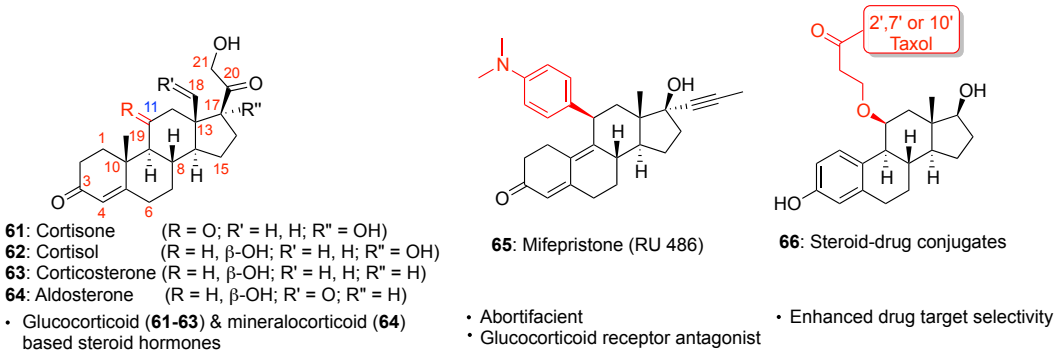


Figure 2.1 C-11 functionalized steroidal hormones and steroidal drugs.

C-11 functionalized hormonal steroids such as the corticosteroids (cortisol in humans and corticosterone in rodents) play an important role in modulation of various physiological functions.[73] An impaired corticosteroid action has been linked with cardio-metabolic diseases such as hypertension, atherosclerosis, hyperlipidemia and diabetes, in addition to some psychiatric disorders.[76] Corticosteroids exert their effects specifically through glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs).

Modulation of their actions by controlling GR signaling[77-78] (through the fine balance of active glucocorticoids by inhibiting 11β-hydroxysteroid dehydrogenases) or blocking MRs[76] (through MR antagonists) is currently under active consideration for treatment of electrolyte disturbances, metabolic diseases and chronic inflammatory disorders. Generally, the physiological human ligands for activating the MRs and the GRs are aldosterone and cortisol, respectively. However, in diverse disorders such as congestive heart failure and essential hypertension, cortisol activates the MRs instead of aldosterone.[79]

11β-Hydroxysteroid dehydrogenases (11β-HSDs), members of the short-chain alcohol dehydrogenase (SDR) family, are responsible for the

interconversion of active glucocorticoid cortisol (**62**) to hormonally inactive cortisone (**61**). Thus, by regulating cortisol levels, 11β-HSDs modulate GR activation (Figure 2.2).[80]

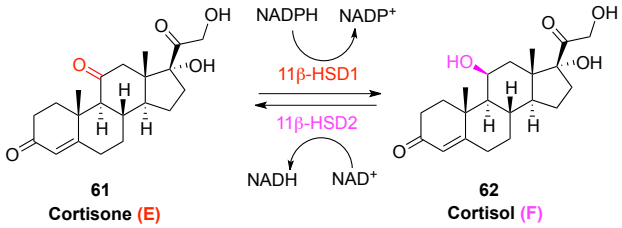


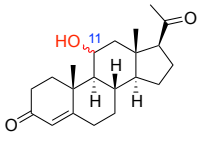
Figure 2.2 Biological equilibrium between cortisone and cortisol.

In humans, NADPH-dependent isozyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) functions as an oxoreductase, converting inactive 11-ketosteroid cortisone (dehydrocorticosterone in rodents) to active cortisol (corticosterone in rodents) in the liver, adipose tissue and skeletal muscle.[78] The reverse reaction is catalyzed by NAD^+-dependent isozyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2). 11β-HSD2 is mainly found in mineralocorticoid (MC) target tissues, such as the kidney, colon, salivary glands, placenta and inflamed tissue.[81]

2.1.1.1 Steroid based inhibitors of 11β-HSDs

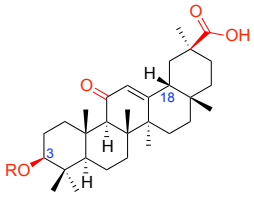
Metabolic syndromes caused by imbalanced levels of cortisone (**61**) and cortisol (**62**) can be treated with both non-steroidal-based[82-84] and steroidal-based non-selective 11β-HSDs inhibitors. The most well-known non-selective steroid hormone-based inhibitors are 11β-hydroxy progesterone (11β-OH-Pro) (**67**)[85] and 11α-hydroxy progesterone (11α-OH-Pro) (**68**).[86] Other inhibitors based on steroid scaffolds include 18β-glycyrrhetinic acid (18β-GA) (**69**), the metabolite of the

liquorice root derivatives, and carbenoxolone (CBX) (**70**), a clinically available derivative of 18β-GA (Figure 2.3).[87] In addition to their glucocorticoid (GC) properties, these non-selective inhibitors act as MR agonists. This results in unwanted side effects such as a fluid-electrolyte imbalance, hypertension and detrimental cardiovascular effects through the increased activation of MR in cardiomyocytes, adipocytes, macrophage and the kidney.[76] This is most likely due to the close structural relationship between GR and MR, and their overlapping signaling pathways. However, MR antagonists such as progesterone improved the morbidity and mortality of patients with myocardial infarction or heart failure.[88] Hence, several pharmaceutical companies are actively developing selective MR antagonists (aldosterone blockers) for the treatment of chronic kidney disease (CKD), fluid-electrolyte imbalance and diabetic nephropathy.



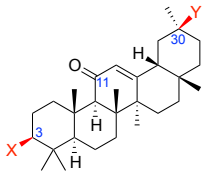
67: 11β-OH-Progesterone
68: 11α-OH-Progesterone

Steroid hormone based non-selective 11β-HSDs inhibitors



69: 18β-Glycyrrhetinic acid
(R = H)
70: Carbenoxolone
(R = CO-CH_2-CH_2-CO_2H)

18β-GA based non-selective 11β-HSDs inhibitors



71
X = alcohol, ether, ester, amine, amide, sulfamide
Y = acid, amide, ether, sulfamide, hydroxamic acid, urea

18β-GA based selective 11β-HSD2 inhibitors

Figure 2.3 Selective and non-selective steroidal inhibitors of 11β-HSDs.

As an alternative to this mineralocorticoid receptor antagonism (MRA), selective inhibition of 11β-HSD1 has also been an active therapeutic area of interest for many pharmaceutical companies in the treatment of metabolic diseases. Selective inhibition of 11β-HSD1 is reported to demonstrate beneficial effects in the treatment of metabolic syndrome disorders such as hyperlipidemia,

type 2 diabetes, atherosclerosis and osteoporosis.[77] On the other hand, selective inhibition of 11β-HSD2 in the kidney results in cortisol-induced MR activation causing sodium and water retention and hypertension.[77] Hence, the development of selective 11β-HSD2 inhibitors has been relatively limited for such an application.

However, the selective inhibition of 11β-HSD2 does offer beneficial effects in the treatment of chronic inflammatory diseases and colon/pituitary tumor cell proliferation.[89-90] Additionally, selective 11β-HSD2 inhibitors are also reported to prevent hyperkalemia in chronic hemodialysis patients by enhancing potassium excretion in the colon.[91] These recent findings have spurred interest in the development of selective 11β-HSD2 inhibitors.[87, 92-95] The research groups of Odermatt,[87] Potter,[92] and Houben[95] have identified several 18β-GA based potent/selective 11β-HSD2 inhibitors by modifying the C-3 and C-30 positions of the parent 18β-GA template (**71**, Figure 2.3). However, concerns regarding the cell membrane permeability of this class of compounds have been raised.[96]

2.1.1.2 11-Hydroxyprogesterone as 11β-HSDs inhibitors & MR agonists

Potter[96] and Jordis[93] have independently demonstrated that the presence of a hydrogen bond acceptor at C-11 of steroids is important for 11β-HSDs inhibition. Dramatic differences in the potency of C-11 modified inhibitors are observed, as this is the position where the 11β-HSD isozymes exert their catalytic action. These results corroborated the findings of Morris and coworkers.[86] They showed that the addition of a hydroxyl group to endogenous inhibitor progesterone, generating either endogenous metabolite 11β-OH-Pro (**67**) or

exogenous metabolite 11α-OH-Pro (**68**), markedly enhanced its inhibitory effect against 11β-HSDs. Upon testing of these metabolites against sheep kidney 11β-HSDs, 11β-OH-Pro (**67**) acted as a competitive inhibitor of 11β-HSD2 (two-fold more potent than **68**). However, 11α-OH-Pro (**68**) acted as a noncompetitive inhibitor of 11β-HSD1 (11-fold more potent than **67**).[86]

Additionally, both diastereomers of 11-OH-Pro (**67** and **68**) are reported to act as MR agonists. Rafestin-Oblin[97] showed that the agonist activity of 11-OH-Pro is attributed to contact between the 11-hydroxy group and Asn 770, a residue of the ligand-binding domain of the human mineralocorticoid receptor (hMR). To study the structure-activity relationship further, we were interested in synthesizing novel analogues of 11β-OH-Pro (**67**) by substituting the 11β-hydroxyl group with 11β-amino substituted derivatives. Furthermore, these facially amphiphilic 11β-aminosteroid based conjugates would likely possess improved cell membrane permeability, water solubility and bioavailability for site-specific drug delivery.

2.1.2 Amorphadiene Sesquiterpenes and Their Conjugates

Structurally similar to amorphane, the amorphadiene sesquiterpenes are a subclass of bicyclic cadinene sesquiterpenes possessing a *cis*-decalin skeleton with the isopropenyl group *trans* to the hydrogen on the ring juncture. Well-known antimalarial drug artemisinin (**11**) (Figure 2.4), isolated from the Chinese plant *Artemisia annua L.* is closely related to this class of sesquiterpenes.[98] A late intermediate in the biosynthesis of artemisinin, artemisinic acid (**72**) [a derivative of amorpha-4,11-diene (**10**)], also belongs to this class of compounds.[99]

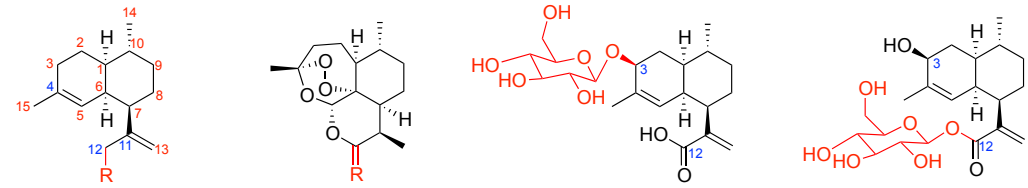


Figure 2.4 Amorphadiene sesquiterpenes and their conjugates.

Artemisinin (**11**), a sesquiterpene lactone with an endoperoxide bridge, is effective against multidrug resistant strains of *Plasmodium falciparum*, the causative agent of malaria.[100] However, problems associated with the therapeutic use of artemisinin include a short plasma half-life, limited bioavailability, poor solubility, and low production from natural sources. These issues have prompted scientists to develop new artemisinin derivatives including amphiphilic artesunate (**73**)[101] and its amino acid[48] and glucose-based[27] conjugates. In addition to treating malaria, these new derivatives of artemisinin are known to be useful in treating hepatitis B, schistosomiasis and a range of cancer cell lines such as breast cancer, human leukemia, colon, small cell lung carcinomas and drug-resistant cancers.[99]

Apart from artemisinin (**11**), artemisinic acid (**72**) has recently gained attention related to the development of conjugates due to its structural simplicity in promoting the conversion into **11** and its abundance in the plant. Although less pharmaceutically important than **11**, acid **72** has a variety of pharmacological properties such as antimalarial activity, anti-tumor activity, antipyretic effect, antibacterial activity, allelopathy effect and anti-adipogenesis effects.[99] The broad biological activity of **72**, in combination with the challenging structure and

relative scarcity of artemisinin **11**, have prompted extensive efforts to identify new amorpha-4,11-diene based artemisinic acid analogues/conjugates.[102]

Recently, Rongming Yu and co-workers[103] isolated new glycosylated amorpha-4,11-diene products including artemisinic acid 3β-*O*-β-D-glucopyranoside (**74**) (Figure 2.4) and 3β-hydroxyartemisinic acid β-D-glucopyranosyl ester (**75**) from a cell culture of *Averrhoa carambola* following the administration of artemisinic acid (**72**). Similar results were obtained using a cell culture of *Artemisia annua L.*[104] Furthermore, biotransformation product **75** exhibited higher anti-tumour activity than **74**, at lower levels, when evaluated against K562 (leukemia cancer) and HeLa (cervical cancer) cell lines.

So far, no other amorpha-4,11-diene based conjugates have been reported. Hence we decided to develop a facially amphiphilic synthetic 12-amorpha-4,11-diene-amino acid conjugate by installing an amino acid moiety at C-12 of amorpha-4,11-diene. It was hoped that this new class of amino acid conjugate might offer interesting biological properties. Additionally, it would also offer a platform to synthesize other peptidyl conjugates based on amorpha-4,11-diene.

2.2 Project Objectives: Synthesis and Evaluation of 11β-Aminoprogesterone Derivatives and Synthesis of 12-Amorpha-4,11-diene-Amino Acid Conjugates

Based on previous 11β-HSDs and MR modulation studies of 11β-OH-Pro (**67**), we decided to synthesize a small library of steroidal hormone analogues by introducing an amino group or amino acid functionalities to the 11β-position of

progesterone. To the best of our knowledge, screening of such synthetic analogues of **67** as 11β-HSDs inhibitors had not been previously reported.

However, stereoselective C-11 functionalization of steroids has remained a long-standing challenge to synthetic organic chemists due to the severe steric hindrance imposed by the C-18 and C-19 angular methyl groups.[105] Only limited examples of 11β-substituted progesterone derivatives have been reported.[75, 106-107] Specifically, the introduction of an 11β-amino functionality on progesterone remains a daunting task, despite it being the basis for an important class of aminosteroid derivatives.[108-109] Syntheses of 10β-, 11α- and 12β-aminoprogesterone have been reported in the literature,[109-111] but the synthesis of 11β-aminoprogesterone has remained unknown. Aminosteroids possess many interesting neuropharmacological activities, and are known to act as anesthetic, antiarrhythmic, fungicidal, antibacterial and immunomodulatory agents.[112]

In addition to synthesizing conjugates with an amino group or amino acid functionality at the C-11β position of progesterone, we have also been interested in exploring means of increasing their chemical and metabolic stability by forming a stable covalent linkage between the terpenoid and amino acid moieties. The most commonly employed chemical linkages, such as glycosidic, amide or ester bonds have an obvious drawback due to their sensitivity towards chemical and enzymatic hydrolysis. Hence an alternate linkage with increased stability *in vivo* and *in vitro* is of interest.[49]

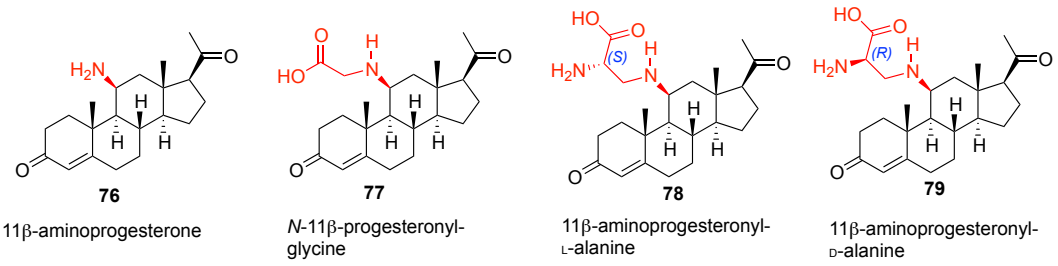
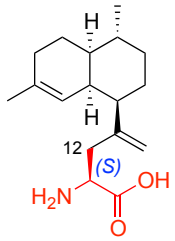


Figure 2.5 Targeted 11β-aminoprogesterone (**76**), and its conjugates with glycine **77**, L-alanine **78**, and D-alanine **79**.

In that regard, we envisioned that a facile construction of a new class of steroid-amino acid conjugates based on 11β-aminoprogesterone (**76**) and glycine as well as L- and D-alanine (**77**, **78**, and **79**) would be of interest (Figure 2.5). Such steroid conjugates have the potential to demonstrate synergistic biological activity arising from the different functionalities (i.e. C-11 substitution, amino and amino acid present in one single molecule). Also, these conjugates could act as a precursor for the synthesis of numerous other novel 11β-aminoprogesterone based conjugates.

Once successfully synthesized, screening these derivatives would further extend our understanding of the favorable or unfavorable interactions at the catalytic site of 11β-HSDs. In that regard, these conjugates were to be screened against 11β-HSDs and MRs through a collaborative effort with Professor Odermatt and co-workers from the University of Basel, Switzerland.[113]

In addition to steroid based conjugates, we also decided to synthesize facially amphiphilic sesquiterpenoid amorpha-4,11-diene based amino acid conjugate **80** by stereoselective installation of glycine at C-12 of amorpha-4,11-diene as shown in Figure 2.6.



80

12-Amorpha-4,11-dienyl-*(S)*-glycine

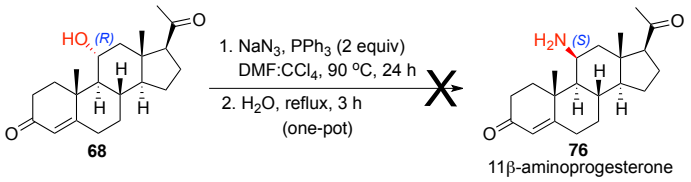
Figure 2.6 Targeted 12-amorpha-4,11-dienyl-(*S*)-glycine conjugate.

2.3 Results and Discussion

2.3.1 Synthesis of 11β-Aminoprogesterone (11β-NH_2-Pro) (76)

Conversion of alcohols to amines is an important reaction for the synthesis of a variety of organic compounds, including the desired progesterone-amino acid conjugates. The conventional approach for preparation of amines involves a three step protocol: i) transforming an alcohol to the corresponding halide or sulfonate, ii) nucleophilic substitution by an azide anion and iii) reduction of the azide to an amine. They can also be prepared by a two step methodology: i) conversion of an alcohol to an azide by the Mitsunobu reaction and ii) reduction of the azide to an amine. In order to avoid handling potentially explosive azide intermediates, Reddy et al.[114] accomplished a new and efficient direct one-pot protocol for the conversion of alcohols to amines using NaN_3 and PPh_3 (2 equiv) avoiding the use of expensive diethyl azodicarboxylate (DEAD) or diisopropyl azodicarboxylate (DIAD). It was proposed that the azides formed *in situ* would react with Ph_3P, forming an iminophosphorane, which in turn transformed to the amine upon treatment with water. Disappointingly, when the same one-step protocol was

applied to 11α-OH-Pro (**68**) (Scheme 2.1) it did not form the desired 11β-NH_2-Pro (**76**).



Scheme 2.1 Attempted synthesis of 11β-aminoprogesterone (**76**) by direct amination on 11α-hydroxyprogesterone (**68**).

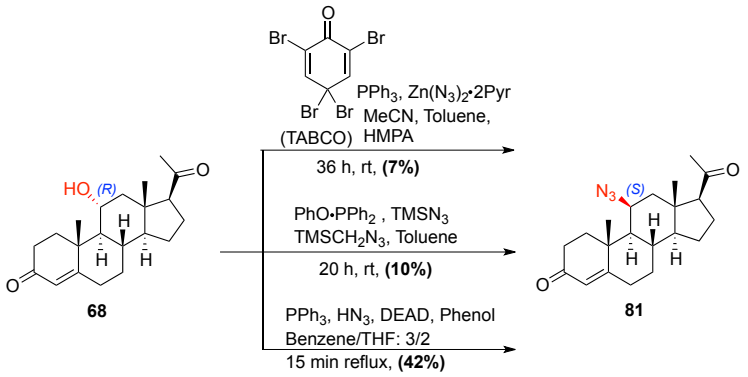
Hence, the two-step methodology of forming alkyl azide 11β-azidoprogesterone (11β-N_3-Pro) (**81**)[115] followed by a chemoselective reduction to amine **76** was explored further.

2.3.1.1 Synthesis of 11β-azidoprogesterone (11β-N_3-Pro) (81)[115]

The most elementary method known for azidation is the Mitsunobu reaction, using hydrogen azide as an azide source. However, due to the limited applicability of this reaction owing to the use of highly toxic and explosive hydrogen azide, we initially sought to adopt an alternative method using a zinc azide/bis-pyridine complex $Zn(N_3)_2$•2Py. In 1997, Tanaka and co-workers[116] demonstrated this new azidation method involving 2,4,4,6-tetrabromo-2,5-cyclohexadienone (TABCO), PPh_3 and $Zn(N_3)_2$•2Py to form azides from alcohols with considerably faster rates compared to those of the most commonly employed for Mitsunobu-type azidation reactions (using PPh_3/DEAD/NaN_3 in toluene). Due to the limited reactivity of the C-11 center of progesterone, we thought to take advantage of this protocol for the synthesis of azide **81**. As shown in Scheme 2.2, when 11α-OH-Pro (**68**) was treated with TABCO, PPh_3 and excess

$Zn(N_3)_2$•2Py[117] in a mixed solvent of CH_3CN/toluene/HMPA, only 7% of 11β-N_3-Pro (**81**) was obtained. Further attempts to synthesize azide **81** using Mitsunobu conditions (i.e. PPh_3/DIAD/$Zn(N_3)_2$•2Py or NaN_3) proved to be futile.

Subsequently, a new azidation method developed by Mukaiyama and Hayashi[118] was considered wherein they performed stereospecific syntheses of *sec*- and *tert*-alkyl azides. These azides were prepared from sterically hindered chiral *sec*- and *tert*-alcohols, through treatment with trimethylsilyl azide ($TMSN_3$) and a new type of oxidation–reduction condensation using phenyl diphenylphosphinite ($PhO•PPh_2$)[119] and trimethylsilylmethyl azide ($TMSCH_2N_3$)[120]. Therefore, 11α-OH-Pro (**68**) was treated with $PhO•PPh_2$, $TMSCH_2N_3$ and $TMSN_3$ (3 equiv of each) in toluene and allowed to stir for 20 h until the reaction was complete (Scheme 2.2). However, only 10% of the desired 11β-N_3-Pro **81** was extracted from the reaction mixture.



Scheme 2.2 Synthesis of 11β-azidoprogesterone (**81**) from 11α-hydroxyprogesterone (**68**).

Finally, adapting the modified Mitsunobu protocol of Loibner and Zbiral[115] using hydrazoic acid (HN_3) allowed for the synthesis of 11β-N_3-Pro (**81**)

in a moderate yield. As shown in Scheme 2.2, refluxing a mixture of PPh_3, DEAD and phenol with freshly prepared hydrazoic acid (HN_3)[121] and 11α-OH-Pro (**68**) in a benzene - THF (3:2) co-solvent system gave the desired azido product **81** in 42% yield, after optimization.

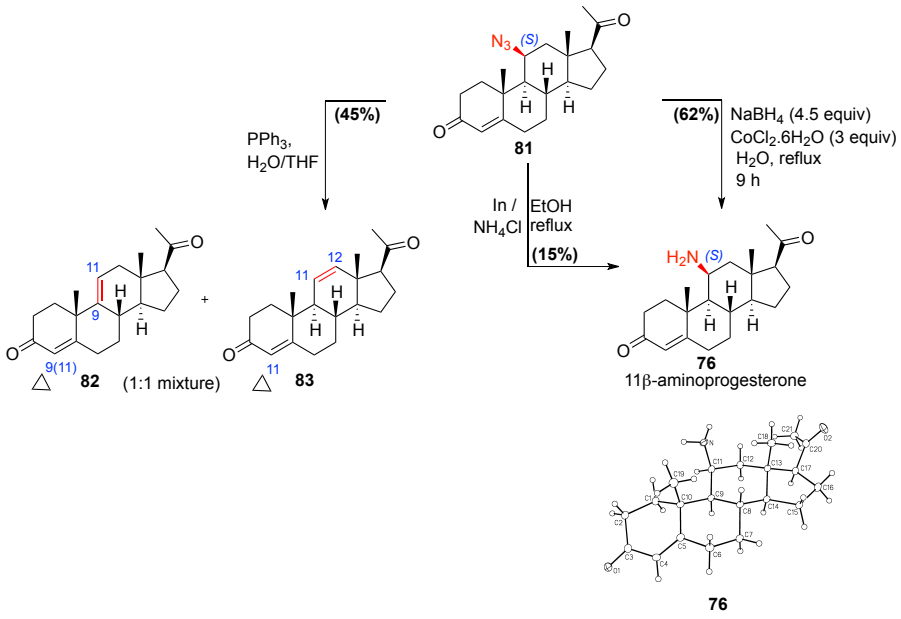
The success of this reaction closely depended on the order of addition of reagents. In that regard, the betaine was initially formed by allowing a mixture of DEAD (3 equiv) and oven-dried PPh_3 (3 equiv) to react for 10 minutes at room temperature in dry THF. To a mixture of a preformed betaine and phenol (0.1 equiv), an excess of HN_3 in benzene (6 equiv) was first added, followed by 11α-OH-Pro (**68**). After refluxing for 15 minutes, the pink-red colored mixture turned to a dark brown indicating the formation of the desired 11β-N_3-Pro (**81**) product and completion of the reaction. The catalytic quantity of phenol was proposed to activate the alcohol towards azidation by HN_3.[122]

2.3.1.2 Reduction of azide 81 to amine 76

Having azide product **81** in hand, a reductive process was further explored to synthesize the desired amine product **76**. Classic procedures for the reduction of azides involve the use of lithium aluminum hydride ($LiAlH_4$) or catalytic hydrogenation (Pd/C, H_2). However, these conditions are not suitable for 11β-N_3-Pro (**81**) due to their potential to transform the unprotected C-20 ketone and C-3 enone of **81**. Hence a chemoselective reduction of azide was required. The Staudinger reduction is a mild and popular method for the chemoselective reduction of an azide to an amine, involving a one-pot hydrolysis of the

iminophosphorane formed when PPh_3 is reacted with the azide in THF, in the presence of an excess of water.

However, when 11β-N_3-Pro (**81**) was exposed to Staudinger reduction conditions[123] (3 equiv of PPh_3, PBu_3 or PMe_3 and 10 equiv of water at either room temperature or reflux), the eliminated products $\Delta^{9(11)}$ **82** and Δ^{11} **83** were obtained in a 1:1 mixture instead of the desired 11β-NH_2-Pro (**76**) (Scheme 2.3). These elimination products were identified based on the reported ^1H and ^{13}C-NMR, and EI HR-MS data.[124-125] Similarly, treatment of azido compound **81** with several other chemoselective reduction conditions reported for the reduction of sterically hindered azides, such as Fe^{3+}/Zn(Ref.126) and $Sn^{4+}/NaBH_4$(Ref.127) also failed to produce the desired amino compound **76**. Alternatively, when azide **81** was treated with In/NH_4Cl[128] in ethanol under reflux, it formed the corresponding amine **76** in a poor yield of 15% (Scheme 2.3).



Scheme 2.3 Reduction of 11β-azidoprogesterone (**81**) to 11β-aminoprogesterone (**76**).

Gratifyingly, after several optimizations, it was found that modifications of the protocols of Fringuelli et al.[129] and Salunke et al.[130] were optimal for the desired transformation. In that regard, when a mixture of azide **81** and $CoCl_2 \cdot 6H_2O$ (3 equiv) was refluxed in heterogeneous phase by adding an aqueous solution of $NaBH_4$ (4.5 equiv), it formed the desired amine **76** with an acceptable yield of 62% (Scheme 2.3). Ganem and co-workers[131] proposed that *in situ* generated cobalt boride/H_2 (Co_2B/H_2) formed by the decomposition of $NaBH_4$ over Co_2B is involved in such heterogeneous reductions.

$NaBH_4$ is a mild reducing agent that does not usually reduce azides to amines. However, using transition metal salts in conjunction with $NaBH_4$ modifies and enhances the properties of these reagents. The combination of $CoCl_2$ with $NaBH_4$ in a protic solvent deposits a finely divided black precipitate of cobalt boride (Co_2B) while evolving hydrogen as shown in equation (2.1).[131]

$$4\ BH_4^- + 2\ Co^{2+} + 9\ H_2O \rightarrow Co_2B + 3\ B(OH)_3 + 4\ NaCl + 12.5\ H_2 \qquad (2.1)$$

The actual structure and mechanism of action of cobalt boride is not clear. However, it is known that the cobalt boride formed has adsorbed hydrogen on its surface. This adsorbed hydrogen is likely responsible for the reduction of a variety of organic functional groups. Further, the cobalt boride activity and the selectivity is highly solvent dependent (e.g. cobalt boride prepared in water is considerably more active).[132]

It is important to note that apart from reducing azides, the Co^{2+}/borohydride mixture is reported to reduce both α,β-unsaturated carbonyl systems and unconjugated carbonyls.[133] However, the reduction of 11β-N_3-Pro

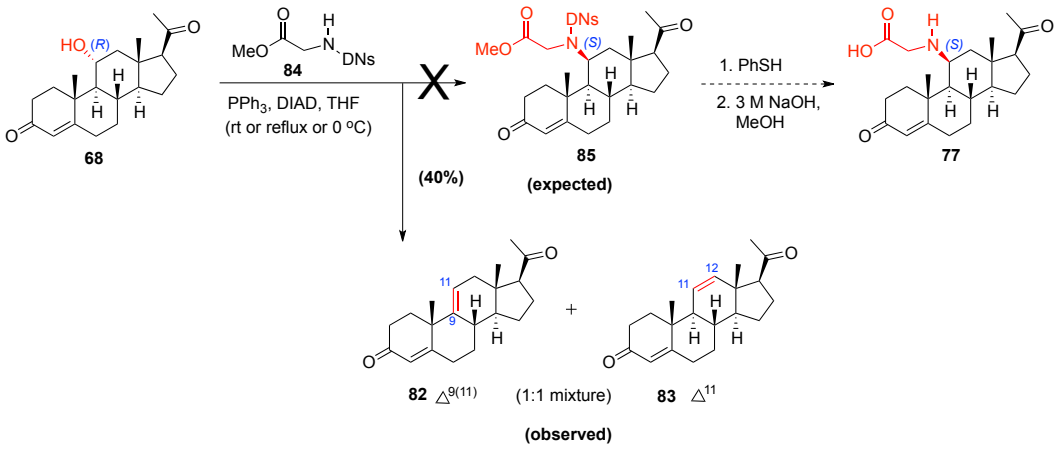
(**81**) to 11β-NH_2-Pro (**76**) with Co^{2+}/borohydride proceeded with excellent chemoselectivity without affecting the unprotected C-20 unconjugated carbonyl and C-3 α,β-unsaturated carbonyl system of azide **81**. Confirmation of the installation of the 11β-amino group, as well as the state of the C-20 carbonyl and C-3 α,β-unsaturated system was obtained by performing X-ray crystallographic analysis (Scheme 2.3) on the crystals of 11β-NH_2-Pro (**76**) grown by a slow evaporation of ether/hexane. To the best of our knowledge, this is the first example in the steroid series wherein an 11β-amino group is directly installed onto a steroid framework in a facile manner leaving the steroid C-20 carbonyl and C-3 enone functionalities unprotected.

2.3.2. Synthesis of *N*-11β-Aminoprogesteronyl Glycine (*N*-11β-NH-Pro-Gly) (77)

We initially hypothesized that the facile synthesis of aminoprogesterone-based amino acid conjugates such as *N*-11β-aminoprogesteronyl-glycine (**77**) could be directly achieved from commercially available 11α-OH-Pro (**68**) using the Fukuyama–Mitsunobu amination strategy, an efficient means of *N*-alkylation of peptides.[134-135] The Fukuyama amine synthesis involves a two-step conversion of primary amines into secondary amines via *ortho*-nitrobenzenesulfonation or 2,4-dinitrobenzenesulfonation in combination with the Mitsunobu reaction, followed by the removal of the *ortho*-nitrobenzenesulfonyl (*o*-Ns) or 2,4-dinitrobenzenesulfonyl (DNs) group with a thiol. It is known that the inversion of a sterically congested alcohol with a carboxylic acid can be effectively done using

the more electron withdrawing DNs group and DIAD instead of *o*-Ns and DEAD.[136]

Accordingly the 2,4-dinitrobenzenesulfonamide-glycine methyl ester (**84**)[137] (1.5 equivalents) was mixed with 11α-OH-Pro (**68**) under Mitsunobu conditions as shown in Scheme 2.4, at either room temperature or reflux. However, it did not afford the expected *N*-alkylated glycine methyl ester (**85**), and unreacted starting material was observed along with a mixture of undesired elimination products $\Delta^{9(11)}$ **82** and Δ^{11} **83**. Performing the reaction at 0 °C did not suppress the formation of these elimination products. Hence, this route was not explored further. It is worth noting that the further treatment of expected ester **85** with a thiophenol followed by a base hydrolysis could have offered target conjugate **77** in a straightforward manner.

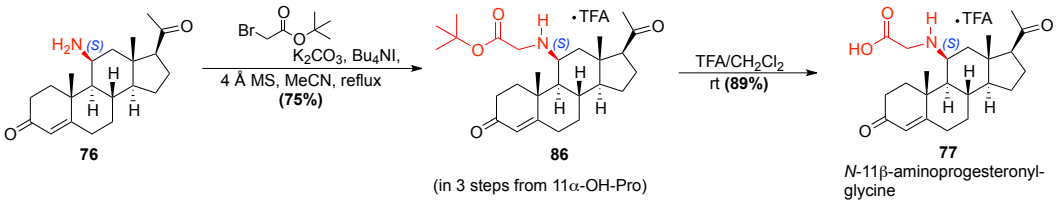


Scheme 2.4 Attempted synthesis of *N*-11β-aminoprogesteronyl-glycine (**77**) by direct Fukuyama–Mitsunobu amination of 11α-OH-Pro (**68**).

It was reasoned that the introduction of a bulkier DNs-protected glycine nucleophile on 11α-OH-Pro (**68**) was likely unfavourable due to the excessive

steric demands introduced by the rigid steroid backbone and the angular C-18 and C-19 methyl groups. Hence, a stepwise protocol involving the synthesis of 11β-NH_2-Pro (**76**) followed by *N*-alkylation of the amino group was envisioned to construct the desired progesterone-amino acid conjugates.

Through retrosynthetic analysis, we envisioned that the target glycine functionalized 11β-aminoprogesteronyl conjugate **77** could be easily prepared by *N*-alkylation of 11β-aminoprogesterone (**76**) with a bromoacetate. However, attempts towards *N*-alkylation of amine **76** in DMF or DCE as well as in the presence of LiOH•H_2O or Et_3N did not form the desired *N*-alkylated product **86**. After optimization, treatment of 11β-NH_2-Pro (**76**) in acetonitrile with 2 equiv of *tert*-butyl bromoacetate, 2 equiv of Bu_4NI and 4 equiv of K_2CO_3 for 9 h at reflux offered the *N*-aminoprogesterone-glycinate conjugate **86** in a 75% yield. Upon acid hydrolysis, **86** gave the target *N*-11β-aminoprogesteronyl glycine (*N*-11β-NH-Pro-Gly) **(77)** in 89% yield as shown in Scheme 2.5.



Scheme 2.5 Synthesis of *N*-11β-aminoprogesteronyl-glycine (**77**) by *N*-alkylation of 11β-OH-Pro (**76**).

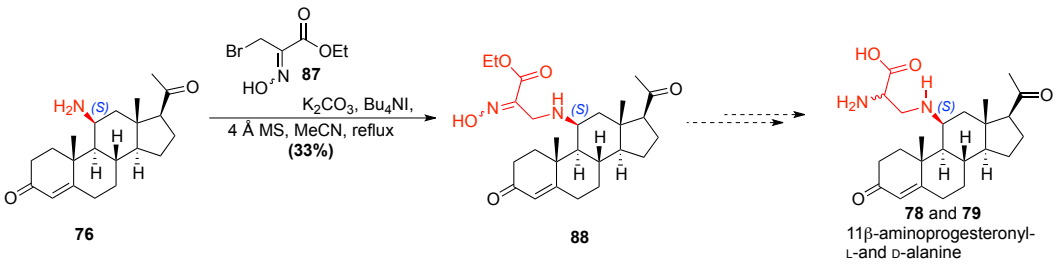
It is worth noting that only three steps were required to access the glycinate derivative of *N*-11β-aminoprogesterone (**86**) from commercially available 11α-OH-Pro (**68**). The only reported synthesis[106-107] of a similar derivative (i.e. an acetyl derivative of *N*-11β-aminoprogesterone) required a total

of 14 steps from 11α-OH-Pro (**68**), and required extensive reduction/oxidation and protection/deprotection chemistry at C-11, the C-20 carbonyl and the C-3 enone of **68**.

2.3.3 Synthesis of *N*-11β-Aminoprogesteronyl L- and D-Alanine [*N*-11β-NH-Pro- L- and D-Ala] (78) & (79)

2.3.3.1 Attempted synthesis by a substrate-controlled reduction of oxime 88

The target conjugates **78** and **79** could be visualized as being derived from a chemoselective and stereoselective oxime reduction of *N*-11β-aminoprogesterone–glycoxylate oxime (**88**) (Scheme 2.6). The oxime **88** could be easily synthesized by the *N*-alkylation protocol developed for 11β-aminoprogesterone. As steroids are generally known to possess inherent substrate selectivity due to their rigid framework and chirality, the reduction of oxime **88** might be expected to proceed in a stereoselective manner. Such a substrate-controlled stereoselective reduction of oxime **88** followed by ester hydrolysis should yield a mixture of *N*-11β-aminoprogesteronyl L- and D-alanine (**78** and **79**) with a great diasteromeric excess of either **78** or **79**. A subsequent treatment of the obtained mixture of **78** and **79** with D-amino acid oxidase should then selectively oxidize the D-alanine based conjugate **79**, leaving the L-alanine based conjugate **78** in pure form for recovery. Similarly, D-alanine based conjugate **79** could be isolated from a mixture of **78** and **79** through the treatment of the mixture with L-amino acid oxidase. To test this hypothesis, synthetic efforts were first directed towards the preparation of oxime **88**.



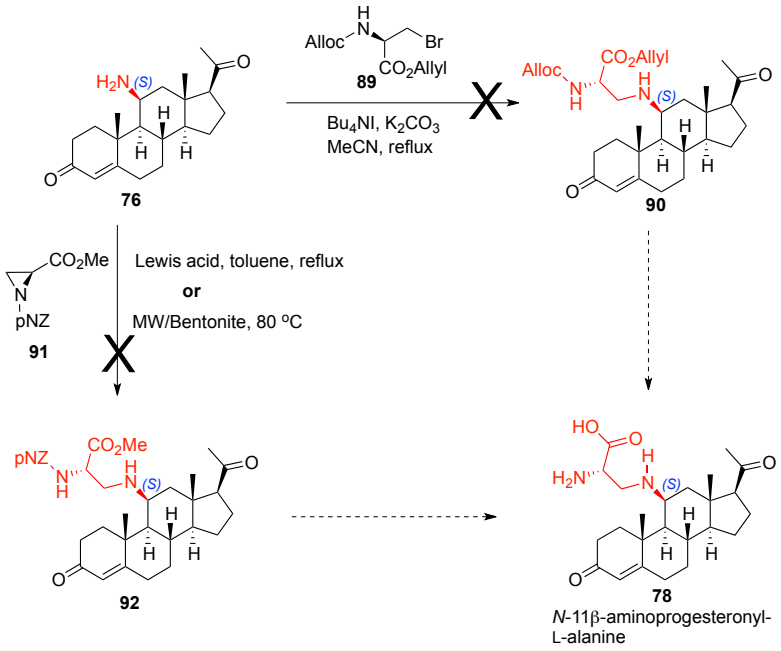
Scheme 2.6 Attempted synthesis of a mixture of *N*-11β-aminoprogesteronyl L- and D-alanine (**78** and **79**) by substrate-controlled reduction of oxime **88**.

Accordingly, following the *N*-alkylation protocol, the reaction of 11β-aminoprogesterone (**76**) with ethyl bromopyruvate oxime **87**[138] in the presence of Bu_4NI and K_2CO_3 in acetonitrile at reflux offered 11β-aminoprogesterone – glycoxylate oxime (**88**) in 33% yield (Scheme 2.6). Subsequent efforts were directed towards achieving a chemoselective oxime reduction of **88** using established reducing systems such as Zn/NH_4HCO_2,[139] $NaBH_4/LiCl/Amberlyst\ 15$,[140] $NaCNBH_3/NH_4OAc/TiCl_3$[141-142] and $NaBH_4$/Ni(0)/5 M NaOH.[143] However, none of these systems reduced the oxime moiety of compound **88**, failing to yield the target conjugates **78** and **79** after hydrolysis. The precise reason for the failure of this reduction remains unknown, but the steric encumbrance of substrate **88** may be a contributing factor.

2.3.3.2 Attempted synthesis by *N*-alkylation of amine 76 using chiral L-alanine synthons derived from β-bromo-L-alanine derivative 89, pNZ-aziridinocarboxylate ester 91 and *N*-trityl-L-serine derivative 94

As an alternative to the above-mentioned substrate-controlled approach, a stereoselective chiral reagent-controlled approach involving *N*-alkylation of aminoprogesterone **76** with a chiral alanine synthon was envisioned to access conjugates **78** and **79** as shown in Scheme 2.7. Accordingly, a protected α-amino

acid bearing a leaving group at the β-position, such as β-bromo-L-alanine derivative **89**,[144] was reacted with 11β-NH_2-Pro (**76**) using the *N*-alkylation protocol, with Bu_4NI and K_2CO_3 in acetonitrile at reflux (Scheme 2.7). However, no *N*-alkylated progesterone-alanine adduct **90** was observed. Instead, amine **76** and a dehydroalanine derivative generated by β-elimination of the β-bromoalanine derivative were obtained. As the β-bromoalanine electrophile **89** is not as activated as the *tert*-butyl bromoacetate or ethylbromopyruvate oxime **87** used previously, a more activated electrophile is likely necessary for the successful *N*-alkylation of amine **76**.



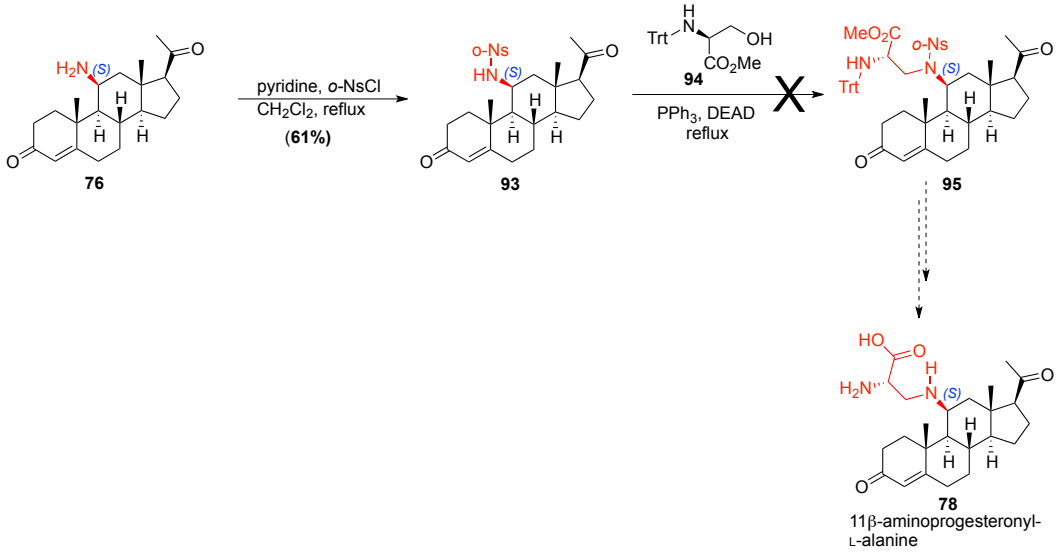
Scheme 2.7 Attempted synthesis of *N*-11β-aminoprogesteronyl L-alanine (**78**) by *N*-alkylation of amine **76** using chiral L-alanine synthons.

Therefore, an activated alanine synthon based on chiral aziridine bearing an electron-withdrawing group (i.e. **91**)[145-146] was sought for the *N*-alkylation of amine **76**. The nucleophilic ring opening of aziridines with nucleophiles[147] such as

amines[148-149] in the presence or absence of Lewis acids is well known. Previously our group has developed a methodology for the ring-opening reaction of an L-serine-derived chiral pNZ-aziridinocarboxylate ester (**91**) with several primary and secondary hydroxyl-based nucleophiles.[146,150] Implementing the same protocol for aziridine ring opening, reaction with an amine-based nucleophile such as 11β-NH_2-Pro (**76**) might offer pNZ-protected *N*-alkylated progesterone adduct **92**. This would yield the target L-alanine-based progesterone conjugate **78** upon removal of the protecting groups (Scheme 2.7). Similarly, the use of a D-serine-derived chiral pNZ-aziridinocarboxylate ester might yield the targeted D-alanine based progesterone conjugate **79**. Nonetheless, refluxing 11β-NH_2-Pro (**76**) with pNZ-aziridinocarboxylate ester (**91**)[146] in the presence of BF_3•OEt_2 or Cs_2CO_3 in toluene did not result in the formation of the desired ring opening adduct **92**, and only starting materials were recovered (Scheme 2.7). Similarly, the reported solvent-free reaction protocol[151] on Bentonite under microwave at 80 °C did not form **92**. It was reasoned that the lack of ring opening products is not limited to the 11β-amino based nucleophile **76**, but most likely due to the sluggish reactivity of the sterically encumbered 11β-progesterone. Commercially available 11β-OH-6α-Me-Pro (not shown here) did not yield any ring opening products under the same conditions.

Aside from the two chiral reagent-based approaches mentioned above, a modified Fukuyama-Mitsunobu procedure for the synthesis of secondary amines could also be explored for the synthesis of conjugates **78** and **79** (Scheme 2.8). For the synthesis of amines from alcohols using the Mitsunobu reaction, the

amines must be activated/protected. Generally the 2-nitrobenzenesulfonamide (*o*-Ns- or *o*-nosyl group) is most commonly used as an activating group in that regard.[152-153] Recently, Wang and co-workers[154] demonstrated effective *N*-alkylation of amines, via *o*-nosyl amine **93**, with commercially available *N*-trityl-L-serine methyl ester (**94**) using the Fukuyama-Mitsunobu reaction protocol. It was proposed that the *o*-nosyl group increased the nucleophilicity of the amine under Mitsunobu conditions, and can be efficiently removed with soft nucleophiles. On the other hand, the trityl group of ester **94** yields the reactive intermediate through the intermolecular coupling and avoids β-elimination, yielding *N*-alkylated products such as **95** in enantiospecific manner without racemization.



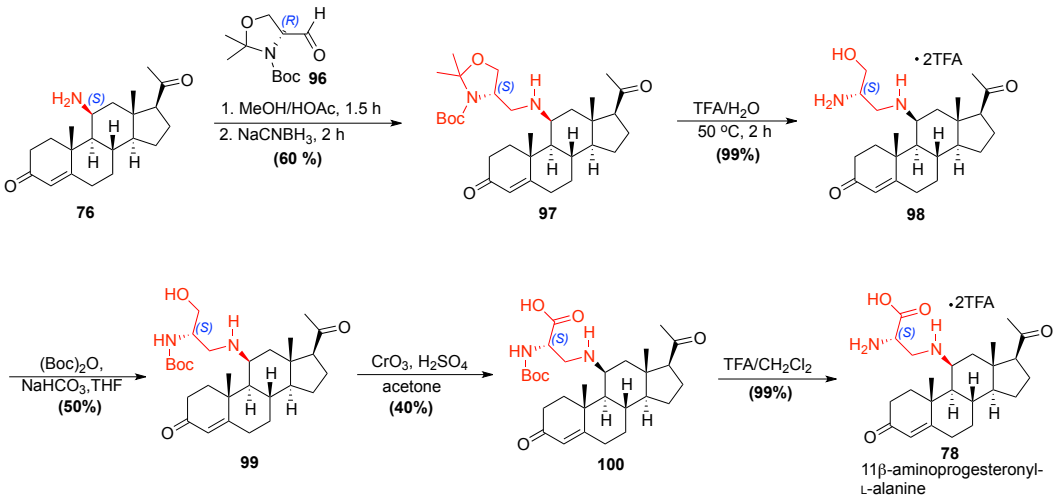
Scheme 2.8 Attempted synthesis of *N*-11β-aminoprogesteronyl L-alanine (**78**) using a L-serine derived chiral L-alanine synthon.

To implement the same protocol, compound **93** was first synthesized by reacting 11β-NH_2-Pro (**76**) with *o*-nosyl chloride in dichloromethane at reflux (Scheme 2.8). Subsequently, when *o*-nosyl amine **93** was treated with *N*-trityl-L-

serine methyl ester (**94**) under Mitsunobu conditions, it did not give the desired *o*-nosyl protected *N*-alkylated product **95**, which upon removal of protecting groups would have given the target L-alanine based conjugate **78**.

2.3.3.3 Synthesis of 78 and 79 by *N*-alkylation of amine 76 using chiral L-alanine synthons derived from (*R*) and (*S*)-Garner's aldehyde

Finally, to avoid the challenges involved in alkylation of amine **76**, reductive amination of primary amines with aldehydes or ketones was explored for the synthesis of secondary amines. It was hypothesized that the protected L- and D-serine-derived α-amino aldehyde (Garner's aldehyde) should act as an activated chiral synthon yielding a single diastereomer of the desired progesterone-glycine based conjugates **78** or **79**, respectively, by preserving the chiral integrity of the newly generated chiral center. Towards that objective, a reductive amination protocol of Chhabra et al.[155] was adapted through collaborative effort with previous Vederas group postdoctoral fellow Dr. David Dietrich. When 11β-NH_2-Pro (**76**) was treated with commercially available (*R*)-Garner's aldehyde (**96**) in the presence of methanol and acetic acid, imine formation was observed. Upon reduction with $NaCNBH_3$, *N*-Boc protected (*S*)-*N*,*O*-acetonide **97** was afforded in 60% yield (Scheme 2.9).



Scheme 2.9 Synthesis of *N*-11β-aminoprogesteronyl L-alanine (**78**) by *N*-alkylation of amine **76** using (*R*)-Garner's aldehyde derived chiral L-alanine synthon.

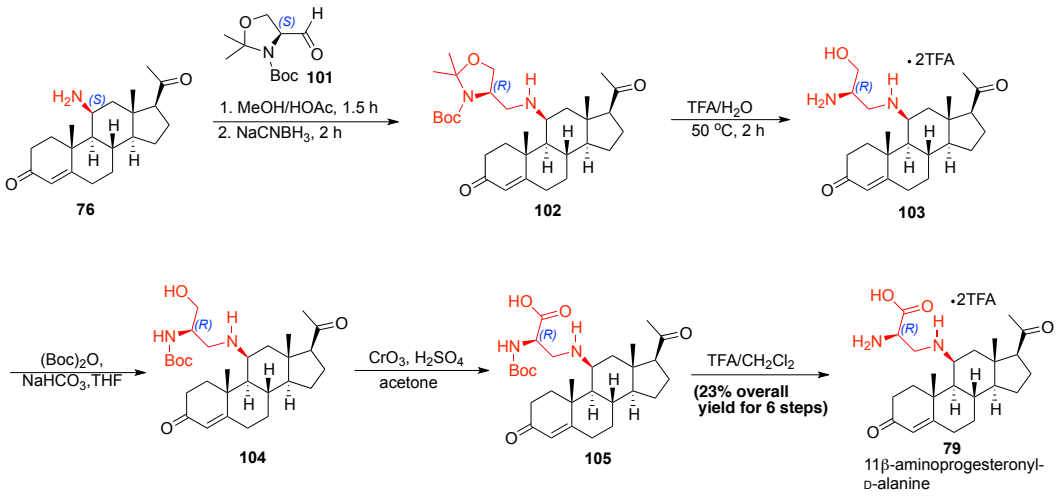
Surprisingly, it was found that when the imine formation reaction was run for longer times at room temperature (greater than 3-4 hours), the subsequent reduction gave the epimerized form of acetonide **97**, i.e. **102**. Upon hydrolysis of this epimerized acetonide **97**, the amino alcohol was obtained that appeared to be a mixture of (*S*)-amino alcohol **98** and (*R*)-amino alcohol **103** in a 1:0.6 ratio (based on HPLC analysis). Similar epimerized products were also obtained when a reductive amination of amine **76** with aldehyde **96** was done in TFE/$NaCNBH_3$ for 15 min at 40 °C.

A direct one-pot conversion of *N*-Boc-(*S*)-*N,O*-acetonide **97** to the target conjugate **78** involving a concurrent cleavage of the *N,O*-acetonide and *N*-Boc protecting groups followed by an oxidation of the *in situ* generated alcohol **98** to the acid **100** was initially attempted using Jones oxidation. However, the desired transformation did not occur, and acetonide **97** was recovered. Hence a step-wise approach of cleaving the *N,O*-acetonide and the *N*-Boc protecting group of **97**,

followed by the oxidation of the resulting free alcohol **98** was attempted. The cleavage of the *N*,*O*-acetonide protecting group of **97** demanded significant experimentation. A treatment of acetonide **97** with excess TMSOTf/CH_2Cl_2, HOAc/H_2O at reflux or 3 M HCl at 85 °C for 11 h did not form the desired amino alcohol **98**. Finally, after optimization, a great excess of the TFA/H_2O system provided a one-pot hydrolysis of the *N,O*-acetonide and *N*-Boc groups of **97** yielding the desired amino alcohol **98** quantitatively upon heating at 50 °C for 2 h (Scheme 2.9).

Conversion of the amino alcohol **98** to amino acid **78** required that the free primary amino group of **98** be re-protected, as the direct oxidation of alcohol **98** to acid **100** failed using Jones oxidation, TEMPO/NaOCl, TEMPO/BAIB, DMP/DCM and PDC/DMF.[156] Accordingly, the amino group of amino alcohol **98** was protected with a Boc group using standard conditions to yield *N*-Boc protected amino alcohol **99** in 50% yield. Subsequent oxidation of this protected amino alcohol **99** to protected amino acid **100** was successfully achieved using Jones oxidation conditions with a 40% yield (Scheme 2.9).

Finally, upon treatment of **100** with TFA, a Boc protecting group was cleaved giving access to the target L-alanine based progesterone conjugate **78** in 20% overall yield, which is in essence a γ-*N*-alkylated 2,3-diaminopropionate (Dap) derivative (Scheme 2.9).



Scheme 2.10 Synthesis of *N*-11β-aminoprogesteronyl D-alanine (**79**) by *N*-alkylation of amine **76** using a (*S*)-Garner's aldehyde derived chiral D-alanine synthon.

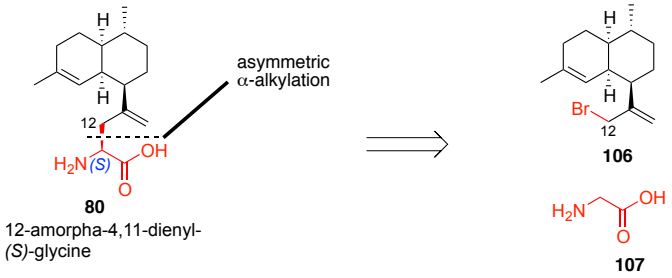
Similarly, following the above-mentioned protocol, reductive amination of 11β-NH_2-Pro (**76**) with commercially available (*S*)-Garner's aldehyde (**101**) gave (*R*)-acetonide **102**, which upon deprotection yielded (*R*)-amino alcohol **103**. Subsequent protection of amino alcohol formed Boc protected (*R*)-amino alcohol **104**, which was oxidized to compound **105**, and deprotected with TFA to form the target D-alanine-based progesterone conjugate **79**, a γ-*N*-alkylated 2,3-diaminopropionate (Dap) derivative, in 23% overall yield (Scheme 2.10).

2.3.4 Synthesis of 12-Amorpha-4,11-dienyl-*(S)*-Glycine Conjugate (80)

2.3.4.1 Retrosynthetic analysis of 80

Unlike previous approaches of synthesizing progesterone-based amino acid conjugates using electrophiles such as Garner's aldehydes and *tert*-butyl bromoacetate, we thought to explore a diversified approach involving a nucleophile such as a glycine enolate for the synthesis of amorpha-4,11-diene

based amino acid conjugate **80**. With this idea in mind, conjugate **80** could be synthesized from the asymmetric α-alkylation of glycine **107** by bromide **106**. This straightforward retrosynthetic strategy (Scheme 2.11) was reasoned to provide the desired stereoisomer with excellent stereoselectivity, as the synthesis of 12-bromoamorpha-4,11-diene (**106**)[157] and asymmetric α-alkylation reactions of glycine (**107**) are both known.[158-159]

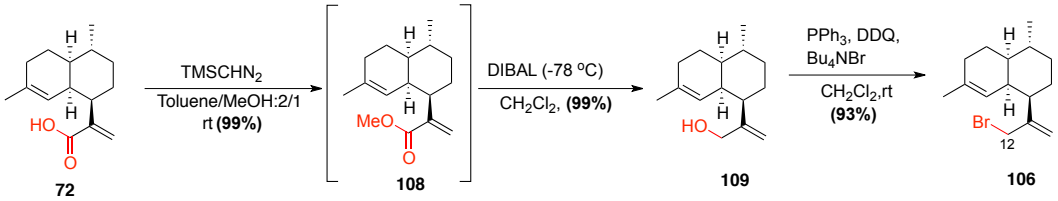


Scheme 2.11 Retrosynthetic analysis of 12-amorpha-4,11-dienyl-(*S*)-glycine (**80**).

2.3.4.2 Synthesis of 12-bromoamorpha-4,11-diene (106)[157]

The synthesis of 12-bromoamorpha-4,11-diene (**106**) was done in three steps from commercially available artemisinic acid (**72**) by altering a protocol of Jung et al.[157] as shown in Scheme 2.12. Acid **72** was first converted to the corresponding methyl ester **108** by treatment with commercially available trimethylsilyldiazomethane ($TMSCHN_2$) at room temperature. This methyl ester was then reduced to the desired allylic alcohol **109** in quantitative yield using excess DIBAL at -78 °C. Upon treatment of this alcohol with a $Ph_3P/DDQ/Bu_4NBr$ system[160] in CH_2Cl_2, the desired allylic bromide **106** was obtained in 93% yield. It was proposed that the mixture of Ph_3P and 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) in CH_2Cl_2 forms a complex, which in the presence of Bu_4NBr converts alcohols into their corresponding alkyl halides by an

S_N2-type displacement. Hence, the order of addition of the reagents in this reaction appreared to be important.



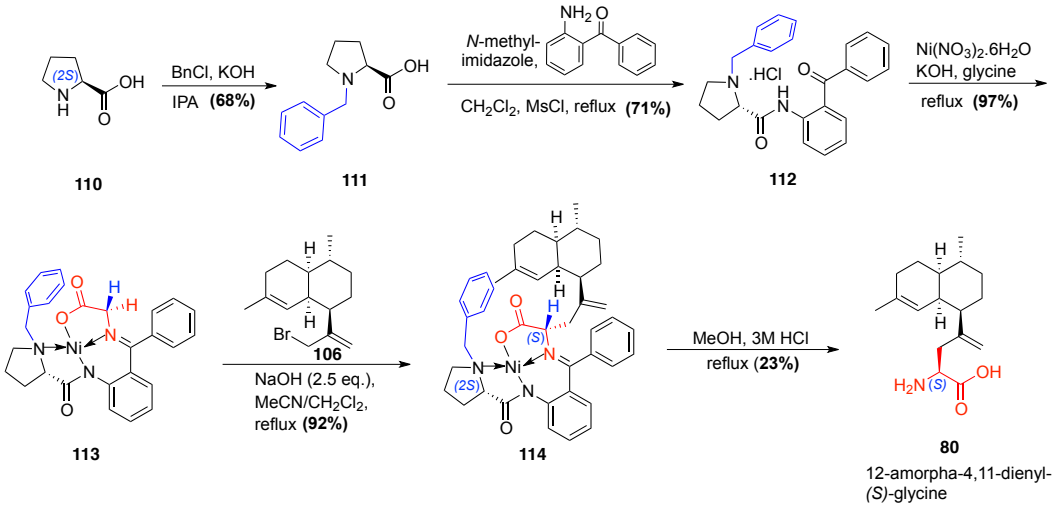
Scheme 2.12 Synthesis of 12-bromoamorpha-4,11-diene (**106**) from artemisinic acid (**72**).

2.3.4.3 Synthesis of 12-amorpha-4,11-dienyl-(*S*)-glycine (80) by asymmetric α-alkylation of Ni(II) based glycine complex

Having synthesized 12-bromoamorpha-4,11-diene (**106**), we then focused on the asymmetric α-alkylation of glycine. Several methods have been developed for this purpose, some of which report the use of the Ni(II) complex **113** (Scheme 2.13).[158-159] The Ni(II) complex-based α-alkylation is reported to proceed with excellent diastereomeric selectivity, and simple flash column chromatography enables product isolation. Moreover, following the alkylation of Gly-Ni(II)-(*2S*)-BPB complex (**113**), the absolute configuration assignment of the α-alkylated Gly-Ni(II)-(*2S*)-BPB product (**114**) (Scheme 2.13) can be easily determined by comparing its proton ^{1}H-NMR chemical shift with well-established similar systems.

Thus, following a modified existing synthetic protocol,[159] (*2S*)-*N*-benzylproline (BP) was synthesized by the reaction of (*S*)-proline **(110)** and benzyl chloride with a 68% yield. The condensation of (*2S*)-BP with 2-aminobenzophenone (**111**), in the presence of MsCl, yielded (*S*)-2-[*N*-(*N'*-benzylprolyl)amino]benzophenone (BPB) (**112**) in 71% yield. The Ni(II) complex

of Schiff base **113** derived from (*2S*)-BPB **(112)** and glycine **107** was prepared using KOH and methanol. The subsequent key alkylation of Gly-Ni(II)-(*2S*)-BPB complex (**113**) with bromide **106** was done by modifying a reported procedure, involving the use of 2.5 equiv of NaOH in MeCN - CH_2Cl_2 (1:1) at reflux, affording the 12-amorpha-4,11-dienyl-*(S)*-Gly-Ni(II)-*(2S)*-BPB complex (**114**) as a single diasteromer in 92% yield (Scheme 2.13). The absolute configuration of the obtained *S(2S)* complex **114** was determined by comparing the ^{1}H-NMR chemical shifts of the *ortho*-protons of the *N*-benzyl and benzophenone groups with those of previously reported analogues of *S*(*2S*) complex **114**.[158] It was reasoned that for all *S*(*2S*)-Ni(II) complexes such as **114**, the *N*-benzyl ring covers the top of Ni(II)-complex plane giving a characteristic ^{1}H-NMR chemical shift for the *ortho*-protons.



Scheme 2.13 Synthesis of 12-amorpha-4,11-dienyl-(*S*)-glycine (**80**) by asymmetric α-alkylation of Ni(II) based glycine complex.

Finally, acid hydrolysis of complex **114** with 3 M HCl/MeOH afforded the desired conjugate **80**, but some side products with polarities similar to that of **80** were difficult to remove by various purification techniques such as extractions,

HPLC, and flash column or ion exchange chromatography. Finally, under a collaborative effort with Dr. David Dietrich, triturating a crude mixture of **80** dissolved in MeOH with diethyl ether at 4 ºC precipitated 23% of the target 12-amorpha-4,11-dienyl-(*S*)-glycine conjugate (**80**) in pure form.

2.3.5 Biological Evaluation of Novel 11β-Aminoprogesterone Derivatives as Inhibitors of 11β-Hydroxysteroid Dehydrogenases (11β-HSDs) and Mineralocorticoid Receptor (MR) Antagonists

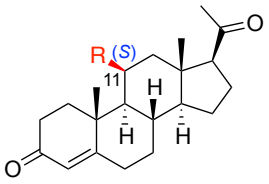
Our collaborators, Professor Odermatt and co-workers from the University of Basel, assessed the inhibitory effect of our 11β-NH_2-Pro derivatives on cortisone reduction by human recombinant 11β-HSD1 and cortisol oxidation by human recombinant 11β-HSD2. These results were compared with those for 11β-OH-Pro (**67**) and 11α-OH-Pro (**68**). The initial inhibition results are shown in Tables 2-1 and 2-2.

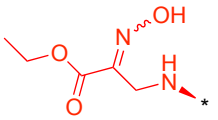
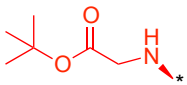
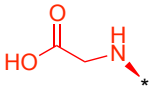
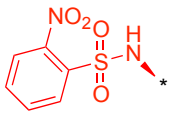
Along with **67** and **68**, our synthesized 11β-aminoprogesterone derivatives were screened at 10 μM against both isozymes. Selected analogues (*i.e.* with >75% inhibition) were examined at lower concentrations and IC_{50} values determined (Table 2-3). As anticipated, the majority of the 11β-NH_2-Pro derivatives tested showed a greater inhibition of 11β-HSD2 over 11β-HSD1, similar to 11β-OH-Pro (**67**). However, the 11β-NH_2-Pro derivatives demonstrated a reduced inhibitory activity and increased selectivity relative to **67**.

Comparing the relative inhibition of alcohol **67**, amine **76** and azide **81**, it suggested that non-protonated functional groups such as the azido group at C-11 fail to provide a favorable interaction with either of the isozymes, offering the

weakest inhibition amongst all of the tested derivatives. Moreover, it also stresses the importance of a strong hydrogen bond acceptor at C-11 to achieve strong inhibition of 11β-HSDs, especially for 11β-HSD1.

Table 2-1 11β-substituted progesterone derivatives and their inhibition of 11β-HSD1 and 11β-HSD2.



Compound	R	% Inhibition of 11-βHSD1 at 10 μM	% Inhibition of 11-βHSD2 at 10 μM
81		14	6
76		50	87
67		85	96
88		93	94
86		52	88
77		53	92
93		33	25

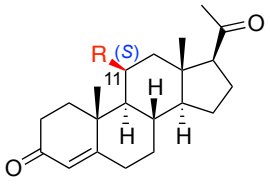
Amine **76** exhibited reduced inhibition of 11β-HSD1 (50% vs. 85%) in comparison to alcohol **67**. However, **76** still maintained relatively potent inhibition of 11β-HSD2 (87%). Thus, unlike alcohol **67,** amine **76** offered a more selective inhibition of 11β-HSD2 over 11β-HSD1. On the other hand, oxime **88**, which demonstrated increased hydrogen-bonding acceptor strength and

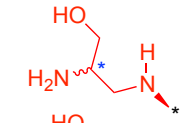
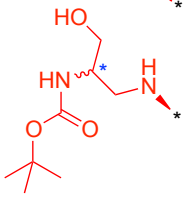
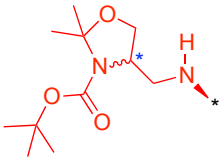
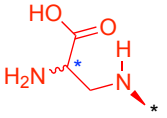
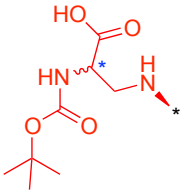
directional electronic capabilities relative to alcohol **67**, showed an increased inhibition of 11β-HSD1 (93%) in comparison to alcohol **67** (85%). Compound **88** maintained a similar potent inhibition of 11β-HSD2 (94%). Oxime **88**, though non-selective like **67**, proved to be the most potent inhibitor amongst all of the tested derivatives for both isozymes, with an IC_{50} of 0.64 ± 0.21 μM and 1.05 ± 0.26 μM for 11β-HSD1 and 11β-HSD2, respectively. These results resemble those obtained for alcohols 11β-OH-Pro **(67)** (0.63 ± 0.13 μM and 0.40 ± 0.08 μM for 11β-HSD1 and 11β-HSD2 respectively), and 11α-OH-Pro **(68)** (1.16 ± 0.21 μM and 0.49 ± 0.06 μM for 11β-HSD1 and 11β-HSD2, respectively) as shown in Table 2.3.

In comparison to oxime **88** with an ethyl ester side chain, the bulkier and more hydrophobic *tert*-butyl ester **86** and the hydrophilic acid **77** exhibited considerably reduced inhibition of 11β-HSD1, while maintaining a comparable potent inhibition of 11β-HSD2. Therefore, unlike oxime **88**, ester **86** and acid **77** offered selective inhibition of 11β-HSD2.

The reduced inhibition of 11β-HSD1 by **86** and **77** relative to oxime **88** is most likely due to an unfavorable electronic interaction of **86** or **77** rather than a hydrophobic interaction. Additionally, *o*-nosyl derivative **93** exhibited poor inhibition of both isozymes as well.

Table 2-2 11β-substituted progesterone based γ-*N*-alkylated 2,3-diamino-propionate (Dap) derivatives and their inhibition of 11β-HSD1 and 11β-HSD2.



Compound	R		% Inhibition of 11β–HSD1 at 10 μM	% Inhibition of 11β–HSD2 at 10 μM
98		(*S*)-isomer	17	33
103		(*R*)-isomer	10	25
99		(*S*)-isomer	22	81
104		(*R*)-isomer	16	41
97		(*S*)-isomer	41	81
102		(*R*)-isomer	21	24
78		(*S*)-isomer	47	58
79		(*R*)-isomer	33	52
100		(*S*)-isomer	40	17
105		(*R*)-isomer	24	55

Furthermore, the screened γ-*N*-alkylated 2,3-diaminopropionate (Dap) based derivatives [the (*S*) and (*R*) isomers of *N*-Boc-acetonide **97/102**, amino alcohol **98/103**, *N*-Boc-amino alcohol **99/104**, *N*-Boc-amino acid **100/105** and free alanine based amino acids **78/79**] also caused an unfavorable interaction, resulting in only a weak non-selective inhibition of 11β-HSD1 (Table 2.2). These findings collectively support that favorable electronic and hydrogen-bonding capabilities

(as of oxime **88**) tightly control the activity of 11β-HSD1. Overall, hydrophobic interactions and stereoisomerism do not have a critical impact for the inhibition of 11β-HSD1.

In contrast to 11β-HSD1, the activity of 11β-HSD2 appears to be greatly influenced not only by hydrogen-bonding and electronic capabilities, but also by stereoisomerism and hydrophobic interactions. Interestingly, the (*S*) stereoisomers of compounds **78**, **97**, **98** and **99** retained higher inhibitory activity compared with the (*R*) forms. For example, (*S*)-amino alcohol **98** showed only a weak (33%) inhibition of 11β-HSD2, while the introduction of a Boc group in *N*-Boc-(*S*)-amino alcohol **99** offered potent (81%) inhibition of 11β-HSD2. Furthermore, *N*-Boc-(*S*)-acetonide **97**, with a rigidified side chain, also offered the same extent (81%) of 11β-HSD2 inhibition. However, *N*-Boc-(*S*)-acetonide **97** offered a relatively reduced (~2.0 fold) selectivity in comparison to *N*-Boc-(*S*)-amino alcohol **99** which has a flexible side chain (~3.7 fold difference, the highest among all the derivatives). This suggests that hydrophobic and electronic capabilities exerted by the Boc group plays a significant role in inhibiting 11β-HSD2. The side chain rigidity appears to have little effect on the extent of inhibition and an unfavorable effect on the selectivity of 11β-HSD2 inhibition.

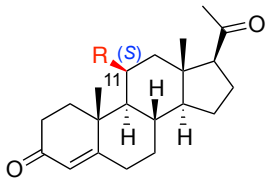
Next, the synthesized (*S*)-amino acid **78** was screened for inhibition of 11β-HSD2. Upon introduction of the carbonyl group in (*S*)-amino alcohol **98**, the resultant (*S*)-amino acid **78** gave an increased but still moderate (58% vs. 33%) inhibition of 11β-HSD2 in comparison to *(S)*-amino alcohol **98**, which is still significantly lower than *N*-Boc-(*S*)-amino alcohol **99** (58% vs. 81%).

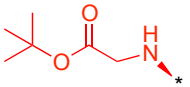
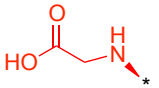
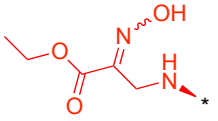
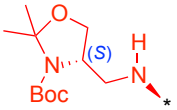
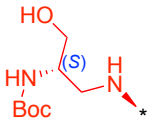
Interestingly, *N*-Boc-(*S*)-amino acid **100** bearing the additive effect of the carbonyl and Boc groups on amino alcohol **98** appeared to cause weaker (17%) inhibition of 11β-HSD2, suggesting a combination of the Boc and carbonyl moieties is sufficient to block key interactions in 11β-HSD2, abolishing inhibition to a significant extent.

This selectivity trend suggests clearly that the presence of Boc (as in **99**) in the side chain of bis amino alcohol **98** provides the most favorable interactions for 11β-HSD2 inhibition. Also, an additive effect of both Boc and carbonyl groups is disadvantageous. However, these findings are in opposition to the effects observed for 11β-HSD1 inhibition using bis amino alcohol-based progesterone derivatives. For example, (*S*)-amino acid **78** offered a moderate 58% inhibition while (*S*)-amino alcohol **98** and *N*-Boc-(*S*)-amino alcohol **99** showed weak (33% and 22% respectively) inhibition of 11β-HSD1.

In summary, activity against 11β-HSD2 can be retained with modifications at the 11β position on the steroid backbone. Even though these 11β-amino derivatives were less active (based on IC_{50} values for compounds with >75% inhibition in Table 3) than alcohols **67** and **68**, many of them (e.g., **76**, **86**, **77**, **97**, **98** and **105**) appeared to be selective and effective inhibitors of 11β-HSD2, providing a basis for the development of more potent inhibitors.

Table 2-3 IC_{50} values for selective 11β-substituted progesterone derived inhibitors of 11β-HSD1 and 11β-HSD2.



Compound	R	IC_{50} [μM] (mean±SD)	
		11β–HSD1	11β–HSD2
68		1.16±0.21	0.49±0.06
67		0.63±0.13	0.40±0.08
76		n.d.	2.20±0.12
86		n.d.	2.41±0.45
77		n.d.	2.69±0.14
88		0.64±0.21	1.05±0.26
97		n.d.	5.85±1.32
98		n.d.	4.21±1.57
78		n.d.	6.76±2.00

Subsequently, alcohol **67** and the 11β-aminosteroid derivatives were tested for direct effects on MR transactivation activity by our collaborators in the group of Professor Odermatt using HEK-293 cells. The reference compound **67** was previously reported to act as an MR agonist in transfected COS-7 cells with an

EC_{50} of about 50 nM.[97] However, it showed weak agonist activity in transfected HEK-293 cells with an EC_{50} of 14 ± 5 μM, and did not act as an antagonist as was previously seen in the COS-7 cell system.

The preliminary screening of 11β-aminosteroid derivatives showed that three compounds modulated MR activity. Compound (*R*)-amino acid **79** activated MR with an EC_{50} of 4.1 ± 1.2 μM but did not act as antagonist, whereas azide **81** and *o*-nosyl **93** inhibited aldosterone-induced MR activation with IC_{50} values of 3.5 ± 0.2 μM and 10.9 ± 0.8 μM, respectively, but did not act as agonists. Interestingly, among all tested derivatives, azide **81** and *o*-nosyl **93** are the only compounds possessing relatively greater inhibition of 11β-HSD1 over 11β-HSD2; both compounds also acted as MR antagonists.

At this point, the reason for the differences in the sensitivity of COS-7 and HEK-293 cells for MR activation remains unknown. However, these results may be rationalized by differences in the experimental procedure and/or the ability of progesterone and its derivatives to enter the cell. However, MR modulation by the three aminoprogesterone derivatives **79**, **81** and **93** was observed at lower concentrations than that of the parental compound **67**, which has been shown to activate sodium absorption in renal cortical collecting duct cells.

2.4 Conclusions and Future Directions

In spite of the imposed steric limitations on the 11β-position of progesterone, we developed a new stereoselective methodology to synthesize the sterically encumbered 11β-aminoprogesterone (**76**) and its glycine and L-/D-alanine-based facially amphiphilic conjugates. These moieties are conjugated by a

metabolically stable linkage and were formed from readily available 11α-hydroxyprogesterone (**68**).

Alkylation of the newly introduced amino functionality was challenging, and only activated unbulky electrophiles were useful in constructing *N*-11β-aminoprogesterone-glycine conjugates (**77**). Additionally, reductive amination proved useful in extending the functionality of the amino group, and allowed for the synthesis of novel γ-*N*-alkylated Dap-based derivatives including 11β-aminoprogesterone-amino acid conjugates **78** and **79**. These amino acid derivatives provide a convenient handle to develop new steroid-drug conjugates or peptidyl steroids. This scaffold may be useful for coupling with biologically active drugs or peptides for the site-specific drug delivery to progesterone rich receptor sites, and for the treatment of gynecological and mammary disorders and cancers.

These conjugates may also be used as a template for the development of second generation 11β-HSD2 inhibitors and MR antagonists. Our results indicate that some of the substituted 11β-aminoprogesterone derivatives exhibited the ability to selectively inhibit 11β-HSD2, and two of those compounds (azide **81** and *o*-nosyl **93**) behaved as MR antagonists. The biological effect of these compounds needs to be further investigated in suitable cell and animal models. Additionally, screening of these amino acid conjugates as amino acid-based synthetically derived bio-surfactants for enhanced oil recovery[161] and antimicrobial agents[40-41] is of interest.

In terms of amorphadiene based conjugate, a new class of facially

amphiphilic 12-amorpha-4,11-diene-based amino acid conjugate **80** was synthesized, and screening its biological profile is of interest. In addition, this methodology should allow for the synthesis of new conjugates possessing interesting biological activities. Currently, this developed methodology is limited to conjugates with a relatively unbulky and reactive electrophilic moiety.

Chapter 3 : Towards the Synthesis of Transition Metal Derived Biometallosurfactant Complexes: Cobalt(III) Complexes of 11β-Aminoprogesterone-Amino Acid Conjugate

3.1 Introduction

The use of transition metal complexes as therapeutic compounds has become more and more pronounced as they have been found to display a broad spectrum of activities. Since the initial discovery of cisplatin – a platinum containing anti-cancer drug, a wide array of bioorganometallic complexes[162-163] (i.e., an organometallic functional group conjugated with biomolecules such as enzymes, DNA, amino acids, carbohydrates, nucleic acids, steroids or oligopeptides) have been reported based on transition metals such as cobalt, gallium, copper, nickel, zinc, manganese, iron, gold and rhodium. These complexes have been found to possess anti-cancer, anti-inflammatory, anti-infective and anti-diabetic properties, amongst many others.[164-168] Bioorganometallic compounds also play vital roles in living systems, such as vitamin B12, chlorophyll, hemoglobin, myoglobin and the cytochromes.[164]

These compounds have vastly different properties than carbon-based compounds because of the intrinsic charged state of their central ions and their ability to adopt more diverse molecular geometries. Further, when a molecule is coordinated to a metal ion, it undergoes several changes that can alter its reactivity. The interactions of such metal complexes with living organisms differ from those of non-metals, hence these complexes show a great diversity of activities.[166, 168]

3.1.1 Bioorganometallic Complexes Derived from Steroids

Having realized the potential of transition metal complexes, Jaouen et al. reported in 1985 the first steroid-based organometallic complexes, **115** and **116**. This is often referred to as the dawn of synthetic bioorganometallic chemistry.[169] These steroidal organometallic markers of carbonyl-metalloimmunoassays (CMIA) have been prepared for the potential application as IR probes in the diagnosis of hormone-dependent cancers. A variety of C-3, C-17 and C-11β substituted steroidal complexes, incorporating organometallic fragments such as $Cr(CO)_3$, $Mo_2Cp_2(CO)_4$, $Mn(CO)_3$, $Co_2(CO)_3$, have been used for this purpose.[163, 170]

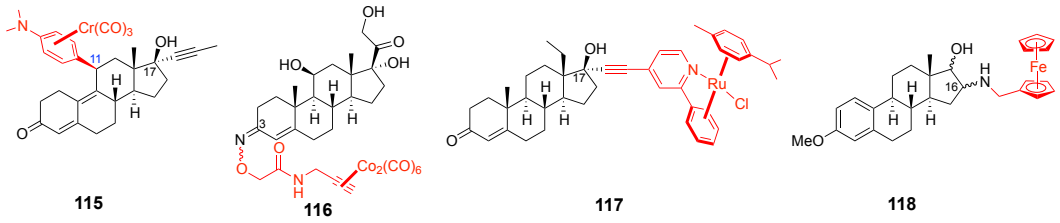


Figure 3.1 Steroid-based bioorganometallic complexes.

Following the groundbreaking work by Jaouen, Ruiz et al.[171] synthesized levonorgestrel **117**, a second-generation synthetic progesterone-based Ru(II) complex. Levonorgestrel targets the predominant sex-steroid nuclear receptor, androgen receptor (AR), which is overexpressed in ~80% of breast cancers, 74–90% of ovarian cancers and substantially in all prostate tumors. Complex **117** bearing a lipophilic steroid showed a very low resistance factor, with about 8-fold higher site-specific antiproliferative activity than cisplatin towards breast cancer cells.[171] The 16-(*N*-ferrocenylmethyl)amino steroid **118** (Figure 3.1) was shown to

exhibit a strong antimicrobial activity against a broad spectrum of test organisms including fungi and multidrug-resistant bacteria.[172] It was reasoned that synergistic effects between the metallic center and the steroidal ligand, generated by conjugating a steroid to an organometallic fragment, was responsible for the high potency of these complexes relative to the otherwise inactive individual components.

Amongst all of the transition metal complexes, cobalt(III)-based complexes have caught the interest of many researchers. This is largely due to their characteristic properties, such as inertness, bio-activatibility, octahedral coordination, diamagnetism and colour.

3.1.2 Cobalt(III)-Derived Coordination Complexes

Cobalt complexes have widely emerged as promising new drug candidates due to their therapeutic uses as tumor imaging agents,[173] antitumor,[174] antimycobacterial,[175] antiischaemic,[176] antiviral,[177] antiparasitic,[178] and anti-inflammatory agents.[179] Until recently, apart from vitamin B12, only a small number of cobalt(III) complexes have been thoroughly exploited for their biological uses. This is likely due to cobalt(III) not being easily available and unstable in water, unlike cobalt(II). However, chelating cobalt(III) with commonly used N or N, O donor ligand systems stabilizes cobalt(III) against reduction to cobalt(II) and increases its utility.[180] One such cobalt(III) complex is hexamine cobalt(III) chloride **119** (Cohex). Compound **119** is a classical Werner complex (Figure 3.2) which both stabilizes Z-DNA and exhibits significant antiviral activity by inhibiting viral structural protein synthesis.[181]

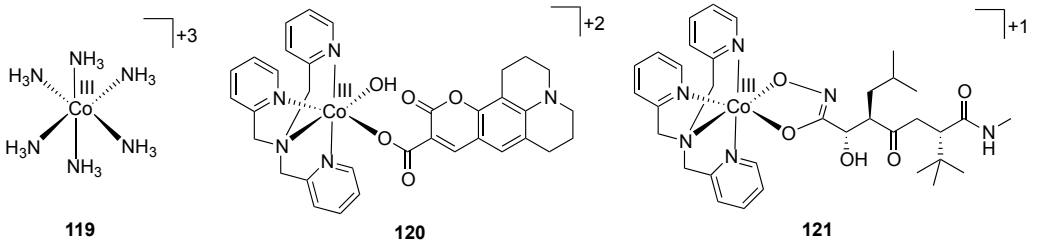


Figure 3.2 Cobalt(III)-derived coordination complexes.

Cobalt(III)-based complexes have recently been investigated as potential hypoxia-activated pro-drugs. High-level hypoxia (i.e., the lack of oxygen) is a characteristic feature of solid tumours, and is an ideal basis for tumour-selective activation of pro-drugs. For a pro-drug to be activated in a hypoxic environment, it must have an inactive prodrug state in which a bioactive molecule is deactivated through coordination to cobalt(III). Subsequently, upon reduction of this cobalt(III) complex to a cobalt(II) state, the bioactive compound gets released at the site of the tumour. Based on this principle, Hambley and co-workers[182] synthesized complexes **120** and **121** wherein cobalt(III) is coordinated to the fluorochrome coumarin-343 or a matrix metalloproteinase (MMP) inhibitor, respectively, for selective delivery to hypoxic target sites.

3.1.2.1 Cobalt(III)-derived antibacterial agents

A large number of stable cobalt(III) complexes with polydentate ligands involving N, O, S and Se donor atoms have been recently found to possess antibacterial properties. Amongst these complexes, those with a rigid and bulky nitrogen-based lipophilic bidentate ligand have demonstrated potent antibacterial activity by blocking metal-binding sites on enzymes.[183] This is likely due to their enhanced bacterial cell membrane diffusion properties. A few notable examples of

such cobalt(III) complexes are shown in Figure 3.3.

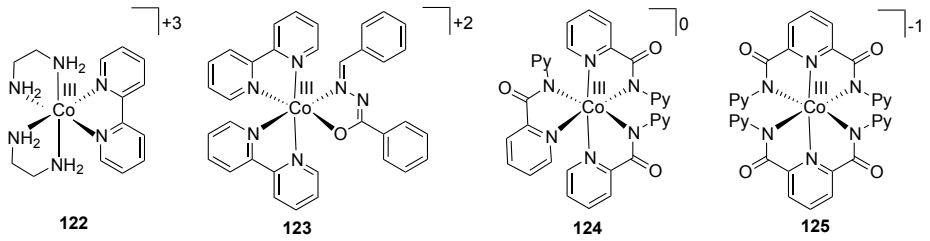


Figure 3.3 Cobalt(III)-derived complexes as antibacterial agents.

In 2006, Nagababu et al.[184] screened numerous bis(ethylenediamine)-based cobalt(III) complexes. These complexes showed significant activity against a variety of Gram-positive and Gram-negative bacteria such as *Escherichia coli*, *Salmonella enterica serovar typhimurium*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Bacillus subtilis*. Amongst these complexes, cation **122** possessed potent activity against *E. coli*, and acted as an efficient photosensitizer for scission of plasmid DNA due to its extended planar π-system. Similarly, Srinivasan et al. synthesized cation **123**, possessing a hydrophobic ligand shell, that exhibited the same biological applications with a higher efficacy.[185]

Gupta and coworkers[186] synthesized a series of complexes with varied charges, including neutral **124** and anionic **125** complexes. All of these complexes showed antibacterial activity, and neutral **124** was especially active against multidrug resistant strains of *Pseudomonas* spp. and *E. coli*, and standard strains of *Shigella* spp. and *Klebsiella* spp.[186] Anionic complex **125** showed activity against *Pseudomonas* spp. Aside from using bulky ligands, another evolving approach for enhancing the lipophilicity of metal complexes is through

functionalization with surfactant-like ligands (i.e. constructing metallosurfactants).

3.1.2.2 Cobalt(III)-based metallosurfactants as antibacterial agents

Surfactants (i.e. surface-active agents) are amphiphilic molecules consisting of a hydrophilic head group and a lipophilic (hydrophobic) tail. Thus, they are able to interact with both polar and non-polar compounds. Due to their solution properties such as detergency, solubilization and surface wetting capabilities, they have found many applications, such as antiseptic agents in cosmetics and as germicides. Among the different classes, cationic surfactants offer improved antibacterial properties and are used as fabric softeners, lubricants, antistatic agents, and as antitumor agents due to their capacity to interact with DNA.[187]

Metallosurfactants (surfactant–metal complexes) are a special type of surfactants, where the portion containing the metal ion acts as the hydrophilic head group, and one or more ligands act as a hydrophobic tail. Like other surfactants, these metallosurfactant complexes also form micelles at a specific concentration known as the critical micelle concentration (CMC) in aqueous solution. Consequently, this increases their bioavailability relative to that of the sparingly soluble lipophilic ligands in water.[188] Due to their promising cytotoxicity against human breast cancer cells and antimicrobial activities, research groups have recently been interested in the synthesis of such complexes.[188-192] Arunachalam and coworkers[188, 190-191] recently reported the synthesis of antibacterial metallosurfactant complexes of cobalt(III) with bidentate ethylenediamine (en) **126**, 2,2'-bipyridyl (bpy) **127** and 1,10-phenanthroline

(phen) **128** chelates (Figure 3.4). These complexes were given surfactant properties through the coordination of a long chain alkyl amine to the metal center. Chander et al.[189, 192] synthesized a series of Schiff base metallosurfactant complexes (**129**) based on tetradentate triethylenetetramine (trien) and a N,O-bidentate ligand derived from the condensation of salicylaldehyde with a variety of long chain alkyl amines.[189]

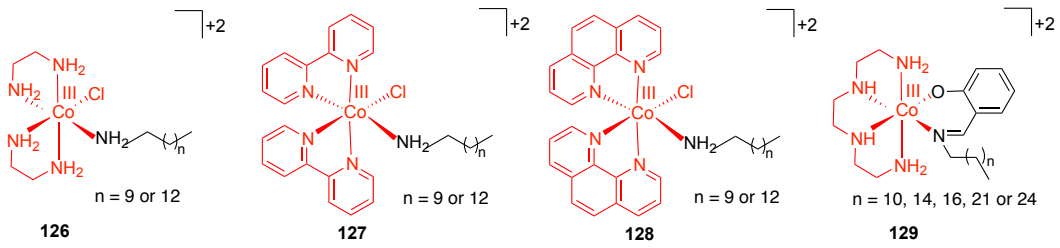


Figure 3.4 Cobalt(III)-based metallosurfactants as antibacterial agents.

These complexes foam in aqueous solution upon shaking and have greater capacity to form aggregates compared to common organic surfactants.[188-192] Upon screening for antibacterial properties, the authors demonstrated significant activity against the Gram-positive bacteria *S. aureus* and *B. subtilis* and the Gram-negative bacteria *E. coli* and *P. aeruginosa*.[188-189] In addition, complex **127** and **128** also exhibited cytotoxic activity towards a human breast cancer cell line by damaging DNA.[190-191]

3.1.3 Predetermination of Geometric Isomers and Induced Chirality at Octahedral Cobalt(III) Centers by N_4 Tetradentate Ligands

When linear tetradentate ligands and bidentate ancillary ligands are complexed at octahedral metal centers such as cobalt(III), three geometrical

isomers (*trans*, *cis*-α and *cis*-β) may be formed. The C_{2v}-symmetric *trans* structure is not chiral at the metal whereas the C_1-symmetric *cis*-β and C_2-symmetric *cis*-α structures (with *cis* oriented co-ligands) have enantiomers with Δ and Λ helicity as shown in Figure 3.5.[193] However, if bidentate ligands mutually differ, either as two different monodentate ligands (e.g. Cl^- and H_2O) or as unsymmetrical bidentate ligands (e.g. the N and O of an aminoacidate chelate), further sub-classes of the chiral *cis* geometric isomers (*cis*-β_1, *cis*-β_2, *cis*-α_1, and *cis*-α_2) are possible. The configuration *cis*-β_1 is labeled where one of the donor atoms of a bidentate ligand bearing a higher CIP priority (e.g. O > N in an aminoacidate) is *trans* to the central donor of tetradentate ligand, whereas in *cis*-β_2, it is *trans* to a terminal donor. [194]

Thus, for a bidentate ligand lacking asymmetric centers, such as glycine, it is possible to get two pairs of enantiomeric complexes designated as Δ,Λ-β_1 and Δ,Λ-β_2. Two isomers are possible here due to enantiomeric arrangement (Δ and Λ) of coordinated ligands about the metal. On the other hand, a chiral amino acid such as L-alanine can give rise to four diasteromeric complexes (i.e. Δ^*-β_1, Λ^*-β_1, Δ^*-β_2, Λ^*-β_2) due to the additional asymmetric carbon atom of the bidentate ligand.[195]

However, coordination of an unsymmetric tetradentate ligand removes the C_2 symmetry, allowing an unsymmetrical bidentate ligand to bind in *cis*-α_1 and *cis*-α_2 configurations. Normally, *cis*-α_1 is defined where a higher-ranking donor atom of a bidentate ligand (e.g. O > N) is *trans* to the higher-ranking central donor (for instance in Figure 3.5, $N^1 > N^2$ based on CIP priority).

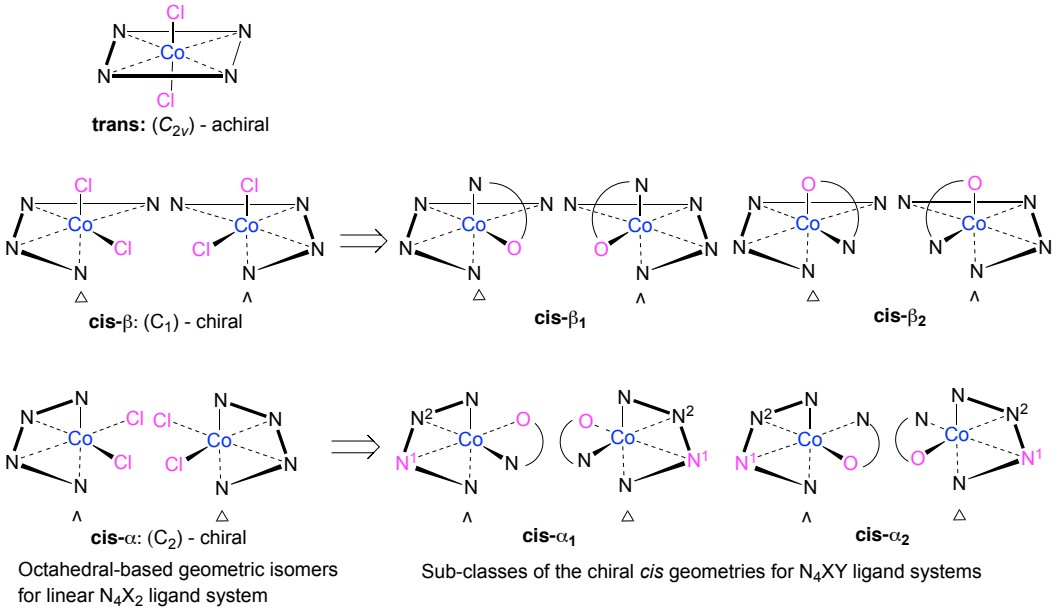


Figure 3.5 Octahedral-based geometric isomers and sub-classes of the chiral *cis* geometries.

In 1925, Morgan and Smith[196] published a cornerstone paper demonstrating the use of resolving agents such as chiral counteranions or chiral aminoacidate ligands to achieve chiral resolution of isomeric mixtures of cobalt(III) complexes through multiple fractional crystallizations. Although, this technique has been successful, it is sometimes poor yielding, and has added complications due to the possible epimerization or isomerization of the isolated enantiomer.

As the design of a tetradentate ligand controls the overall handedness of a complex, several researchers have synthesized a variety of ligands and complexed them with cobalt(III).[193] This was done with the hope of minimizing geometric isomers by predetermining the chirality-at-metal using chiral nonracemic ligands. This approach was reasoned as an alternative to chiral resolution through multiple fractional crystallizations using resolving agents.

Some of the earliest examples of octahedral complexes involved ligands with a bipyridine backbone.[197] Replacement of the bipyridine unit with alkylamines allows for enhanced flexibility with the possibility of *cis* coordination. A significant amount of research has been directed towards designing a variety of alkylamino ligands. The most widely studied alkylamino ligands are trien (**130**) and its derivatives (Figure 3.6). Basolo[198] and Sargeson[199] reported that most cobalt(III) complexes with trien preferentially adopted nonspecific binding with Δ,Λ-*cis*-α and Δ,Λ-*cis*-β coordination and were purple in colour. Increasing the chain lengths between the amino groups made the ligands more flexible, resulting in preferential adoption of *trans* coordination, which was green in colour.

In contrast, Gibson and McKenzie[200] showed that a structural analogue of trien bearing more rigid and sterically demanding pyridine rings (e.g. picen **131**) showed either Δ,Λ-*cis*-α or Δ,Λ-*cis*-β geometry upon coordination with cobalt(III). No evidence of *trans* isomers was observed even by increasing the flexibility of diamine backbones. Furthermore, upon *N,N'*-dimethylation of picen to picenMe_2 (**132**), Vagg and coworkers[201] observed stereospecific Δ,Λ-*cis*-α and Λ-*cis*-α geometries with two identical monodentate Cl^- ligands and an asymmetrical bidentate chiral (*S*)-phenylalanine ligand respectively.

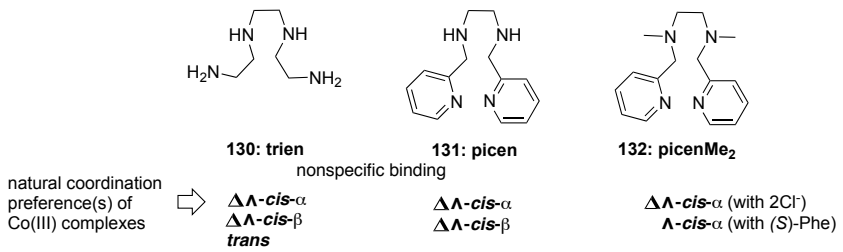


Figure 3.6 Natural coordination preference(s) of N_4 tetradentate based cobalt(III) complexes bearing achiral diamine backbones.

Besides chiral bidentate ligands, such predetermination of chirality-at-metal complexes can also be introduced more efficiently by chiral tetradentate ligands (Figure 3.7). For example, cobalt(III) complexes of chiral tetradentate (*R,R*)-piccyhxn (**133**) adopted the stereospecific *cis*-β geometry, and Λ enantioselectivity with symmetrical or unsymmetrical bidentate ligands.[202] However, its *N,N'*-dimethylated derivative, (*R,R*)-piccyhxnMe_2 (**134**), enforced Δ-*cis*-α geometry.[203]

This outcome is in agreement with molecular models suggesting that replacement of the protons on the secondary nitrogen atoms of the trien backbone with bulky methyl groups favored an α topology. The observed *cis* outcome is favored due to rigid and sterically demanding pyridine ligands. Such stereospecificity may be attributed to a vicinal substituent effect involving repulsion of the two vicinal methyl groups along the C-N bond. Additional enantioselectivity (Δ or Λ) was observed due to the (*R,R*) or (*S,S*) configuration of the chiral bidentate ligand, respectively.[193] Other examples of the (*R*)-picpn (**135**) series demonstrating the effect of substituents in predetermining chirality-at-metal are shown in Figure 3.7.

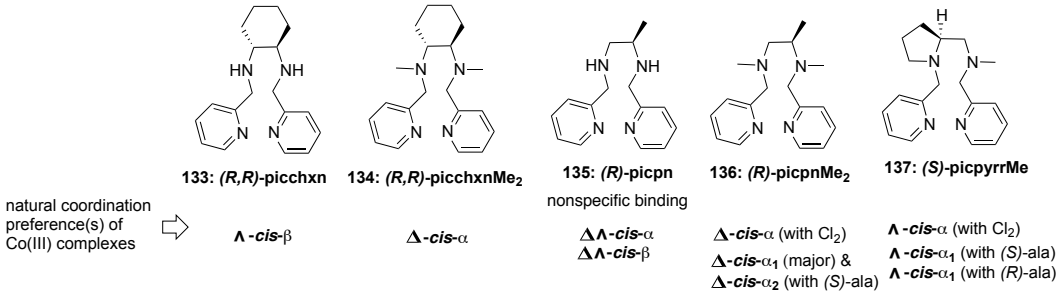


Figure 3.7 Natural coordination preference(s) of N_4 tetradentate based cobalt(III) complexes bearing a chiral diamine backbone.

The less constrained (*R*)-picpn (**135**) ligand has been shown to be nonspecific on coordination to cobalt(III) due to its flexible diamine backbone, and the added chirality has almost no influence on outcome.[204] Methylation of the secondary amino groups of **135** yielded ligand (*R*)-picpnMe_2 (**136**) which coordinated stereoselectively to cobalt(III) with an absolute configuration of Δ-*cis*-α.[205] Moreover, due to the unsymmetrical nature of ligand **136**, two isomers (major Δ-*cis*-α_1 and minor Δ-*cis*-α_2) were observed when amino acids such as *(S)*-alanine and (*S*)-phenylalanine were coordinated as co-ligands.[205-206]

Using ligand (*S*)-picpyrrMe **137** (a relatively inflexible variant of **136**), which was formed by incorporating a (*S*)-pyrrolidine group as part of the secondary amine backbone, Vagg and co-workers observed only the Λ-*cis*-α_1 isomer of cobalt(III) complex upon coordination with amino acids such as (*S*)- and (*R*)-alanine.[207] It would be of interest to perform cobalt(III) complexation with a further rigidified version of ligand **137** by incorporating (*S,S*)-2,2'-bispyrrolidine as a part of the chiral secondary diamine backbone.

3.2 Project Objective: Studies Towards the Synthesis of Cobalt(III)-Based Biometallosurfactants Derived from 11β-Aminoprogesterone-Amino Acid Conjugate

Developing new classes of metallosurfactants has recently been a growing area of research. So far, all of the reported cationic cobalt(III)-based metallosurfactant complexes have used long flexible aliphatic alkyl chains as a hydrophobic tail. We reasoned that constructing a new class of cobalt(III)-based biometallosurfactants (such as cations **138** and **139**; Figure 3.8) containing rigid 11β-aminoprogesterone-based steroids as lipophilic (hydrophobic) ligands would be of interest due to their added facial amphiphilicity. These steroid-based cobalt(III) complexes may also exhibit novel physical and chemical properties with interesting modulation of androgen and progesterone receptors. In addition, they may possess antimicrobial activities, like other metallosurfactants **126-129** and surfactants such as cetylpyridinium chloride (CPC; **140**).

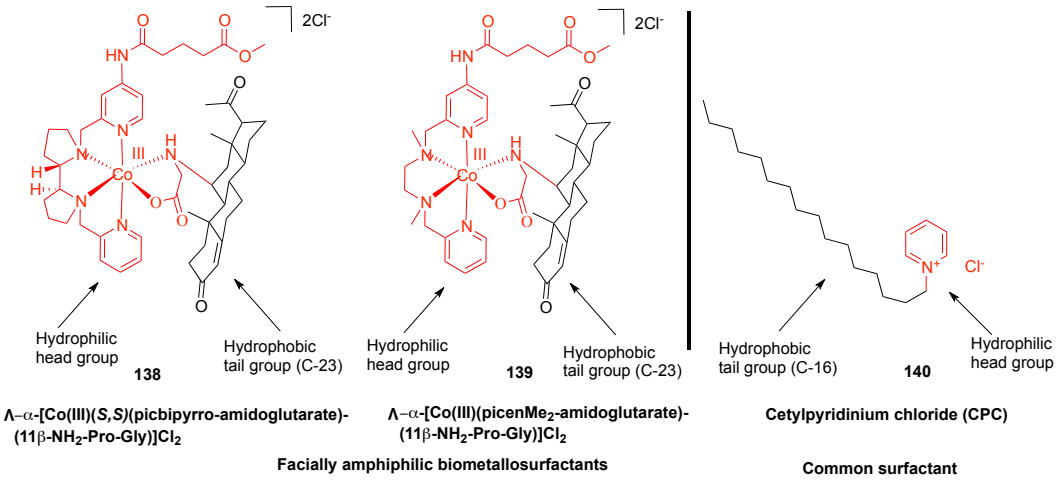


Figure 3.8 Targeted facially amphiphilic cobalt(III)-based biometallosurfactants **138** and **139**, containing a rigid 11β-aminoprogesterone-glycine conjugate.

Accordingly, the previously prepared 11β-aminoprogesterone-glycine conjugate **(77)** (synthesis described in chapter 2) was chosen as an N,O-bidentate ligand. Rigidified asymmetrical (*S,S*)-2,2'-bipyrrolidine derived ligand **141** [(*S,S*)-picbipyrro] and relatively flexible achiral picenMe_2 derived ligand **142** bearing an amidoglutarate tether were chosen as tetradentate N_4 ligands towards the synthesis of cobalt(III)-based complexes **138** and **139** (Figure 3.9). It is worth pointing out that the manganese(II) and iron(II)-based C_2 symmetrical chiral non-heme complexes of *(S,S)*-picbipyrro ligand (i.e. [Mn(II)(*S,S*)(picbipyrro)$(CF_3SO_3)_2$] and [Fe(II)(*S,S*)(picbipyrro)$(CH_3CN)_2$]$(SbF_6)_2$) were efficiently used by Bryliakov[208] and White[209] for the selective epoxidation of olefins and the oxidation of inert aliphatic C-H bonds respectively. These iron(II) and manganese(II) complexes of (*S,S*)-picbipyrro are reported to adopt *cis*-α topology. However, no cobalt(III) complexes based on this ligand or its derivatives have been reported.

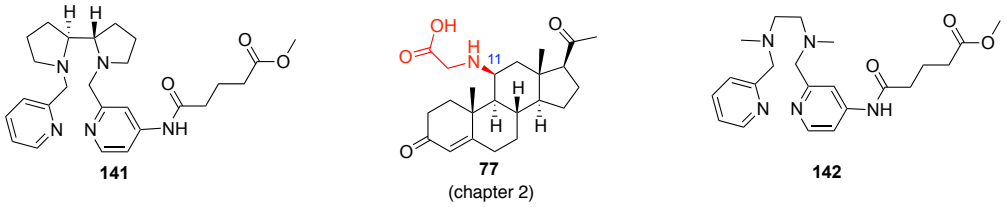


Figure 3.9 Targeted tethered tetradentate ligands: (*S,S*)-picbipyrro-amidoglutarate (**141**), picenMe_2-amidoglutarate (**142**) and bidentate ligand: *N*-(11β-NH-Pro)-Gly (**77**).

It was reasoned that *N,N'*-alkylated ligands **141** and **142** should induce a predetermination of chirality-at-metal giving a stereospecific binding with Λ-*cis*-α geometry upon coordination with cobalt(III). Additionally, due to the unsymmetrical nature of ligands **141** and **142**, either one or a mixture of both geometric isomers (i.e. Λ-*cis*-α_1 or/and Λ-*cis*-α_2) may be observed when amino

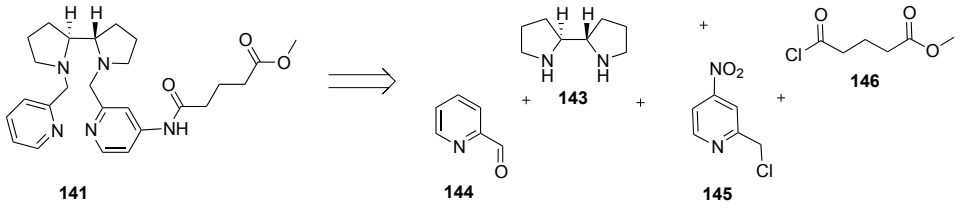
acids were coordinated as co-ligands. The appended amidoglutarate tether should allow further conjugation of these complexes with other biologically important molecules to exhibit site-specific physical and chemical properties.

3.3 Results and Discussion

3.3.1 Synthesis of *(S,S)*-[*N*-(2-Picolyl) - *N'*-(2-picolyl-4-amidoglutarate]-2, 2'-bipyrrolidine *(S,S*-picbipyrro-amidoglutarate) (141)

3.3.1.1 Retrosynthetic analysis of 141

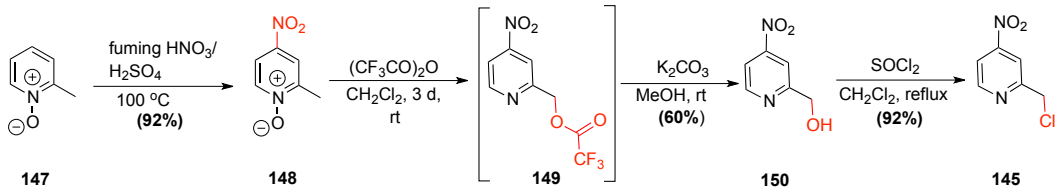
As depicted in the retrosynthesis (Scheme 3.1), the targeted amidoglutarate-tethered ligand **141** could be easily synthesized using *(S,S)*-2,2'-bipyrrolidine (**143**), 2-pyridine carboxaldehyde (**144**), 4-nitro-2-(chloromethyl)pyridine (**145**) and methyl glutaryl chloride (**146**). Amongst these, **143**, **144** and **146** are commercially available, whereas a chloromethyl derivative of nitropyridine **145** was synthesized following a literature protocol.[210-211]



Scheme 3.1 Retrosynthetic analysis of the (*S,S*)-picbipyrro-amidoglutarate ligand (**141**)

3.3.1.2 Synthesis of 4-nitro-2-(chloromethyl)pyridine (145)[212]

The preparation of 4-nitro-2-(chloromethyl)pyridine (**145**)[212] was accomplished in four steps starting from 2-picoline-*N*-oxide (**147**) as shown in Scheme 3.2. Nitration of *N*-oxide **147** was realized with fuming HNO_3 and H_2SO_4 to afford 4-nitro-2-picoline-*N*-oxide (**148**) in 92% yield. Subsequently, the resulting *N*-oxide **148** was exposed to Katada[213] or Boekelheide rearrangement conditions[214] using trifluoroacetic anhydride (TFAA) at 25 °C for 3 days for the *in situ* generation of 4-nitro-2-trifluoroacetoxymethylpyridine (**149**). This general reaction is commonly used for selective oxidation of electron-deficient alkylated pyridines.[215-216] Once ester **149** formed, it was directly saponified in a methanol solution of K_2CO_3 to give 4-nitro-2-pyridylmethanol (**150**) in 60% yield. Finally, the hydroxyl group of alcohol **150** was replaced with chloride upon reaction with thionyl chloride to yield 4-nitro-2-(chloromethyl)pyridine (**145**) in 92% yield.

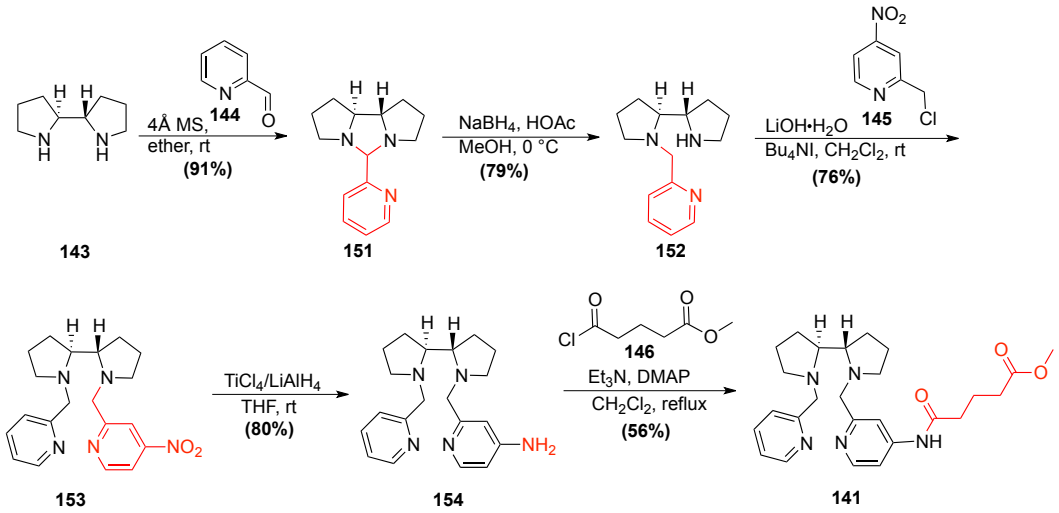


Scheme 3.2 Synthesis of 4-nitro-2-(chloromethyl)pyridine (**145**).

3.3.1.3 Synthesis of N_4 tetradentate 141

Having the four required components, a synthesis of the desired tetradentate ligand (*S,S*)-picbipyrro-amidoglutarate (**141**) was done by bis-alkylation of (*S,S*)-2,2'-bis-pyrrolidine (**143**) (Scheme 3.3). The first step involved

formation of aminal **151** by reacting unalkylated (*S,S*)-2,2'-bis-pyrrolidine (**143**) with 2-pyridine carboxaldehyde (**144**). Subsequently, treatment of aminal **151** with $NaBH_4$ and acetic acid in methanol formed the monoalkylated diamine (*S,S*)-*N*-2-picolyl-bis-pyrrolidine (**152**) in 79% yield.

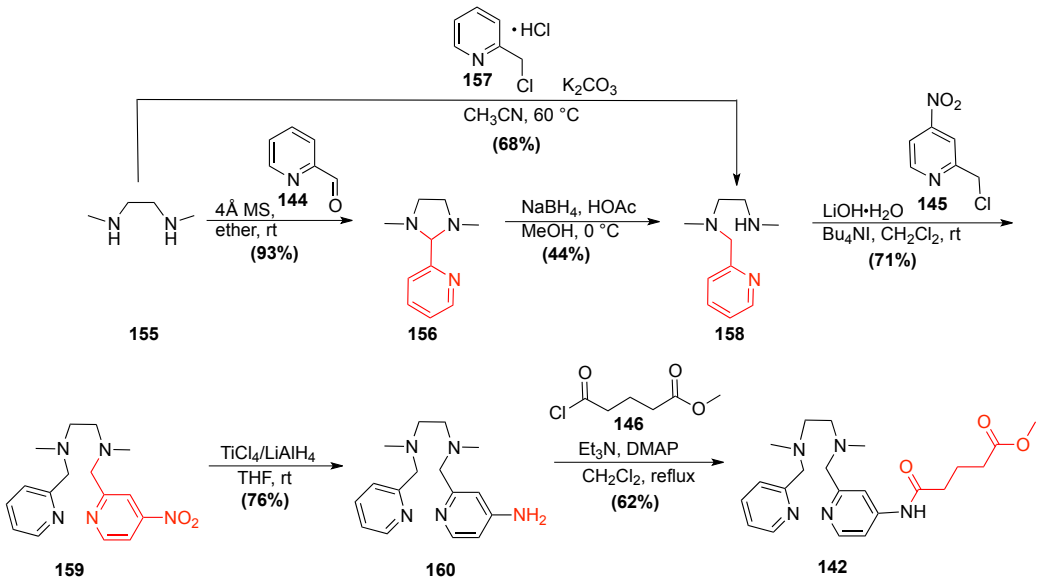


Scheme 3.3 Synthesis of tetradentate ligand (*S,S*)-picbipyrro-amidoglutarate (**141**).

Monoalkylated diamine **152** was further alkylated with 4-nitro-2-(chloromethyl)pyridine (**145**) to furnish bis-alkylated nitroamine **153** in 76% yield. The nitro group of (*S,S*)-picbipyrro-NO_2 (**153**) was reduced to amine (*S,S*)-picbipyrro-NH_2 (**154**) using a low valent Ti(0) slurry generated *in situ* from $TiCl_4$ and $LiAlH_4$. Finally, acylation of amine **154** with methyl glutaryl chloride (**146**) in the presence of DMAP and NEt_3 afforded the tethered N_4 tetradentate ligand **141** with 56% yield.

3.3.2 Synthesis of *N*, *N'*-Dimethyl-[*N*-(2-picolyl)- *N'*-(2-picolyl-4-amido-glutarate]ethane-1,2-diamine (picenMe$_2$-amidoglutarate) (142)

A tethered ethylenediamine-based tetradentate ligand **142** was synthesized according to the protocol described above for the synthesis of ligand **141**. Initially, *N*-alkylation of *N*,*N'*-dimethylethylenediamine (**155**) was explored using a two-step reductive amination procedure (Scheme 3.4). Accordingly, diamine **155** was condensed with 2-pyridine carboxaldehyde (**144**) to yield cyclic aminal **156** in 93% yield. Subsequent reduction of aminal **156** was accomplished using a large excess of $NaBH_4$/AcOH to give the desired *N*-alkylated diamine **158**[217] in only 44% yield. No improvements in yield were obtained through further optimization, or the use of other reducing systems such as $LiClO_4$/$NaBH_4$ and $NaBH(OAc)_3$/AcOH. Alternatively, *N*-alkylated diamine **158** was obtained in 68% yield by heating an excess of diamine **155** with 2-picolyl chloride (**157**) and K_2CO_3 in acetonitrile.[218]

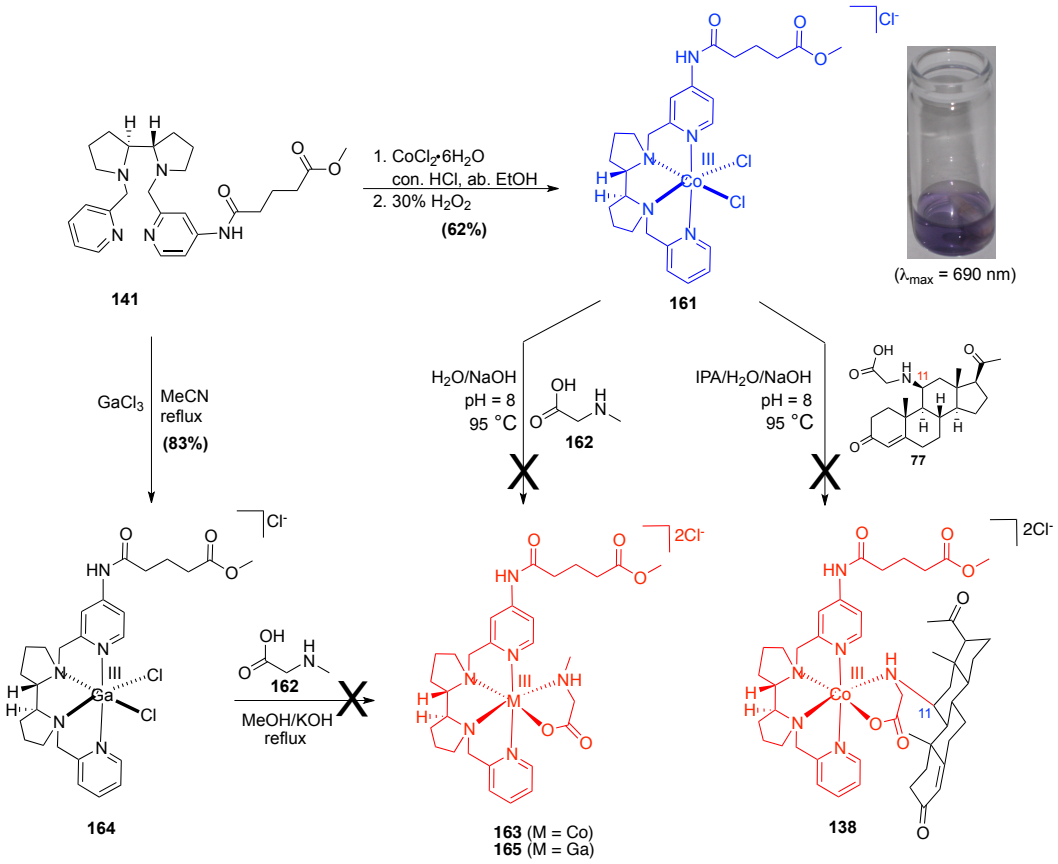


Scheme 3.4 Synthesis of tetradentate ligand picenMe$_2$-amidoglutarate (**142**).

N'-alkylation of *N*-alkylated diamine **158** with 4-nitro-2-(chloromethyl) pyridine (**145**) using lithium hydroxide and tetrabutylammonium iodide followed by a reduction of nitro compound **159** with $TiCl_4/LiAlH_4$ in THF formed amine **160** in 76% yield. Finally, the reaction between methyl glutaryl chloride (**146**) and amine **160** afforded the desired tethered N_4 tetradentate ligand **142** in 62% yield.

3.3.3 Complexation Studies Towards the Synthesis of Cobalt(III)-Derived Biometallosurfactants: Co[(*S,S*-picbipyrro-amidoglutarate)(11β-NH_2-Pro-Gly)]$^{2+}$ (138)

Having amidoglutarate-tethered N_4 tetradentate ligand **141** and N,O bidentate ligand *N*-(11β-NH-Pro)-Gly (**77**) in hand, the desired cobalt(III) derived biometallosurfactant **138** could be obtained in two steps as shown in Scheme 3.5. The first step involves a synthesis of the dichloro cobalt(III) complex **161** of tetradentate ligand **141**, and the second step involves a substitution of the two monodentate chloride ligands of the resultant dichloro cobalt(III) complex with bidentate steroid-amino acid based ligand **77**. Accordingly, the sky blue *cis*-alpha dichloro cobalt(III) complex **161** was formed in 62% yield from the reaction of the tetradentate (*S,S*)-picbipyrro-amidoglutarate (**141**) and cobalt(II) chloride hexahydrate in the presence of excess chloride (i.e. con. HCl) and absolute ethanol using hydrogen peroxide as the oxidant. The presence of excess water produced the highly soluble, characteristically purple, chloro-aquo species instead of dichloro complex (not shown in Scheme 3.5).



Scheme 3.5 Attempts towards the synthesis of cobalt(III) and gallium(III)-derived complexes based on tethered (*S,S*)-picbipyrro-amidoglutarate (**141**) and *N*-substituted amino acids.

The absolute configuration of the Λ-α-Co[(*S,S*-picbipyrro-amidoglutarate)Cl_2]$^+$ (**161**) was assigned on the basis of its NMR and circular (CD) spectra, in comparison with similar complexes such as Λ-α-Co[(*S*-picpyrrMe)Cl_2]$^+$ [207] and Λ-α-Co[(*S,S*-picchxnMe$_2$)Cl_2]$^+$ [219] for which the structural determinations are reported in literature. ^{1}H NMR and CD data of **161** are illustrated in the later part of the chapter. Subsequently, the coordination of the bidentate steroid-amino acid ligand **77** with the Λ-α-Co[(*S,S*-picbipyrro-amidoglutarate)Cl_2]$^+$ (**161**) moiety was attempted in an IPA/H_2O co-solvent system.

In that regard, a *cis*-α-dichoro complex **161** was initially dissolved in water to yield the purple chloro-aquo species, and the system was made slightly alkaline (pH = 8) through the addition of 2 M NaOH. To this solution, steroid-amino acid-based bidentate ligand **77** was added, and the reaction was heated at 95 °C for 2 h. However, no reaction of amino acid **77** was observed. Several unsuccessful attempts with varying amounts of amino acid **77**, pH, temperature and reaction time did not result in the formation of the desired cobalt(III) derived hexadentate complex **138**. This might be due to the relative inflexibility of the bispyrrolidine rings, as well as the steric hindrance caused by a bulky *N*-alkyl amino acid (i.e. the bidentate ligand *N*-(11β-NH-Pro)-Gly **77**). To ease the overall steric demand of the complexation process, the coordination of a relatively small *N*-alkyl amino acid sarcosine (**162**) with the *cis*-α-dichoro complex **161** was attempted. However, this also failed to yield the desired complex.

Besides *N*-alkyl amino acids, replacement of the cobalt(III) metal center was also explored to confirm that the cobalt(III) was not contributing to the failure of the hexadentate complexation with sarcosine or a steroid-amino acid conjugate. Hence, a hexadentate gallium(III)-based *cis*-dichloro complex Λ-α-Ga[(*S,S*-picbipyrro-amidoglutarate)Cl_2]$^+$ (**164**) was synthesized in 83% yield by refluxing tetradentate (*S,S)*-picbipyrro-amidoglutarate ligand (**141**) with $GaCl_3$ in acetonitrile (Scheme 3.5). The geometric isomerism and absolute configuration of the complex **164** were assigned on the basis of its NMR and CD spectra in comparison with other known gallium(III) complexes.[220-221] When gallium(III)-derived dichloro complex **164** was treated with sarcosine (**162**) under non-

aqueous conditions, no hexadentate complex **165** with sarcosine (**162**) was observed. Further, decomposed tetradentate ligand **141** and unreacted **162** were seen by NMR and HR-MS. Similar results were obtained when the gallium(III)-derived dichloro complex was treated with steroid-amino acid conjugate **77** (not shown in Scheme 3.5).

3.3.4 Model Studies on Synthesis of Cobalt(III)-Derived BioMetallosurfactants: Cobalt(III) Complexation of Ligands picenMe_2 (132), (*S,S*)-piccyhxnMe_2 (166), (*S,S*)-picbipyrro (167) and (*S,S*)-picbipyrro-NH_2 (154) with Amino Acids

Initial complexation attempts towards coordinating (*S,S*)-picbipyrro-amidoglutarate with *N*-(11β-NH-Pro)-Gly (**77**) or sarcosine (**162**) at a cobalt(III) metal center led us to believe that the failure to obtain the desired cobalt(III) based complexes **138** and **163** might be due to one of the following impeding factors: (i) the highly rigid bispyrrolidine-based tetradentate ligand **141**; (ii) the appended amidoglutarate tether of ligand **141** or (iii) the *N*-alkylation of the bidentate amino acid ligand **77** or **162**.

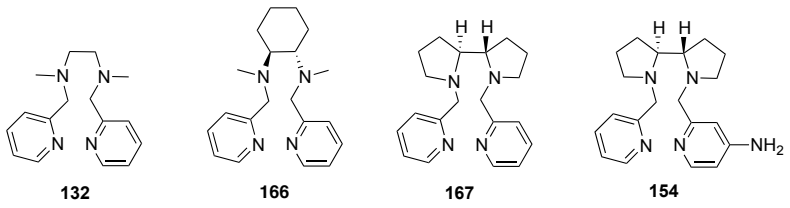
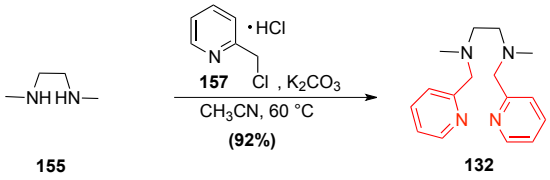


Figure 3.10 Untethered N_4 tetradentate ligands for model studies on cobalt(III) complexation with amino acids.

Accordingly, further model studies on cobalt(III) complexation with untethered symmetrical tetradentate ligands **132**, **166**, **167** and unsymmetrical ligand **154** (Figure 3.10) with *N*-alkyl substituted or *N*-unsubstituted amino acids were undertaken to identify the impeding factor for such complexation.

3.3.4.1 Synthesis of C_2-symmetrical picenMe_2 (132), (*S,S*)-piccyhxnMe_2 (166) and (*S,S*)-picbipyrro (167) ligands

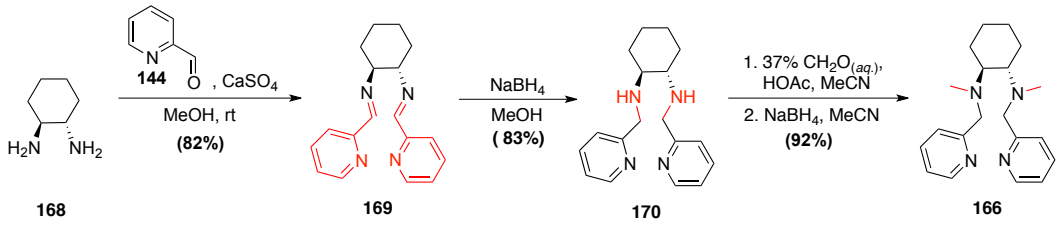
Among the four ligands chosen for model studies, the synthesis of ligand **154** has already been described in Scheme 3.3, whereas ligands **132**, **169** and **170** were synthesized following the literature procedures.[222-224] Achiral C_2-symmetrical picenMe_2 ligand (**132**)[222] bearing a relatively flexible diamine backbone was synthesized with 92% yield by reacting 2 equivalents of 2-picolyl chloride hydrochloride (**157**) with *N,N*'-dimethylethylenediamine (**155**) in the presence of K_2CO_3 (Scheme 3.6).[223]



Scheme 3.6 Synthesis of achiral C_2-symmetrical untethered ethylenediamine-derived N_4 tetradentate ligand picenMe_2 (**132**).

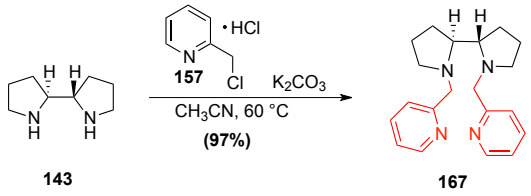
A chiral C_2-symmetrical (*S,S*)-piccyhxnMe_2 ligand (**166**)[224] bearing a semi-rigid cyclohexane diamine backbone was synthesized by performing two sequential reductive aminations on commercially available (*S,S*)-1,2-cyclohexanediamine (**168**) (Scheme 3.7). In that regard, pyridine-2-carboxaldehyde (**144**) was condensed with diamine **168** to yield diimine adduct **169** in 82% yield. **169** was further reduced with sodium borohydride to offer

bis(pyridine-2-ylmethyl)-substituted secondary diamine (*S,S*)-piccyhxn (**170**) in 83% yield. Adaptation of the general method of Borch and Hassid[225] for the methylation of secondary diamine **170** using a large excess of 37% formaldehyde and sodium borohydride in acetonitrile provided the desired (*S,S*)-piccyhxnMe_2 (**166**) ligand in 92% yield.



Scheme 3.7 Synthesis of chiral C_2-symmetrical untethered cyclohexane-derived N_4 tetradentate ligand (*S,S*)-piccyhxnMe_2 (**166**).

Following a similar procedure as for picenMe_2 (**132**), a chiral C_2-symmetrical (*S,S*)-picbipyrro (**167**) ligand bearing a rigid (*S,S*)-2,2'-bipyrrolidine (**143**) backbone was synthesized by the bisalkylation of diamine **143** with 2-picolyl chloride hydrochloride (**157**) in 97% yield (Scheme 3.8).[209]



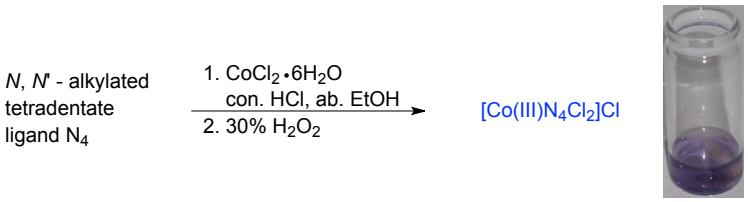
Scheme 3.8 Synthesis of chiral C_2-symmetrical untethered bispyrrolidine-derived N_4 tetradentate ligand (*S,S*)-picbipyrro (**167**).

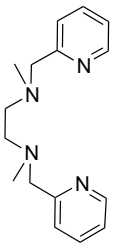
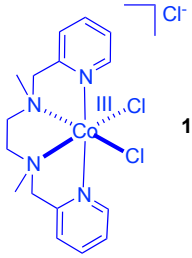
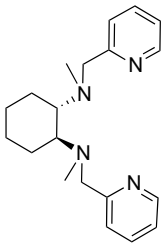
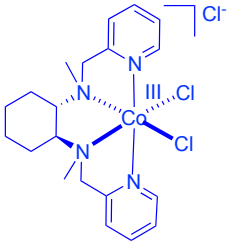
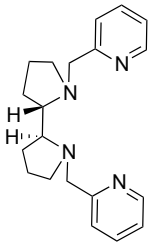
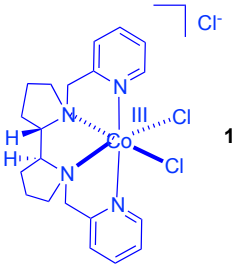
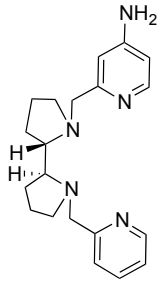
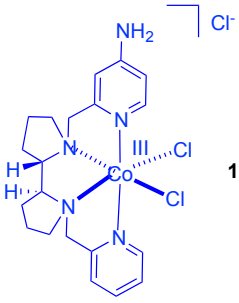
3.3.4.2 Synthesis and characterization of *cis*-α-Co[N_4Cl_2]$^+$ complexes based on N_4 tetradentate picenMe_2 (132), (*S,S*)-piccyhxnMe_2 (166), (*S,S*)-picbipyrro (167) and (*S,S*)-picbipyrro-NH_2 (154) ligands

3.3.4.2.1 Synthesis of *cis*-α-Co[N_4Cl_2]$^+$ complexes 171,[201] 172,[219] 173 and 174

Having synthesized all of the required *N,N'*-alkylated untethered N_4 tetradentate ligands, the first step towards model complexation studies involved the stereospecific syntheses of cobalt(III)-derived dichloro complexes **171**, **172**, **173** and **174** with ligands **132**, **166**, **167** and **154**, respectively. Adopting the same procedure as was used for the previously synthesized Λ-α-Co[(*S,S*-picbipyrro-amidoglutarate)Cl_2]$^+$ (**161**), the *cis*-α-dichloro intermediates **171-174** were prepared in moderate yields (48% to 68%, Table 3-1) from the reaction of the tetradentate ligands with cobaltous chloride.

Table 3-1 Synthesis of cobalt(III)-derived dichloro complexes **171**, **172**, **173** and **174** based on untethered N_4 tetradentate ligands.



ligand N_4		$[Co(III)N_4Cl_2]Cl$ product complex	yield (%)
	**132**	 **171:** Δ Λ-α–Co[(picenMe_2)Cl_2]Cl	**65**
	**166**	 **172:** Λ-α–Co[(*S*,*S*-piccyhxnMe_2)Cl_2]Cl	**68**
	**167**	 **173:** Λ-α–Co[(*S*,*S*-picbipyrro)Cl_2]Cl	**60**
	**154**	 **174:** Λ-α–Co[(*S*,*S*-picbipyrro-NH_2)Cl_2]Cl	**46**

3.3.4.2.2 Characterization of untethered and tethered *cis*-α-$Co[N_4Cl_2]^+$ complexes 171, 172, 173, 174 and 161

Tetradentate ligands with three continuous five-membered metal chelate rings preferentially adopt *cis* topologies, either *cis* α or *cis* β, over *trans* structures to prevent unfavorable in-plane nonbonding interactions between *ortho* hydrogens on pyridyl rings.[226] For such tetradentate ligands in which two internal donor groups are secondary amines, *cis* β complexes or mixtures of *cis* α and *cis* β isomers are generally obtained. Nonetheless, when these internal donor groups are alkylated to yield tertiary amines (as in this case), *cis* α structures are preferred.[227]

3.3.4.2.2.1 Proton NMR spectra

The *cis* geometry of the synthesized untethered dichloro cobalt(III) complexes **171**, **172**, **173**, **174** and tethered dichloro complex **161** could be easily deduced by their violet color, the characteristic color for all known *cis*-dichloro-tetramine cobalt cations.[228] In addition to this qualitative analysis, an assignment of the *cis*-α topology was made on the basis of ^1H NMR features (Figure 3.11).

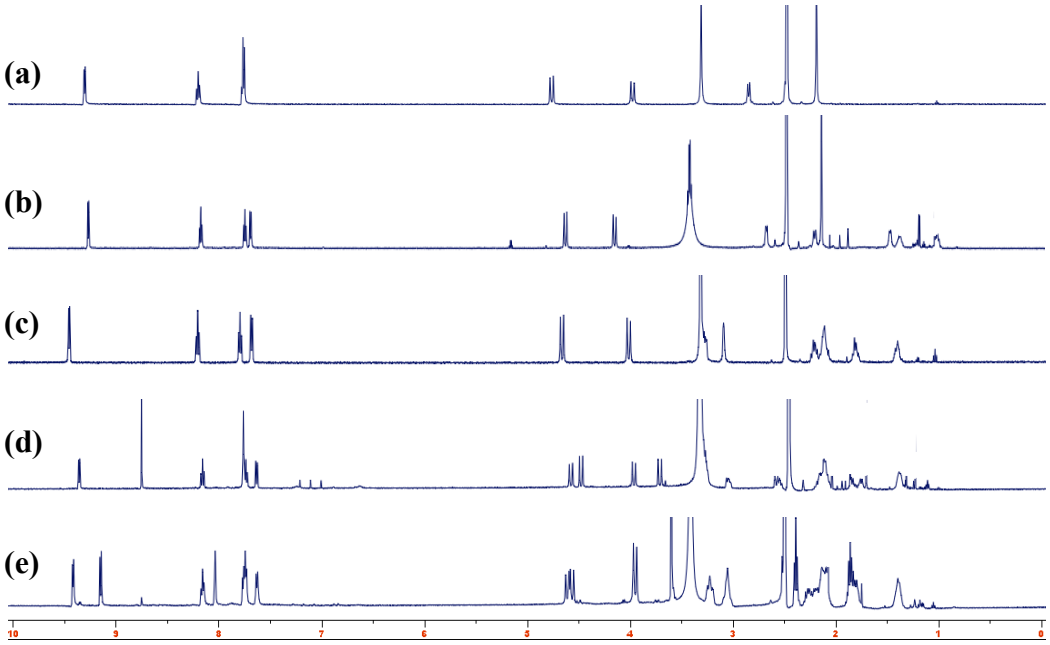


Figure 3.11 ^{1}H NMR spectra of the cobalt(III) dichloride complexes in DMSO-d_6: **(a)** Δ,Λ-α-Co[(picenMe_2)Cl_2]$^+$ (**171**); **(b)** Λ-α-Co[(*S,S*-piccyhxnMe_2)Cl_2]$^+$ (**172**); **(c)** Λ-α-Co[(*S,S*-picbipyrro)Cl_2]$^+$ (**173**); **(d)** Λ-α-Co[(*S,S*-picbipyrro-NH_2)Cl_2]$^+$ (**174**); **(e)** Λ-α-Co[(*S,S*-picbipyrro-amidoglutarate)Cl_2]$^+$ (**161**).

The cobalt(III) complex of such *N*, *N'*-alkylated tetradentate ligands, containing two identical monodentate ligands or one symmetrical bidentate ligand would have C_2 symmetry with a simple ^{1}H NMR spectrum. The observed simplicity in the aromatic region of ^{1}H NMR spectra of the cobalt(III) based dichloro complexes **171-174** and **161** suggested that they each adopted an α topology (Figure 3.11).[204]

As seen by ^{1}H NMR, both ortho protons of the two pyridyl residues were deshielded. This is the opposite for a β-isomer for which the ortho proton of one of the pyridyl rings is oriented towards the π-cloud of the second pyridyl group (Figure 3.12), which would be expected to resonate at higher field in a proton NMR spectrum.[204] The data obtained matches the reported data for **171**[201] and **172**[219], whereas that of **173**, **174** and **161** is closely comparable with the reported

data for *(R)*-picpnMe$_2$ and (*S*)-picpyrrMe-based analogues.[206-207]

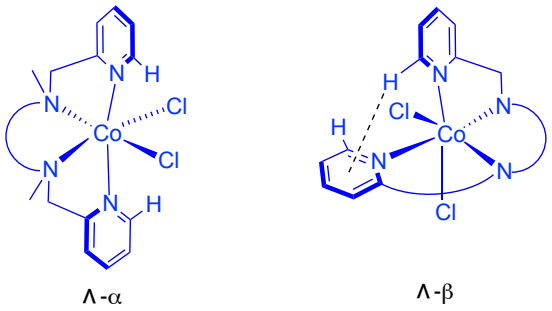


Figure 3.12 Effects of *cis*-α and *cis*-β geometric isomerism on the ortho protons of pyridyl rings of cobalt(III)-derived complexes.[204]

3.3.4.2.2.2 UV-visible spectra

The absolute configuration/chirality-at-metal of the cobalt(III)-derived α-dichloro complexes **171**, **172**, **173**, **174** and **161** was examined by comparing their CD spectra with those of analogous complexes whose structural determinations were confirmed by X-ray diffraction studies. The observed d-d band maxima of the electronic spectra for all of the cobalt(III)-based α-dichloro complexes are listed in Table 3-2. The obtained UV-visible spectra for all these complexes showed two absorption bands of approximately the same intensity at the wavelengths of 662 nm and 690 nm, which are in close agreement with the data obtained for **171**, **172** and structural analogues such as Λ-α-[Co(*S*-picpyrrMe)Cl$_2$]$^+$.[207] Furthermore, the obtained molar absorptivity or molar extinction coefficient (ε) value also resembled that of structurally similar cobalt(III)-based α-dichloro complexes.

Table 3-2 Electronic spectral data for cobalt(III)-derived dichloro complexes **171**, **172**, **173**, **174** and **161**

cis-dichloro cobalt(III) complex (Solvent: conc. HCl)	c x 10^{-3} (M)	λ (nm)	ε x 10^{-3} (dm^2 mol^{-1})	Δε (dm^2 mol^{-1})
171: Δ Λ-α–[Co(picenMe_2)Cl_2]$^+$	1.2	690	1.87	-
		662	1.87	
172: Λ-α–[Co(*S,S*-piccyhxnMe_2)Cl_2]$^+$	1.3	690	1.32	
		663	1.32	
		653		- 0.33
		575		+ 0.39
		428		+ 0.74
		347		- 2.45
173: Λ-α–[Co(*S,S*-picbipyrro)Cl_2]$^+$	1.0	690	2.16	
		663	2.15	
		635		- 0.76
		548		+ 1.35
		418		+ 0.67
		341		- 2.70
174: Λ-α–[Co(*S,S*-picbipyrro-NH_2)Cl_2]$^+$	1.1	690	1.96	
		662	1.96	
		635		- 0.80
		554		+1.55
		404		- 0.17
		333		- 2.34
161: Λ-α–[Co(*S,S*-picbipyrro-amidoglutarate)Cl_2]$^+$	0.8	690	2.41	
		662	2.35	
		634		- 0.66
		624	1.81	
		554		+ 1.19
		428		+ 0.40
		347		- 1.53

3.3.4.2.2.3 CD spectra

The CD spectral data are given in Figure 3.13. The dominant positive and negative extrema at the higher wavelength in the CD spectra of the chiral ligand-based cobalt(III) complexes is correlated to retention of the Λ-and Δ-configurations respectively.[229] The (*S,S*)-piccyhxnMe_2, (*S,S*)-picbipyrro, (*S,S*)-picbipyrro-NH_2 and (*S,S*)-picbipyrro-amidoglutarate-derived *cis*-α-cobalt(III)

dichloro complexes **172**, **173**, **174** and **161** exhibited the dominant positive CD band at the higher wavelength in the visible region of the spectrum along with a small negative absorption at longer wavelengths.

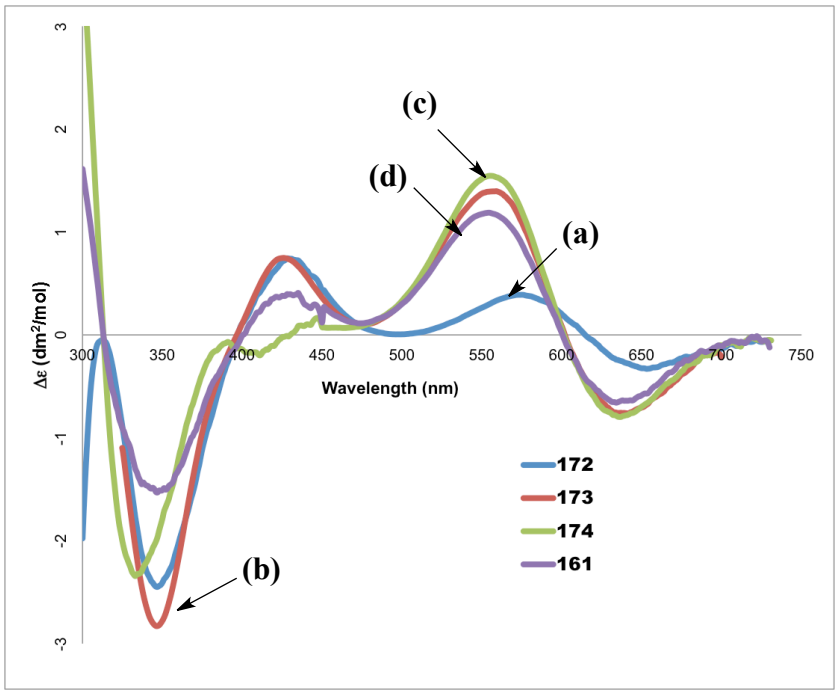


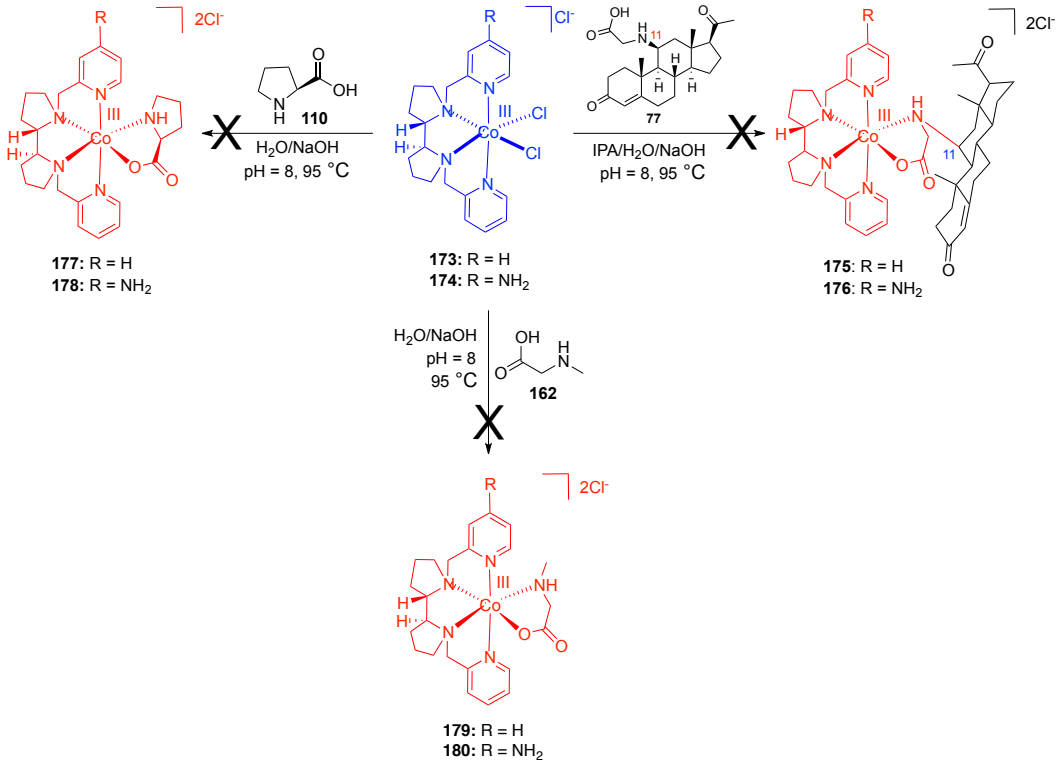
Figure 3.13 The CD spectra of the cobalt(III) dichloride complexes in con. HCl: **(a)** Λ-α-Co[(*S,S*-piccyhxnMe$_2$)Cl$_2$]$^+$ (**172**); **(b)** Λ-α-Co[(*S,S*-picbipyrro)Cl$_2$]$^+$ (**173**); **(c)** Λ-α-Co[(*S,S*-picbipyrro-NH$_2$)Cl$_2$]$^+$ (**174**); **(d)** Λ-α-Co[(*S,S*-picbipyrro-amidoglutarate)Cl$_2$]$^+$ (**161**).

The obtained CD spectral features in the visible region closely match those of Λ-α-[Co(*S,S*-picpyrrMe)Cl$_2$]$^+$ [(Ref. 207)] and Λ-α-[Co(*S,S*-piccyhxnMe$_2$)Cl$_2$]$^+$ [(Ref. 219)], whose absolute configurations are known from crystallographic studies. Thus, the absolute configurations of complexes **172**, **173**, **174** and **161** have been assigned as Λ, and the values of molar circular dichroism (Δε) values are shown in Table 3.2. It should be noted that due to the racemic nature (mixture of Λ and Δ

configurations) of the achiral ligand picenMe_2-based cis-α cobalt(III) dichloro complex **171**, it did not generate a CD spectrum.

3.3.4.3 Attempted complexation between varyingly rigid untethered *cis*-α-Co[N_4Cl_2]$^+$ complexes and *N*-substituted amino acids

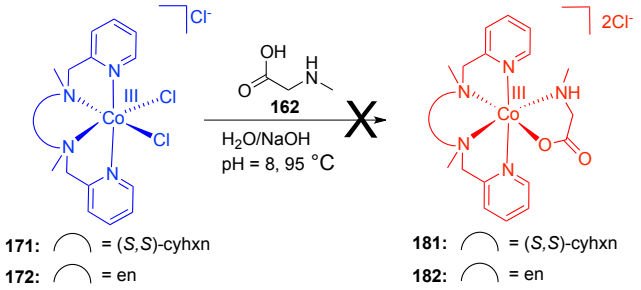
As a part of the model studies performed to elucidate the factors impeding the complexation of Λ-α-Co[(*S,S*-picbipyrro-amidoglutarate)Cl_2]$^+$ (**161**) with *N*-substituted amino acids, we first decided to determine if the rigid (*S,S*)-bipyrrolidine-based ligand system or the appended amidoglutarate tether had a negative impact on complexation. Accordingly, the coordination of the C_2-symmetrical untethered Λ-α-Co[(*S,S*-picbipyrro)Cl_2]$^+$ (**173**) and the amino-substituted untethered Λ-α-Co[(*S,S*-picbipyrro-NH_2)Cl_2]$^+$ (**174**) complexes with *N*-substituted amino acids was tested following the same complexation protocol as previously used (Scheme 3.9).



Scheme 3.9 Attempts towards the synthesis of cobalt(III) complexes based on rigid untethered (*S,S)*-picbipyrro-derived ligands and *N*-substituted amino acids.

The attempted complexations of **173** and **174** with an excess of steroid-amino acid conjugate **77** resulted in a dark orange-red solution. This solution was diluted with water for the purpose of purification using a column of CM-Sephadex®-C25 cation exchange resin. The adsorbed material was washed with water and eluted stepwise with 0.1 M to 0.3 M NaCl. The initially eluted pale yellow band and the subsequently eluted pink-orange bands gave complex NMR spectra. Upon further purification with high resolution LC-MS, this yielded an unidentified mixture of products, lacking the desired hexadentate cobalt(III) complexes **175** and **176** . Similarly, attempts to coordinate **173** and **174** with (*S*)-proline (**110**) or sarcosine (**162**) failed to offer the desired complexes **177** and **178**, or **179** and **180** respectively.

Subsequently, we diverted our attention to complexing *N*-substituted amino acids with C_2-symmetric relatively flexible Δ,Λ-α-Co[(picenMe$_2$)Cl$_2$]$^+$ (**171**) and semi-rigid Λ-α-Co[(*S,S*-piccyhxnMe$_2$)Cl$_2$]$^+$ (**172**) complexes (Scheme 3.10). However, several attempts to coordinate **171** and **172** with sarcosine (**162**) did not give the desired complexes **181** and **182** respectively. These findings led us to believe that the amidoglutarate tether and rigid (*S,S*)-bispyrrolidine based systems were not likely the source of problems for the complexation of Λ-α-Co[(*S,S*-picbipyrro-amidoglutarate)Cl$_2$]$^+$ (**161**) with *N*-substituted amino acids, as even untethered and relatively less rigid ligand systems also failed to coordinate. These observations left us to speculate that the failure of the desired hexadentate complexation must be due to an undesired steric interaction of the *N*-alkyl group of the *N*-substituted amino acid with one of the pyridine rings of the tetradentate ligand.



Scheme 3.10 Attempts towards the synthesis of cobalt(III) complexes based on non-rigid and semi-rigid untethered N_4 tetradentate ligands and *N*-substituted amino acids.

3.3.4.4 Synthesis and characterization of untethered and tethered Λ-α-Co[N_4(AA)]$^{2+}$ complexes 185, 186, 190, 191 and 192 derived from *cis*-α-Co[N_4Cl_2]$^+$ and *N*-unsubstituted amino acids

3.3.4.4.1 Synthesis of Λ-*cis*-α-Co[N_4(AA)]$^{2+}$ complexes 185, 186, 190, 191 and 192

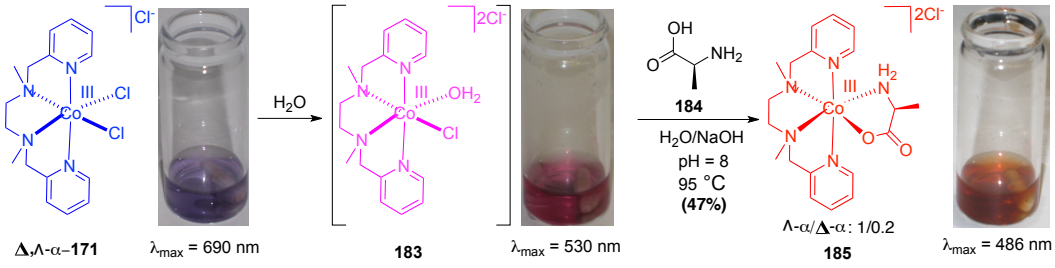
To verify the hypothesis that *N*-alkyl group of the *N*-substituted amino acid indeed prevented the desired hexadentate complexation, the complexation of untethered cobalt(III)-derived *cis*-α-dichloro compounds with *N*-unsubstituted amino acids was initially tested.

3.3.4.4.1.1 Synthesis of untethered Λ-α-Co[N_4(AA)]$^{2+}$ complexes 185, 186, and 190 based on varyingly rigid C_2 symmetrical *cis*-α-Co[N_4Cl_2]$^+$ and *N*-unsubstituted amino acids

The coordination of the C_2-symmetric and relatively non-rigid achiral Δ,Λ-α-Co[(picenMe_2)Cl_2]$^+$ (**171**) complex was attempted with *N*-unsubstituted amino acids such as (*S*)-alanine (**184**) following the same procedure that was tested for *N*-substituted amino acids (Scheme 3.11). Upon dissolution of the sky blue dichloro complex **171** (λ_{max} = 690 nm) in water, analogous to all the previous attempts for complexation, the highly soluble purple chloro-aquo species **183** (λ_{max} = 530 nm) was produced. Subsequent heating of the slightly alkaline solution (pH = 8) in the presence of excess (*S*)-alanine resulted in a dark red-orange solution. This was then passed through a column of CM-Sephadex®-C25 cation exchange resin and eluted with 0.3 M NaCl. A minor pale yellow band was

initially obtained, followed by a pink-orange mobile band. However, these fractions were found not to contain the desired product by NMR and discarded.

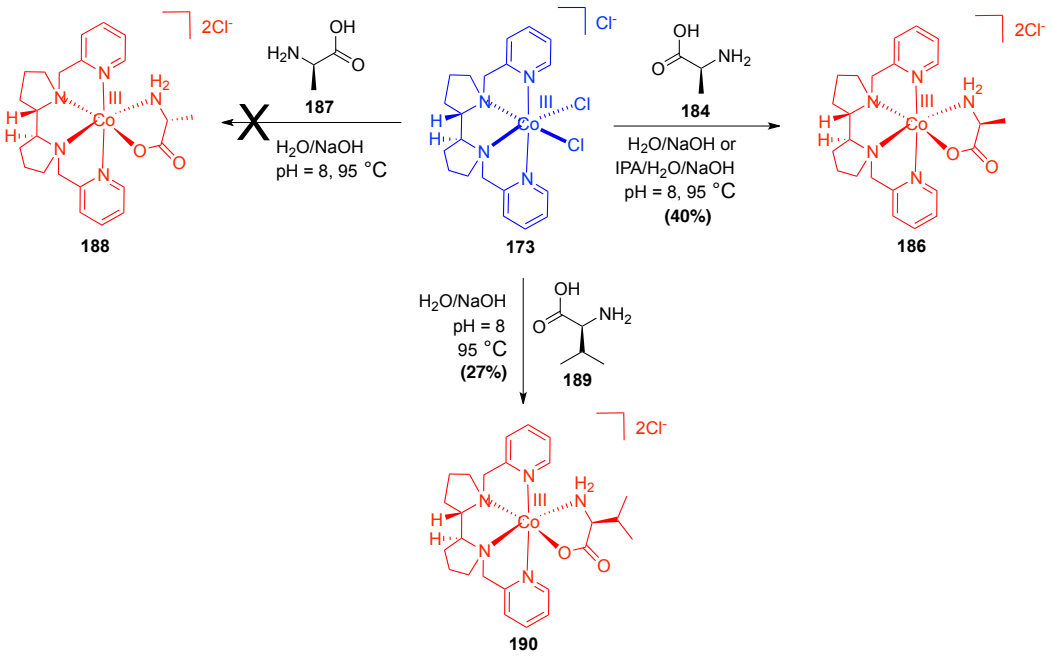
Eventually, the primary slow moving red-orange band (λ_{max} = 486 nm) was collected, giving 47% of desired octahedral complex **185**. NMR (Figure 3.17), CD (Figure 3.19) and electronic absorption spectra (Table 3-3) of isolated **185** indicated that it was a mixture of two diastereomers (Λ-α/Δ-α : 1/0.2). The major isomer was Λ-α-Co[(picenMe$_2$)(*S*-ala)]$^{2+}$ (**185**), most likely due to the presence of (*S*)-alanine as a chiral label. Similar isomeric mixture was reported when Δ,Λ-α-Co[(picenMe$_2$)Cl$_2$]$^+$ (**171**) was reacted with (*S*)-phenylalanine.[201] However, the authors were subsequently able to crystallize the major Λ-α-Co[(picenMe$_2$)(*S*-Phe)]$^{2+}$ isomer.



Scheme 3.11 Synthesis of cobalt(III)-derived hexadentate complex based on C_2-symmetrical non-rigid untethered picenMe$_2$ and *N*-unsubstituted amino acid: Λ-α-Co[picenMe$_2$(*S*-ala)]$^{2+}$ (**185**) – major isomer.

Having achieved a successful coordination of an *N*-unsubstituted bidentate amino acid with flexible achiral picenMe$_2$ ligand-based cobalt(III) dichloro complex **171**, coordination of (*S*)-alanine was then attempted with the rigid (*S,S*)-picbipyrro ligand-based chiral cobalt(III) dichloro complex **173** (Scheme 3.12). Gratifyingly, the reaction of Λ-α-Co[(*S,S*-picbipyrro)Cl$_2$]$^+$ (**173**) with excess (*S*)-alanine (**184**)

yielded the desired Λ-α-Co[(*S,S*-picbipyrro)(*S*-ala)]$^{2+}$ (**186**) in 40% yield. A similar result was obtained when the complexation was done in an IPA/H_2O co-solvent system at 95 °C. This suggests that such a co-solvent system could be used for the complexation of amphiphilic *N*-unsubstituted steroid-amino acids. However, none of the desired octahedral complex Co[(*S,S*-picbipyrro)(*R*-ala)]$^{2+}$ (**188**) was obtained when complex **173** bearing a chiral (*S,S*)-2,2' bipyrrolidine based rigid backbone was mixed with (*R*)-alanine (**187**).



Scheme 3.12 Synthesis of cobalt(III)-derived hexadentate complexes based on C_2-symmetrical rigid untethered (*S,S*)-picbipyrro and *N*-unsubstituted amino acids: Λ-α- Co[(*S,S*-picbipyrro)(*S*-ala)]$^{2+}$ (**186**) and Λ-α-[Co[(*S,S*-picbipyrro)(*S*-val)]$^{2+}$ (**190**).

These observations are in line with the results reported for a semi-rigid Λ-α-Co[(*S,S*-piccyhxnMe$_2$)Cl$_2$]$^+$ (**172**) system, which exhibited a discriminatory behavior in coordinating only with the (*S*) form of sterically demanding proline but coordinated to both enantiomers of alanine.[203, 219] Based on complex

molecular modeling, it was reasoned that the relative positioning of the terminal pyridyl groups of complex **172** severely hindered the coordination of (*R*)-proline, and exhibited a minor preference for the relatively small methyl group substituent of (*S*)-alanine. Upon coordination, the (*R*)-alaninate chelate ring was severely flattened to minimize a steric interaction by orienting the methyl group into an equatorial position. This is unlike the preferred (*S*)-alaninate chelate ring, which adopted a relatively unstrained puckered conformation.[203] This flattening of the chelate ring was also evident from X-ray crystallographic studies of the analogous Δ-α-Co[(*R*-picpnMe_2)(*S*-ala)]$^{2+}$ [(Ref. 206)] and (*S*)-pyrrolidine-based Λ-α-Co[(*S*-picpyrrMe)(*R*-ala)]$^{2+}$,[(Ref. 207)] presumably due to the same reason.

Extrapolating from these findings, in the case of a further rigidified (*S*,*S*)-2,2' bispyrrolidine-based Λ-α-Co[(*S,S*-picbipyrro)Cl_2]$^{+}$ (**173**), the unsuccessful complexation of **173** with (*R*)-alanine might be due to an undesired steric interaction between the ortho proton of one of the pyridyl rings and the alanine methyl group as shown in a steric blocking model (Figure 3.14). Likely due to a rigidified ligand system, even a severe flattening of (*R*)-aminoacidate ring was not enough to alleviate the undesired methyl group interaction, causing a steric hindrance for complexation. This might be a reason for the discriminatory behavior of **173** in coordinating only with (*S*)-alanine.

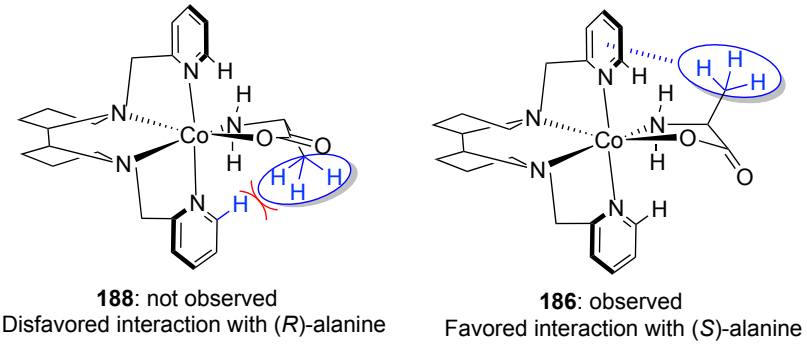


Figure 3.14 Proposed steric blocking complexation models showing a stereoselective interaction of Λ-α-Co[(*S,S*-picbipyrro)Cl_2]$^+$ (**173**) with (*R*)-alanine and (*S*)-alanine.

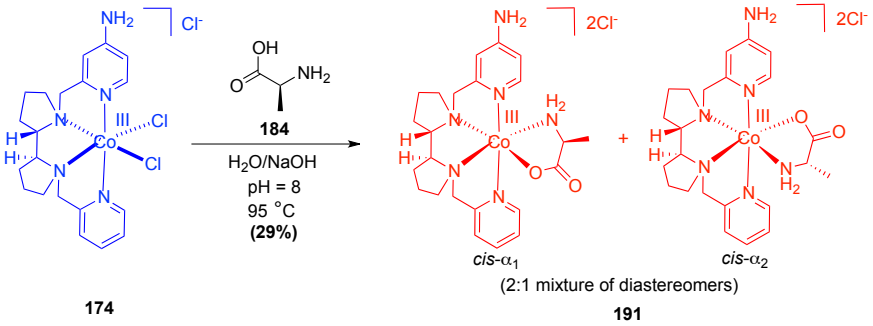
Furthermore, the observed preference for isomer **186** bearing the methyl group under the pyridyl rings may be rationalized in terms of electronic and steric reasons. Toscano et al.[195] proposed that the stability of such an isomer might be attributable to hydrophobic and/or attractive van der Waals' interactions between the methyl and pyridyl groups by having the methyl moiety under the electron-deficient pyridyl ring. It may be the reason why even the sterically encumbered amino acid (*S*)-valine (**189**) successfully coordinated with complex **173**, giving 27% of Λ-α-Co[(*S,S*-picbipyrro)(*S*-val)]$^{2+}$ (**190**) (Scheme 3.12).

3.3.4.4.1.2 Synthesis of untethered complex Λ-α$_1$-α$_2$-Co[(*S,S*-picbipyrro-NH_2)(*S*-ala)]$^{2+}$ (191) based on unsymmetrical Λ-α-Co[(*S,S*-picbipyrro-NH_2)Cl_2]$^+$ (174) and (*S*)-alanine

Next, it was of interest to explore the coordination of the unsymmetrical tetradentate complex Λ-α-Co[(*S,S*-picbipyrro-NH_2)Cl_2]$^+$ (**174**) with an *N*-unsubstituted amino acid (Scheme 3.13). It is worth mentioning that the coordination of the unsymmetric tetradentate **174** with two different unidentate ligands (e.g. H_2O and Cl, generated after dissolution in water) or unsymmetrical

bidentate ligands [e.g. (*S*)-alanine] removes formal C_2 symmetry. Thus it allows for coordination in two senses, *cis*-α_1 and/or *cis*-α_2, depending on the disposition of the carboxylate unit of a bidentate ligand in relation to the internal donor tertiary amine bearing the amino-substituted pyridine.

The NMR spectra (Figure 3.17) of purified (*S*)-aminoacidate complex **191**, obtained in 29% yield after the complexation of unsymmetric dichloro complex **174** with (*S*)-alanine (**184**) (Scheme 3.13), appeared to be a mixture of *cis*-α_1 and *cis*-α_2 geometric isomers, with a two fold isomeric excess of either α_1 or α_2. Presumably due to only small structural differences between the α_1 and α_2 forms of complex **191**, CM-Sephadex®-C25 cation exchange chromatography did not provide a discriminative separation of these alaninato α-isomers. At this point, without suitable X-ray quality crystals in hand, it is unclear which is the major isomer.

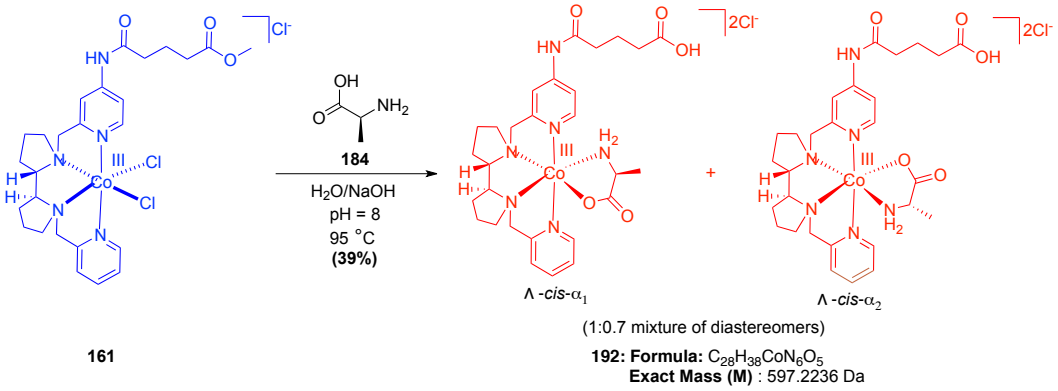


Scheme 3.13 Synthesis of Λ-α_1-α_2-Co[(*S,S*-picbipyrro-NH_2)(*S*-ala)]$^{2+}$ (**191**) complex based on unsymmetrical tetradentate N_4 and *N*-unsubstituted amino acid.

A similar isomeric mixture (α_1/α_2 : 2/1) was observed for the alaninato complex derived from the analogous unsymmetrical Δ-α-Co[(*R*)-picpnMe_2]$^{3+}$,[205] whereas only the α_1 isomer was observed for unsymmetrical Λ-α-Co[(*S*)-picpyrrMe]$^{3+}$.[207]

3.3.4.4.1.3 Synthesis of tethered complex Λ-α$_1$-α$_2$-Co[(*S,S*-picbipyrro-amidoglutaric acid)(*S*-ala)]$^{2+}$ (192) based on unsymmetrical Λ-α-[Co(*S,S*-picbipyrro-amidoglutarate)Cl$_2$]$^+$ and (*S*)-alanine

Having realized that an *N*-unsubstituted amino acid was required to obtain the desired hexadentate complexation with untethered cobalt(III)-based dichloro compounds, the ongoing model studies were further extended to the complexation of the amidoglutarate tethered cobalt(III)-derived *cis*-α-dichloro **161** with (*S*)-alanine (**184**) (Scheme 3.14).



Scheme 3.14 Synthesis of the cobalt(III) complex based on unsymmetrically tethered (*S,S*)-picbipyrro ligand and *N*-unsubstituted amino acid: Λ-α$_1$-α$_2$-Co[(*S,S*-picbipyrro-amidoglutaric acid)(*S*-ala)]$^{2+}$ (**192**).

Accordingly, complex **161** was reacted with an excess of (*S*)-alanine using the complexation protocol as described above. Purification of the resulting red-orange solution was attempted using CM-Sephadex®-C25 cation exchange chromatography. The major red-orange band, consisting of 38% of the amidoglutaric acid-tethered hexadentate cobalt(III) complex **192**, was collected upon elution with 0.3 M NaCl. ^{1}H NMR analysis (Figure 3.17) of this major band

appeared to be relatively complex in nature, featuring a mixture of *cis*-α_1 and *cis*-α_2 geometric isomers based on the presence of two sets of broad doublets belonging to the alanine methyl protons.

To confirm the formation of **192** as a mixture of two isomers, it was analyzed by liquid chromatography on a short C18 reverse phase column coupled with high-resolution mass spectrometry. The HR-LC-MS profile of **192** [M: $C_{28}H_{38}CoN_6O_5$] showed two major peaks at 3.2 minutes [m/z of 598.2302 $(M+H)^+$, 299.1288 $(M+2H)^{2+}$] and 3.9 minutes [m/z of 598.2303 $(M+H)^+$, 299.1161 $(M+2H)^{2+}$], indicating the formation of **192** as a mixture of two geometrical isomers in a 1 : 0.7 ratio (Figure 3.15). Again without X-ray crystallographic studies, it is unclear which isomer is the major one.

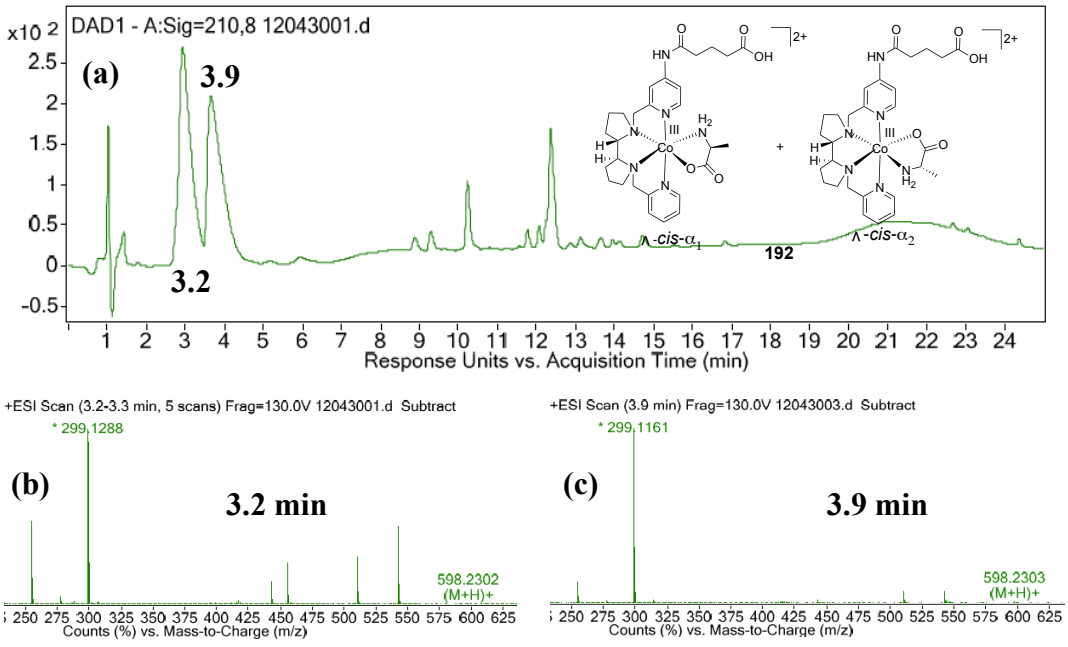


Figure 3.15 HR-LC-MS profile of Λ-α_1-α_2-Co[(*S,S*-picbipyrro-amidoglutaric acid)(*S*-ala)]$^{2+}$ (**192**): **(a)** Chromatogram of Λ-α_1-**192** and Λ-α_2-**192**; **(b)** HR-MS data of **192** for the peak at 3.2 minutes; **(c)** MS data of **192** for the peak at 3.9 minutes.

3.3.4.4.2 Characterization of untethered and tethered Λ-*cis*-α-Co[N_4(AA)]$^{2+}$ complexes 185, 186, 190, 191 and 192

The synthesized cobalt(III)-amino acid complexes were characterized based on their proton NMR (Figure 3.17, 3.18), infrared (Table 3-3), UV-visible (Table 3-4) and CD (Figure 3.19) spectra. These data are in close agreement with those reported for structurally analogous cobalt(III) derived (*S*)-aminoacidate complexes. [201, 203, 205-207]

3.3.4.4.2.1 Proton NMR spectra

The ^{1}H NMR spectra of (*S*)-aminoacidate complexes **185**, **186**, **190**, **191** and **192** are shown in Figure 3.17. These data are closely comparable with those of structural analogous such as Λ-α-Co[(*S*-picpyrrMe)(*S*-ala)]$^{2+}$, Λ-α-Co[(picenMe_2)(*S*-phe)]$^{2+}$ and Δ-α-Co[(*R*,*R*-piccyhxnMe_2)(*S*-ala)]$^{2+}$ for which the structural determinations have been reported in the literature.[201, 203, 207] It appears that the chloride donor atoms undergo substitution by (*S*)-aminoacidate with full retention of Λ-α-topology as demonstrated by the chiroptical properties of the complexes. The simplicity of the NMR spectra, especially in the aromatic region, indicated α topology for each of the synthesized (*S*)-aminoacidate complexes **185**, **186**, **190, 191** and **192 (**Figure 3.17).

In comparison to C_2-symmetrical *cis*-α-dichloro complexes such as Δ,Λ-α-Co[(picenMe_2)Cl_2]$^+$ (**171**) and Λ-α-Co[(*S*,*S*-picbipyrro)Cl_2]$^+$ (**173**), the NMR spectra of their corresponding aminoacidate complexes Δ,Λ-α-Co[(picenMe_2)(*S*-ala)]$^{2+}$ (**185**), Λ-α-Co[(*S*,*S*-picbipyrro)(*S*-ala)]$^{2+}$ (**186**) and Λ-α-Co[(*S*,*S*-

picbipyrro)(*S*-val)]$^{2+}$ (**190**) exhibit two general characteristic features (Figure 3.11 vs. 3.17):

(i) The spectra of aminoacidate complexes are somewhat more complex as the coordinated unsymmetrical bidentate amino acid resulted in the formation of *cis*-α complexes with *pseudo* C_2 symmetry.[206] This is seen in the resonances attributable to the pyridyl H atoms that are grouped in pairs, whereas the *N*-methylene groups in **185**, **186** and **190**, *N*-methyl groups in **185** and (*S*)-valine methyl groups in **190** did not appear in groups.

(ii) Considerable chemical shift differences (~ δ 9.4 vs. ~ δ 8.5) are seen for the ortho protons of both pyridyl rings. These approximately 1 ppm differences are consistent with those in literature for analogous protons in Λ-α-Co[(picenMe_2)(*S*-phe)]$^{2+}$, Δ-α-Co[(*R*-picpnMe_2)(*S*-phe)]$^{2+}$ and Λ-α-Co[(*S*-picpyrrMe)(*S*-ala)]$^{2+}$.[(Ref. 201, 206-207)] If both pyridyl rings experience nonequivalent magnetic environments, these differences are rationalized. Generally the ortho proton of the pyridyl ring in vicinity of the amino group of the aminoacidate bidentate ligand is more deshielded (~ δ 9.4 – 9.0) than the other ortho proton (~ δ 8.5 – 8.1), which is situated over the carboxylate group (Figure 3.16). [206]

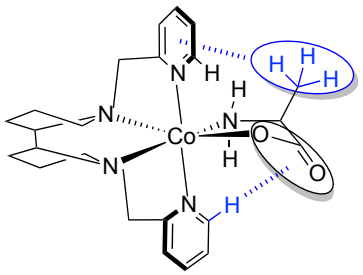


Figure 3.16 Stereochemical model of complex **186** demonstrating ortho protons of two pyridyl rings experiencing nonequivalent magnetic environments.

Furthermore, the ^{1}H NMR spectrum of (*S*)-alaninato complex Λ,Δ-α-Co[(picenMe$_2$)(*S*-ala)]$^{2+}$ (**185**) showed an integration of the two doublet resonances at 1.60 and 1.30 ppm - attributable to the (*S*)-alanine methyl group protons, in a ratio of 1.0:0.2 for the Λ and Δ diastereomers of **185** respectively (Figure 3.17). The Λ absolute configuration of major isomer **185** was assigned by comparing its CD spectral features with those of analogous complex Λ-α-Co[(picenMe$_2$)(*S*-phe)]$^{2+}$ whose structure has been confirmed by X-ray crystallographic studies.[201]

Similarly, the ^{1}H NMR spectrum of the unsymmetrical (*S*,*S)*-picbipyrro-NH_2 ligand-based complex **191** showed a comparative integration of the two doublet resonances, at 1.33 and 1.30 ppm attributable to the (*S*)-alanine methyl group protons, in a ratio of 2:1 for the Λ-α_1 and Λ-α_2 geometric isomers of **191**. Electronic and NMR spectral results alone were not enough to determine the preferred coordination mode of **191**, and X-ray studies would be required to identify the major geometric isomer. The ^{1}H NMR spectrum **(**Figure 3.17) of Δ,Λ-α-Co[(picenMe$_2$)(*S*-ala)]$^{2+}$ (**185**) and Λ-α_1,α_2-Co[(*S*,*S*-picbipyrro-NH_2)(*S*-ala)]$^{2+}$ (**191**) showed nearly perfect resonance overlap for both isomers in the aromatic region.

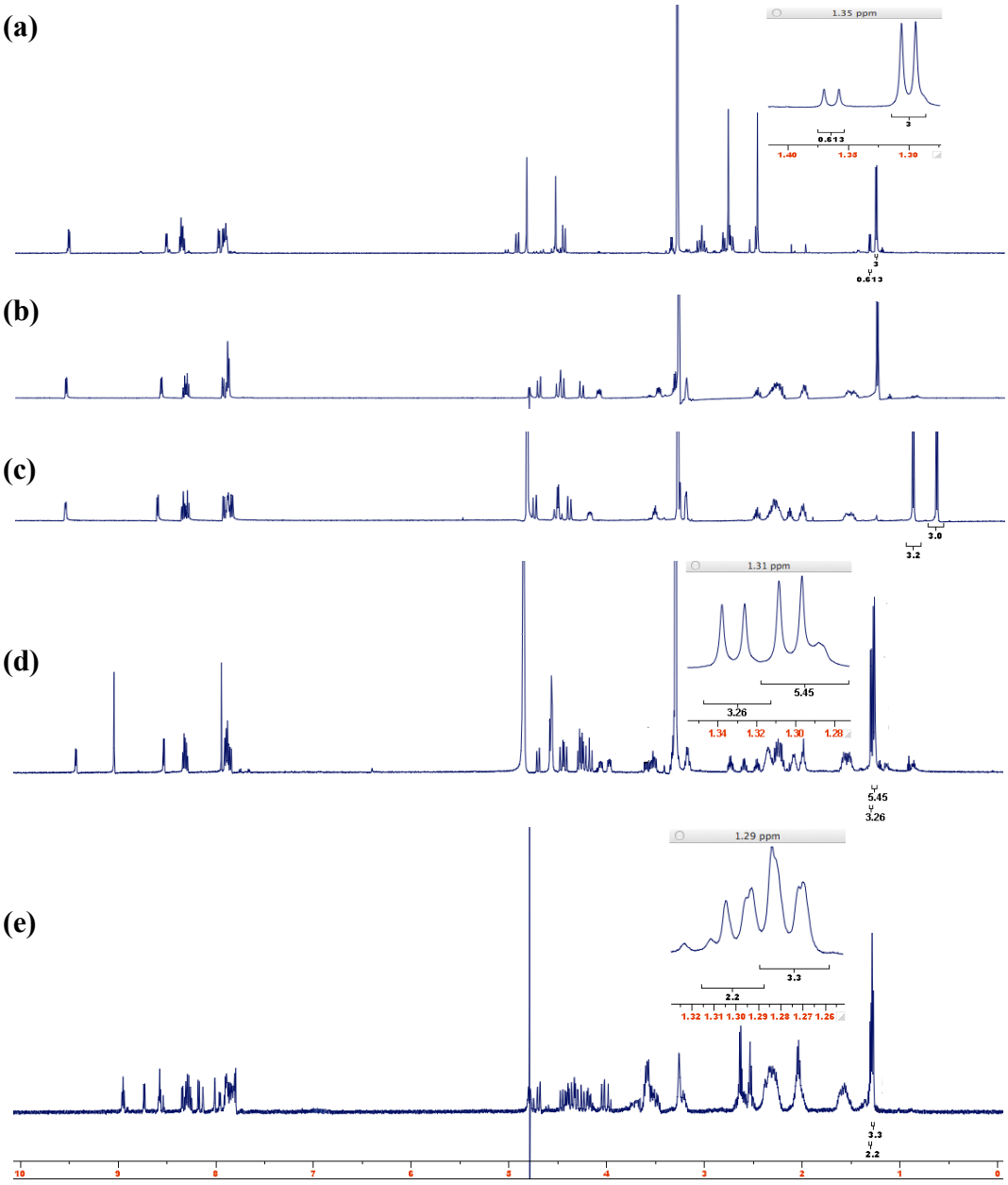


Figure 3.17 The ^{1}H NMR spectra of the cobalt(III)-derived amino acid complexes (in CD_3OD unless mentioned otherwise): **(a)** Δ,Λ-α-Co[(picenMe_2)(*S*-ala)]$^{2+}$ (**185**), Λ/Δ:1:0.2; **(b)** Λ-α-Co[(*S,S*-picbipyrro)(*S*-ala)]$^{2+}$ (**186**); **(c)** Λ-α-Co[(*S,S*-picbipyrro)(*S*-val)]$^{2+}$(**190**); **(d)** Λ-α_1-α_2-Co[(*S,S*-picbipyrro-NH_2)(*S*-ala)]$^{2+}$ (**191**); **(e)** Λ-α_1-α_2-Co[(*S,S*-picbipyrro-amido-glutaric acid)(*S*-ala)]$^{2+}$(**192**), solvent D_2O.

Curiously, the methyl resonance of (*S*)-aminoacidate bidentate ligands in the N_4 tetradentate – N,O bidentate-based octahedral complexes appears to have an upfield shift relative to that of the free amino acids in their NMR spectra. For example, the coordinated (*S*)-alanine methyl group protons of Λ-α-Co[(*S*,*S*-picbipyrro)(*S*-ala)]$^{2+}$ (**186**) resonate at δ 1.27 ppm in D_2O, whereas the methyl protons of externally added free (*S*)-alanine appear at δ 1.50 ppm (Figure 3.18 a, b). This is also visible in the ^{1}H NMR spectra of complexes **185** and **191** where the alanine methyl group of the major isomer resonates at a higher field than that of the minor isomer (Figure 3.17). The upfield shift of methyl resonances in the preferred isomers suggests that the alanine methyl group is situated in the shielding region of an aromatic ring, i.e., under the pyridyl ring of the tetradentate ligand (Figure 3.16).[206]

Additionally, the isomer obtained of complex **186** appears to be kinetically stable, as no exchange of the coordinated (*S*)-alanine appeared when a mixture of **186**, 0.3 equivalent of free (*S*)-alanine and an excess of deuterated free (*S*)-alanine was heated at 50 °C for 5 h, after incubation at room temperature for 24 h. It was reasoned that any exchange of the coordinated (*S*)-alanine with the excess of free deuterated (*S*)-alanine would be clearly reflected in the ^{1}H NMR spectrum of the above-mentioned mixture. If the amino acid were not complexed very strongly to the metal center, the integration of the coordinated (*S*)-alanine methyl group would be expected to be reduced, accompanied with an increase in the integration of the free alanine methyl signal. However, the ^{1}H NMR peaks of complex **188** remained unchanged in the presence of free alanine (Figure 3.18c).

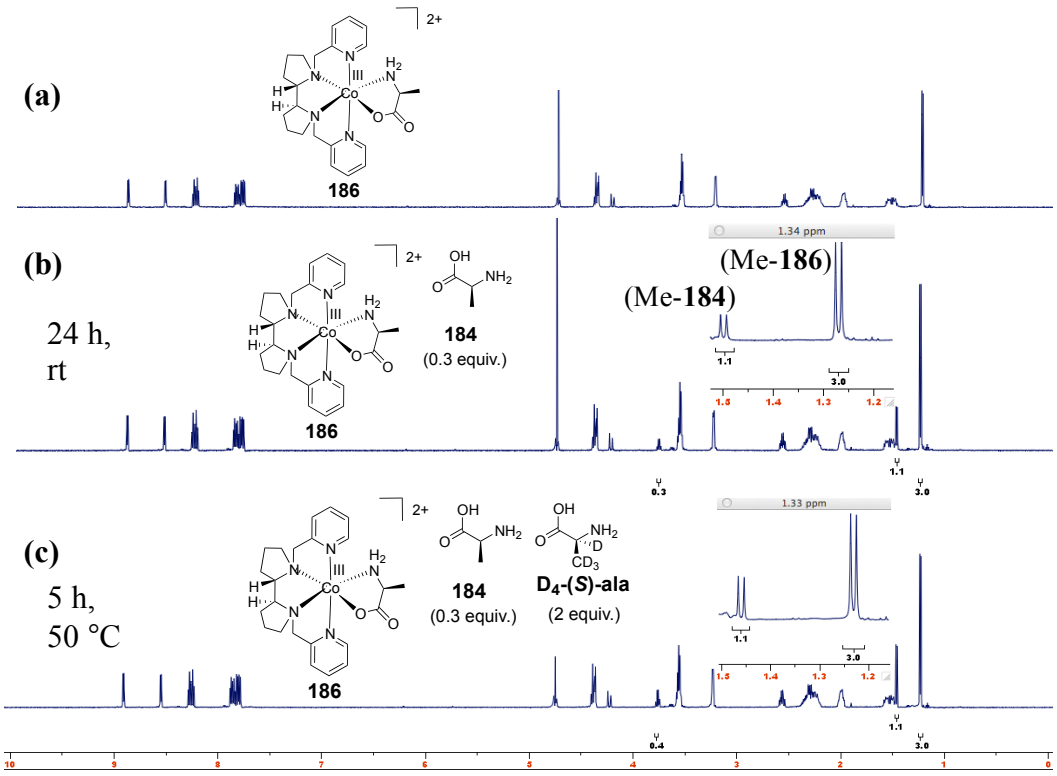


Figure 3.18 Assessing kinetic stability of the (*S,S)*-picbipyrro cobalt(III)-derived amino acid complex by inspecting ^{1}H NMR spectra in D_2O: **(a)** Λ-α-Co[(*S,S*-picbipyrro)(*S*-ala)]$^{2+}$ (**186**); **(b)** Λ-α-Co[(*S,S*-picbipyrro)(*S*-ala)]$^{2+}$ (**186**) with 0.3 equiv of free (*S*)-alanine, after 24 h at rt; **(c)** Λ-α-Co[(*S,S*-picbipyrro)(*S*-ala)]$^{2+}$ (**186**) with 0.3 equiv of free (*S*)-alanine and 2 equiv of deuterated free (*S*)-alanine, after 5 h at 50 °C.

3.3.4.4.2.2 Infrared spectra

Some important IR bands of the synthesized cobalt-amino acid complexes are shown in Table 3-3. The locations of two key IR bands (i.e. –COO$^-$ and N-H) indicate the bonding of the aminoacidate ligands with the central metal ion. The 3000 cm^{-1} region of the IR spectra for the cobalt-amino acid complexes indicated that the N-H stretching vibration was changed considerably to ~ 3350 cm^{-1} upon formation of the nitrogen to metal bond. For uncoordinated aminoacidate ligands, the N-H stretch of amino acid -NH_2 vibrates at approximately 3000-3100 cm^{-1}.[230]

The increased N-H stretching frequencies in the Co-aminoacidate system, in comparison to the aminoacidate itself, is attributable to the enhanced inductive effect on the nitrogen atom and the increased stability of the N-H bond. [231]

Table 3-3 Characteristic IR bands (cm^{-1}) for cobalt(III)-derived (*S*)-aminoacidate complexes **185**, **186**, **190, 191** and **192**.

cis-amino acid-N_4 cobalt(III) complex	υ (NH_2)	υ (COO^-)
185: Λ,Δ -α–Co[picenMe_2)(*S*-ala)]$^{2+}$ Λ / Δ : 1/0.2	3387	1665
186: Λ-α–Co[(*S,S*-picbipyrro)(*S*-ala)]$^{2+}$	3378	1669
190: Λ-α–Co[(*S,S*-picbipyrro)(*S*-val)]$^{2+}$	3381	1668
191: Λ-α_1–α_2–Co[(*S,S*-picbipyrro-NH_2)(*S*-ala)]$^{2+}$	3324	1665
192: Λ-α_1–α_2–Co[(*S,S*-picbipyrro-amidoglutaric acid)(*S*-ala)]$^{2+}$	3392	1721, 1665

Stretching frequencies for the carboxyl groups were established as criteria for differentiating between protonated carboxyl groups (1700-1750 cm^{-1}) and coordinated carboxyl groups (1600-1700 cm^{-1}). For uncoordinated aminoacidate ligands, the carboxylate groups appear at approximately 1550-1600 cm^{-1}.[230] All of the synthesized cobalt-aminoacidate complexes showed a strong absorption around 1665 cm^{-1}, in the COO^- stretching region. The lack of absorption between 1700-1750 cm^{-1} suggested that the carboxyl groups of aminoacidate ligands were coordinated to the central cobalt(III) ion. Complex **192** gave an additional COO^- stretch at 1721 cm^{-1} due to a tethered free carboxylic acid, whereas an amide carbonyl stretch was likely buried under the broad COO^- stretch at 1665.

3.3.4.4.2.3 UV-visible spectra

The UV-visible spectra of these (*S*)-aminoacidate complexes showed a single absorption band at 486 nm with very little dependence on the identity of the amino acids. This is in agreement with the reported absorption band at 497 nm for structural analogues such as Λ-α-[Co(*S*-picpyrrMe)(*S*-ala)]$^{2+}$ and Δ-α-[Co(*S,S*-piccyhxnMe$_2$)(*S*-ala)]$^{2+}$.[203, 207] Additionally, the molar absorptivity value (ε) also resembled those of the structurally similar cobalt(III)-based (*S*)-aminoacidate complexes (Table 3-4).

Table 3-4 Electronic spectral data for cobalt(III)-derived (*S*)-aminoacidate complexes **185**, **186**, **190, 191** and **192**.

cis-amino acid-N_4 cobalt(III) complex (Solvent: H_2O)	c x 10^{-3} (M)	λ (nm)	ε x 10^{-3} (dm^2 mol^{-1})	Δε (dm^2 mol^{-1})
185: Λ,Δ-α–Co[(picenMe$_2$)(*S*-ala)]$^{2+}$	1.0	501		+0.37
		486	0.53	
Λ / Δ : 1/0.2		358		- 0.09
186: Λ-α–Co[(*S,S*-picbipyrro)(*S*-ala)]$^{2+}$	1.0	498		+2.61
		487	1.54	
		358		- 0.21
		337	3.42	
		300	17.60	
190: Λ-α–Co[(*S,S*-picbipyrro)(*S*-val)]$^{2+}$	1.0	492		+0.77
		485	0.62	
		359		- 0.09
191: Λ-α_1–α_2–Co[(*S,S*-picbipyrro-NH_2)(*S*-ala)]$^{2+}$	1.0	489		+0.87
		488	0.96	
		368		- 0.24
192: Λ-α_1–α_2–Co[(*S,S*-picbipyrro-amidoglutaric acid)(*S*-ala)]$^{2+}$	1.0	495		+1.05
		480	1.50	
		421		- 0.44
		369		- 0.38

3.3.4.4.2.4 CD spectra

The CD spectral data are given in Figure 3.19. The spectral data of all of the cobalt(III)-(*S*)-aminoacidate complexes showed a dominant positive extrema at the highest wavelength along with a minor negative band at a lower wavelength. The observed CD spectral features in the visible region closely match with those for Λ-α-Co[(*S*-picpyrrMe)(*S*-ala)]$^{2+}$, Λ-α-Co[(*S*,*S*-piccyhxnMe$_2$)(*S*-ala)]$^{2+}$ and Δ-α-Co[(*R*-picpnMe$_2$)(*S*-phe)]$^{2+}$, whose absolute configurations are known from crystallographic studies.[203, 205, 207] This confirms the Λ absolute configuration for (*S*)-aminoacidate complexes **185**, **186**, **190** and **191**.

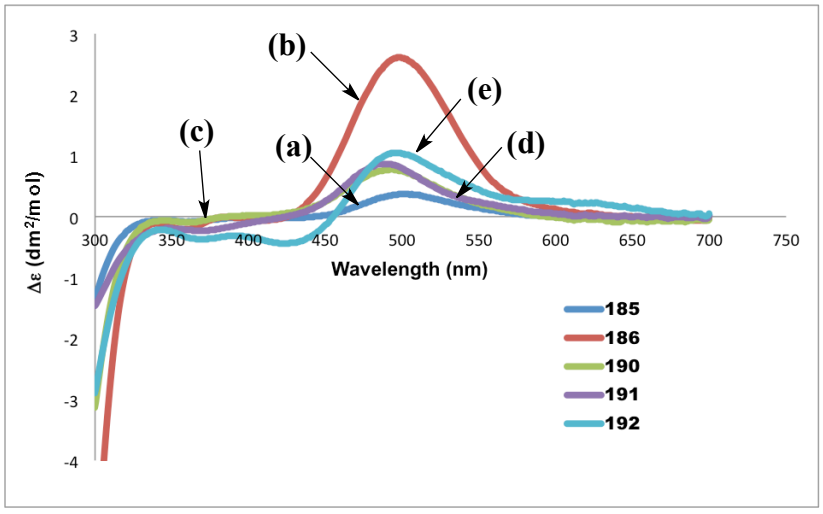


Figure 3.19 The CD spectra of the cobalt(III) aminoacidate complexes in H_2O: **(a)** Λ,Δ-α-Co[(picenMe$_2$)(*S*-ala)]$^{2+}$ (**185**), Λ/Δ:1/0.2; **(b)** Λ-α-Co[(*S*,*S*-picbipyrro)(*S*-ala)]$^{2+}$ (**186**); **(c)** Λ-α-Co[(*S*,*S*-picbipyrro)(*S*-val)]$^{2+}$ (**190**); **(d)** Λ-α$_1$-α$_2$-Co[(*S*,*S*-picbipyrro-NH$_2$)(*S*-ala)]$^{2+}$ (**191**); **(e)** Λ-α$_1$-α$_2$-Co[(*S*,*S*-picbipyrro-amidoglutaric acid)(*S*-ala)]$^{2+}$ (**192**).

A significantly lower positive extrema value of molar circular dichroism (Δε) for Λ,Δ-α-Co[(picenMe$_2$)(*S*-ala)]$^{2+}$ (**185**) (Table 3-4) suggested it to be a

mixture of Λ and Δ isomers, with Λ being the major isomer. This is in agreement with its ^{1}H NMR, which demonstrated a mixture of two isomers.

3.4 Conclusions and Future Directions

Studies were done towards developing a new class of cationic cobalt(III)-based biometallosurfactant complexes (**138** and **139**; Figure 3.8) bearing N_4-tetradentate and N,O-bidentate ligands based on hydrophobic steroid-based *N*-substituted amino acid **77**. In that regard, tethered N_4-tetradentate ligands (*S,S*)-picbipyrro-amidoglutarate (**141**) and picenMe_2-amidoglutarate (**142**) were synthesized. Initial attempts towards achieving N_5,O-hexadentate cobalt(III) complexations of the synthesized dichloro complex Λ-α-Co[(*S,S*-picbipyrro-amidoglutarate)Cl_2]$^+$ (**161**) with 11β-aminoprogesteronyl glycine (**77**) appeared to be futile for forming **138**. Changing the metal center from cobalt(III) to gallium(III) {i.e. using Λ-α-Ga[(*S,S*-picbipyrro-amidoglutarate)Cl_2]$^+$ (**164**)} also did not result in a successful hexadentate complexation.

In order to troubleshoot the failure of the desired N_5,O-hexadentate complexation, systematic model studies involving the complexation of varyingly rigid C_2-symmetrical/untethered Δ,Λ-α-Co[(picenMe_2)Cl_2]$^+$ (**171**), Λ-α-Co[(*S,S*-piccyhxnMe_2)Cl_2]$^+$ (**172**), Λ-α-Co[(*S,S*-picbipyrro)Cl_2]$^+$ (**173**), and unsymmetrical Λ-α-Co[(*S,S*-picbipyrro-NH_2)Cl_2]$^+$ (**174**) with a variety of *N*-alkyl substituted (e.g. steroid-amino acid, sarcosine, proline) and *N*-unsubstituted amino acids (e.g. alanine, valine) were performed.

In contrary to a variety of *N*-substituted amino acids, *N*-unsubstituted amino acids such (*S*)-alanine yielded the N_5,O-hexadentate complexes **185** and

186 upon coordination of C_2-symmetrical/untethered non-rigid and rigid cobalt(III) chloride complexes **171** and **173** respectively. These findings go on to suggest that the relative positioning of the pyridyl rings of the cobalt(III) chloride ligands **161**, **164** and **171**-**174** caused a severe steric interaction with the amino acid *N*-alkyl groups, causing the complexation using the initially chosen N,O-bidentate ligand (i.e. *N*-substituted steroid-amino acid **77**) to be unsuccessful.

Moreover, rigid chiral (*S*,*S*)-picbipyrro-based cobalt(III) system **173** exhibited a discriminatory behavior, coordinating only to the (*S*) isomer of alanine in preference to the (*R*)-isomer. This is most likely due to an undesired steric interaction of the (*R*)-alanine methyl group with an ortho proton of one of the pyridyl rings. The preferred formation of a hexadentate complex with (*S*)-alanine also seemed to be benefitted by a methyl group being under one of the electron deficient pyridyl rings. It was reflected in ^{1}H NMR analysis (Figure 3.17) of the complex, wherein methyl group protons of the coordinated (*S*)-alanine resonated at a higher field than those of uncoordinated (*S*)-alanine. Subsequently, a complex bearing a sterically encumbered (*S*)-valine was synthesized successfully. The synthesized N_5,O-hexadentate complex **186** also appeared to be kinetically stable, and no dissociation or exchange of the (*S*)-aminoacidate bidentate ligand was observed upon heating the complex at 50 °C for 5 h (Figure 3.18).

Besides using the C_2-symmetrical ligands **171** and **173**, the similar N_5O-hexadentate complexation of (*S*)-alanine was also achieved using unsymmetrical and tethered ligands for cobalt(III) dichloro complexes **174** and **161** respectively. Accordingly, untethered/unsymmetrical Λ-α_1-α_2-Co[(*S,S*-picbipyrro-NH_2)(*S*-

ala)]$^{2+}$ (**191**) and tethered/unsymmetrical Λ-α_1-α_2-Co[(*S,S*-picbipyrro-amidoglutaric acid)(*S*-ala)]$^{2+}$ (**192**) were synthesized as a mixture of α_1 and α_2 isomers. Without X-ray crystallographic studies it was not possible to determine which of α_1 or α_2 was the major isomer. However, from their ^{1}H NMR spectra (Figure 3.17), it is clear that both complexes yield the isomer bearing the (*S*)-alanine methyl group under one of the pyridyl rings of the ligand as the major isomer (i.e., the integration of the upfield methyl proton doublet is higher).

All of the synthesized cobalt(III) dichloro **161**, **171**, **172**, **173** and **174** and (*S*)-aminoacidate complexes **185**, **186**, **190**, **191** and **192** enforced the strict formation of octahedral complexes having *cis*-α geometry and Λ helicity, except for a complex derived from achiral ligand **171** which consisted of a mixture of Λ,Δ helicity. The *cis*-α geometry of cobalt(III) complexes was evident from a simplified aromatic region of ^{1}H NMR spectra of the complexes as well as from the deshielded ortho protons of both pyridyl rings. The CD spectral features a dominant positive extrema at higher wavelengths, which confirmed Λ helicity. Additionally, the shifting of the –COO^- and –NH_2 IR stretching frequencies in the complexes, when compared to their corresponding aminoacidate ligands, indicated the coordination of ligands to the metal ion. These results parallel the characterization of structurally similar cobalt(III) complexes for which X-ray crystallographic studies have been reported.

Based on the model complexation studies, the impeding factor for the desired N_5,O-hexadentate complexation has been clearly identified. It is of interest to extend these complexation studies towards developing novel

biometallosurfactants (**193**) and (**194**) based on a modified N,O-bidentate *N*-unsubstituted steroid-amino acid ligand, e.g., a 11β-aminoprogesteronyl-(*S*)-alanine conjugate (**78**) (Figure 3.20).

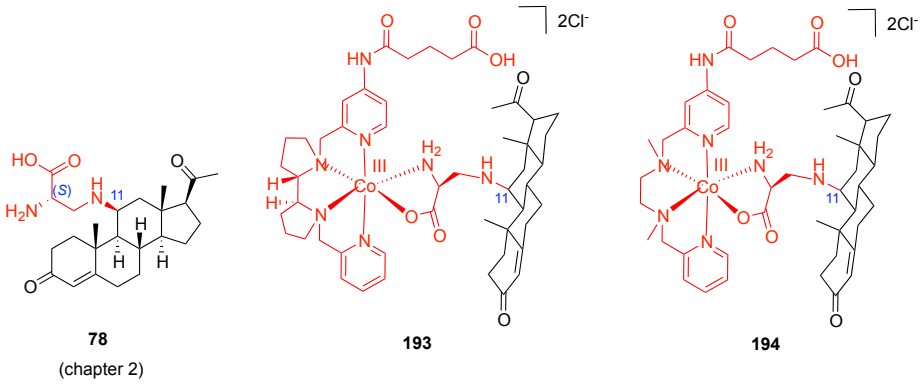


Figure 3.20 Newly designed biometallosurfactants **193** and **194** containing amidoglutaric acid tethered chiral and achiral N_4 tetradentate and *N*-unsubstituted N,O-bidentate ligands based on 11β-aminoprogesteronyl-(*S*)-alanine conjugate (**78**).

Chapter 4 : Experimental Procedures

4.1 General Experimental Methods

4.1.1 Reagents, Solvents and Purifications

All commercially available reagents were acquired from Sigma-Aldrich Canada Ltd., Fisher Scientific Ltd. or Alfa Aesar Ltd., and used without further purification unless stated otherwise. Solvents, of American Chemical Society (ACS) grade, were purchased from Fisher and used without further purification unless otherwise stated. All anhydrous reactions were done using flame-dried glassware and under an atmosphere of argon, unless stated otherwise. When anhydrous solvents were required, they were dried and distilled as follows: dichloromethane, methanol and toluene were distilled over calcium hydride, while tetrahydrofuran and diethyl ether were distilled over sodium with benzophenone as an indicator. Acetonitrile and hexanes were of HPLC grade, and used without further purification. A Milli-Q reagent water system (Millipore Co., Milford, MA) was used to deionize water. Commercially available ACS grade solvents (>99.0% purity) were used for column chromatography without further purification. Flash chromatography was performed using Silicycle, 240-400 mesh silica gel. Preparative thin layer chromatography (TLC) purification was done using plates purchased from Analtech (1000 or 500 microns). Ion-exchange chromatography was performed using Sephadex CM-25 cation exchanger resin, with a capacity of 4.5 ± 0.5 meq/g and particle size of 40 – 120 μm. Sephadex CM-25 was purchased from Pharmacia Fine Chemicals. All reactions and fractions from silica

gel column chromatography were monitored by thin layer chromatography (TLC) using glass plates with a UV fluorescent indicator (normal SiO_2, Merck 60 F_{254}). One or more of the following methods was used for visualization: UV absorption by fluorescence quenching, staining with phosphomolybdic acid in ethanol (10 g/100 mL), ninhydrin (ninhydrin : acetic acid : *n*-butanol/ 0.6 g : 6 mL : 200 mL) or permanganate ($KMnO_4$: K_2CO_3 : NaOH : H_2O/ 1.5 g : 10 g : 0.12 g : 200 mL). The organic volatiles were removed *in vacuo* via evaporation under reduced pressure using a Büchi rotary evaporator.

Preparative scale high performance liquid chromatography (HPLC) was performed on the following systems: a Gilson chromatograph equipped with model 322 pump heads, a model UV/VIS-156 detector, a GX-271 liquid handler and a Rheodyne 7725i injector fitted with a 1000 μL sample loop. The column used was a Phenomenex C_{18} (5 μm, 21.2 x 250 mm). All HPLC solvents were filtered through a Millipore filtration system under vacuum prior to use.

4.1.2 Characterization

A Perkin Elmer 241 polarimeter with a microcell (10 cm, 1 mL) was used to measure optical rotations at ambient temperature, with values reported in units of 10^{-1} deg cm^2 g^{-1}. All reported optical rotations were referenced against air and measured at the sodium D line (λ = 589.3 nm).

A Hewlett Packard 8453 UV-VIS Spectrophotometer was used to measure UV-VIS absorbance of metal complexes. CD data for metal complexes were recorded using an Olis DSM 17 Circular Dichroism spectrophotometer and a microcell (1 mm to 1 cm).

Infrared spectra (IR) were recorded using a Nicolet Magna 750 FT-IR spectrometer or a Nic-Plan FT-IR microscope. The term thin film refers to the evaporation of a solution on a NaCl plate.

Varian Inova 600, Inova 500, Inova 400 or Unity 500 spectrometers were used to record nuclear magnetic resonance (NMR) spectra at 27 °C. For ^{1}H (400, 500 or 600 MHz) spectra, δ values were referenced to residual proteated solvent as: $CDCl_3$ (7.26 ppm), CD_2Cl_2 (5.32 ppm), CD_3OD (3.30 ppm), DMSO-d_6 (2.50 ppm), or D_2O (4.79 ppm) and for ^{13}C (100, 125 or 150 MHz) spectra, δ values were referenced to $CDCl_3$ (77.0 ppm), CD_2Cl_2 (53.8 ppm), CD_3OD (49.0 ppm), or DMSO-d_6 (39.5 ppm). Reported splitting patterns are abbreviated as s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet.

Mass spectra (MS) were recorded on an Agilent Technologies 6220 oaTOF or a Kratos AEIMS-50.

4.1.3 HPLC Purification Methods

For analytical purposes, some of the compounds were purified using the following method to get a single peak in a HPLC chromatogram.

System A:

0-5 min: 5% CH_3CN/95% H_2O (0.1% TFA)

5-55 min: 40%-60% CH_3CN ramp (0.1% TFA)

55-58 min: 60%-90% CH_3CN ramp (0.1% TFA)

58-60 min: 90% CH_3CN/5% H_2O (0.1% TFA)

60-63 min: 90%-5% CH_3CN ramp (0.1% TFA)

63-68 min: 5% CH_3CN/95%H_2O (0.1% TFA)

Column: Phenomenex Luna® 5 µm C18(2) 100 Å, LC Column 250 x 21.2 mm, temperature RT

Flow rate: 10 mL/min

λ = 245, 250 nm

System B:

0-2 min: 1% CH_3CN/99% H_2O (0.1% TFA)

2-15 min: 1%-25% CH_3CN ramp (0.1% TFA)

15-20 min: 25%-95% CH_3CN ramp (0.1% TFA)

20-23 min: 95%-98% CH_3CN ramp (0.1% TFA)

23-24 min: 98% CH_3CN/2% H_2O (0.1% TFA)

24-25 min: 98%-1% CH_3CN ramp (0.1% TFA)

25-27 min: 1% CH_3CN/99% H_2O (0.1% TFA)

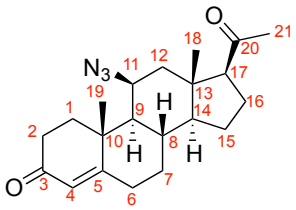
Column: Phenomenex Luna® 3 µm C18(2) 100 Å, LC Column 50 x 2.0 mm, temperature 35 °C

Flow rate: 0.2 mL/min

λ = 210, 280 nm

4.2 Synthesis and Characterization of Compounds

11β-Azidopregn-4-ene-3,20-dione (81):[115]



The title azido compound **81** was synthesized by modifying an existing literature procedure.[115] diethyl azodicarboxylate (0.80 mL, 4.6 mmol, 3 equiv) was added dropwise to a stirred solution of vacuum-oven dried triphenylphosphine (1.2 g, 4.6 mmol, 3 equiv) and phenol (0.03 g, 0.3 mmol, 0.2 equiv) in anhydrous THF (2.4 mL) under argon at room temperature. Stirring was continued for 2 minutes. To this stirred mixture, hydrazoic acid[232] (3.85 mL of 2.4 M solution in benzene, 9.2 mmol, 6 equiv) was added, followed by 11α-hydroxyprogesterone (**68**) (0.5 g, 1.53 mmol, 1 equiv). The reaction mixture was then heated at reflux for 20 minutes. A color change from pink to dark brown indicated the completion of the reaction. After the reduced pressure evaporation of solvent, the crude product was chromatographed twice on a silica gel column (first column eluents; DCM:ethyl ether, from 9.5:0.5 to 9:1; and second column eluents; diethyl ether:hexane, from 1:1 to 4:1) to yield 11β-azidoprogesterone (**81**) (0.23 g, 42 %) as a colorless solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.67 (d, *J* = 1.7 Hz, 1H, H-3), 4.17-4.15 (m, 1H, H-11), 2.51-2.35 (m, 4H, H-6β, H-12β, CH_2-2, H-17), 2.24-2.18 (m, 2H, H-6α, H-1β), 2.18-2.09 (m, 4H, H-16β, CH_3-21), 2.05-1.93 (m, 1H, H-7β), 1.91-1.75

(m, 2H, H-8, H-1α), 1.74-1.65 (m, 2H, H-15α, H-16α), 1.41 (s, 3H, CH_3-19), 1.39-1.23 (m, 2H, H-15β, H-12α), 1.17-0.96 (m, 3H, H-14, H-7α, H-9), 0.90 (s, 3H, CH_3-18).

^{13}C NMR (400 MHz, $CDCl_3$): δ 208.7, 199.3, 171.4, 122.4, 63.5, 57.6, 57.4, 55.1, 42.5, 42.4, 39.0, 35.0, 33.6, 32.3, 31.7, 31.5, 31.2, 24.1, 22.6, 20.4, 14.4.

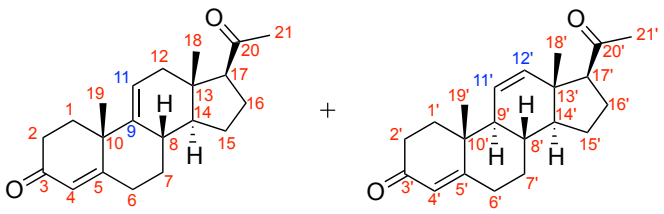
HRMS (ESI) *(m/z)*: Calc'd for $C_{21}H_{29}N_3O_2Na$ $[M+Na]^+$: 378.2152, found 378.2142.

FTIR (thin film) cm^{-1}: 3244, 3015, 2987, 2091, 1710, 1676, 1479.

$[\alpha]_D$: 274 ($_C$ 0.87, CH_2Cl_2).

TLC (50% diethyl ether, 50% hexane), **R_f**: 0.5 (UV, $KMnO_4$).

Pregn-4,9(11)-diene-3,20-dione (82) & Pregn-4',11'-diene-3',20'-dione (83):[124-125]



The unexpected title compounds were obtained through modification of the following literature procedures:

Protocol 1: [123]

To a stirred solution of azide **81** (0.05 g, 0.14 mmol, 1 equiv) in THF (1.4 mL) was added tributylphosphine (64 µL, 0.42 mmol, 3 equiv) and water (25 µL, 1.4 mmol, 10 equiv). The resulting solution was stirred at room temperature for 3

h. The solvent was removed and the residue was purified by silica gel flash column chromatography (eluents; diethyl ether:hexane, 8:2) to give an inseparable mixture of title compounds **82** and **83** (20 mg, 45%) as a colorless solid, instead of the desired 11β-aminopregn-4-ene-3,20-dione **76**.

Protocol 2:[137]

To an ice-cooled stirred solution of *N*-2-nitrobenzenesulfonamide(DNs)-glycine methyl ester **84**[137] (0.058 g, 0.18 mmol, 1 equiv), triphenylphosphine (0.048 g, 0.18 mmol, 1 equiv) and 11α-OH-Pro **68** (0.06 g, 0.18 mmol, 1 equiv) in dry THF (2 mL) was added diethyl azidodicarboxylate (DEAD) (33 μL, 0.18 mmol, 1 equiv) dropwise over a period of 10 minutes. The reaction mixture was allowed to warm to room temperature and stirred overnight. The solvent was removed and the residue was purified by silica gel flash column chromatography (eluents; diethyl ether:hexane, 4:1) to give an inseparable mixture of title compounds **82** and **83** (18 mg, 40%) as a colorless solid.

The obtained title compounds were in accordance with the spectroscopic data available in literature.[124-125]

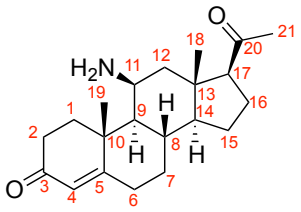
^{1}H NMR (600 MHz, $CDCl_3$): δ (Mixture of isomers) 6.25 (dd, *J* = 10.2, 2.8 Hz, 1H, H-12'), 5.78 (br s, 1H, H-4'), 5.75 (d, *J* = 1.4 Hz, 1H, H-4), 5.55 (d, *J* = 10.2, 1.6 Hz, 1H, H-11'), 5.54 – 5.52 (m, 1H, H-11), 2.70 (t, *J* = 9 Hz, 1H), 2.60 (t, *J* = 9.6 Hz, 1H), 2.57 – 2.42 (m, 6H), 2.41 – 2.20 (m, 6H), 2.19 (s, 3H, CH_3-21'), 2.14 (s, 3H, CH_3-21), 2.26 – 2.20 (m, 2H), 1.92 – 1.83 (m, 3H), 1.82 – 1.68 (m, 6H), 1.65 – 1.58 (m, 2H), 1.46 – 1.36 (m, 3H), 1.34 (s, 3H, CH_3-19), 1.28 – 1.20 (m, 3H), 1.17 (s, 3H, CH_3-19'), 0.76 (s, 3H, CH_3-18'), 0.62 (s, 3H, CH_3-18).

^{13}C NMR (125 MHz, $CDCl_3$): δ (Mixture of isomers) 209.5, 209.4, 199.7, 199.6, 170.0, 169.9, 145.5, 137.8, 125.6, 125.1, 124.5, 118.7, 63.8, 60.0, 56.5, 53.3, 53.2, 46.1, 42.7, 41.4, 41.1, 39.2, 37.7, 35.6, 34.7, 34.5, 34.2, 34.1, 33.7, 33.2, 32.5, 31.8, 31.6, 31.0, 26.5, 25.8, 23.5, 23.4, 23.3, 18.2, 17.9, 13.4.

HRMS (EI) *(m/z)*: Calc'd for $C_{21}H_{28}O_2$ [$M^{+\bullet}$]: 312.20892, found 312.20897.

TLC (80% ethyl ether, 20% hexane), $\mathbf{R_f}$: 0.5 (UV, CAM).

11β-Aminopregn-4-ene-3,20-dione (76):



To a suspension of azide **81** (1.9 g, 5.4 mmol, 1 equiv) and $CoCl_2{\cdot}6H_2O$ (3.8 g, 16.1 mmol, 3 equiv) in water (10 mL) at 25 °C, a solution of $NaBH_4$ (0.92 g, 24.1 mmol, 4.5 equiv) in H_2O (25 mL) was added dropwise (to subside excessive foaming) with stirring. The appearance of a black precipitate indicated the formation of a cobalt boride species. The mixture was then stirred at reflux for 3.5 h, and upon completion of the reaction, the solution was allowed to cool and ethyl acetate (30 mL) was added. The resulting biphasic mixture was filtered through Celite and the aqueous layer was separated. The collected aqueous phase was extracted several times, first with ethyl acetate (3 x 30 mL) and then with CH_2Cl_2 (3 x 30 mL). The combined organic phases were dried (Na_2SO_4) and concentrated under reduced pressure, and further purified on silica gel by flash column chromatography (eluents; ethyl acetate followed by CH_2Cl_2:MeOH,

9.5:0.5) to give the pure amine **76** (1.1 g, 62%) as a colorless solid. X-ray quality crystals were grown by a slow evaporation of **76**, dissolved in a hexane/ethyl ether co-solvent system.

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.67 (d, *J* = 1.7 Hz, 1H, H-4), 3.62 (br s, 1H, H-11), 2.51-2.34 (m, 5H, H-12β, H-6β, CH_2-2, H-17), 2.26-2.21 (m, 1H, H-6α), 2.20 – 2.09 (m, 5H, H-1β, H-16β, CH_3-21), 2.07 – 2.00 (m, 1H, H-7β), 1.97 – 1.88 (m, 2H, H-8, H-1α), 1.79 – 1.68 (m, 2H, H-15α, H-16α), 1.44 (s, 3H, CH_3-19), 1.35 – 1.28 (m, 2H, H-15β, H-12α), 1.15 – 1.03 (m, 3H, H-14, H-9, H-7α), 0.92 (s, 3H, CH_3-18).

^{13}C NMR (125 MHz, $CDCl_3$): δ 209.0, 199.3, 172.2, 122.2, 64.4, 58.4, 56.2, 48.7, 43.1, 39.2, 35.0, 33.9, 32.9, 32.0, 31.4, 31.1, 29.8, 24.4, 22.6, 21.6, 16.6.

HRMS (ESI) *(m/z)*: Calc'd for $C_{21}H_{32}NO_2$ $[M+H]^+$: 330.2428, found 330.2426.

FTIR (thin film) cm^{-1}: 3381, 3323, 2928, 2874, 1701, 1667, 1446, 1388.

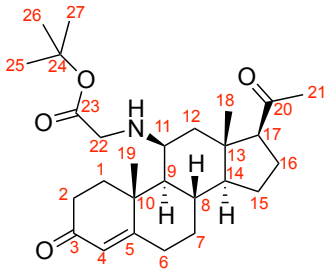
$[\alpha]_D$: 208 ($_C$ 0.93, CH_2Cl_2).

TLC (10% methanol, 90% DCM), **Rf**: 0.5 (UV, CAM).

HPLC: t_R = 33.5 min (System A).

MP: 142-145 °C.

***N*-[11β-aminopregn-4-enyl-3,20-dione]-*O-tert*-butyl glycinate (86):**



To a mixture of amine **76** (0.100 g, 0.3 mmol, 1 equiv), *tert*-butyl bromoacetate (88 μL, 0.6 mmol, 2 equiv), tetra butyl ammonium iodide (0.222 g, 0.6 mmol, 2 equiv) and 4 Å MS in acetonitrile (5 mL) was added K_2CO_3 (0.166 g, 1.2 mmol, 4 equiv) under argon at room temperature. The mixture was then stirred at reflux for 9 h. The resulting mixture was filtered through Celite and the filtrate was evaporated under reduced pressure. The obtained residue was dissolved in CH_2Cl_2 (10 mL) and extracted several times with water (2 x 5 mL) followed by brine (1 x 5 mL). The combined organic phase was dried (Na_2SO_4), concentrated under reduced pressure, and further purified by silica gel flash column chromatography (eluents: ethyl acetate:hexane, 1:1) to give the pure title compound **86** (0.101 g, 75%) as an off-white solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ 5.67 (s, 1H, H-4), 3.42 (d, J = 16.8 Hz, 1H, H-22a), 3.29 – 3.10 (m, 2H, H-11, H-22b), 2.52 – 2.35 (m, 5H, H-6β, H-2β, H-17, H-2α, H-12β), 2.25-2.20 (m, 1H, H-6α), 2.19 – 2.15 (m, 1H, H-1β), 2.15 – 2.13 (m, 4H, H-16β, CH_3-21), 2.08-2.03 (m, 1H, H-7β), 1.99 – 1.90 (m, 1H, H-8), 1.90 – 1.85 (m, 1H, H-1α), 1.79 – 1.73 (m, 1H, H-15α), 1.72 – 1.66 (m, 1H, H-16α), 1.56 (s, 3H, CH_3-19), 1.48 (s, 9H, $C(CH_3)_3$-25, 26, 27), 1.35 – 1.28 (m, 1H, H-

15β), 1.18 (dd, J = 11.4, 4.5 Hz, 1H, H-9), 1.14 – 1.02 (m, 3H, H-14, H-12α, H-7α), 0.92 (s, 3H, CH_3-18).

^{13}C NMR (125 MHz, $CDCl_3$): δ 209.1, 199.6, 172.5, 171.8, 121.8, 81.4, 64.2, 58.5, 56.1, 55.0, 50.6, 43.3, 40.7, 39.2, 34.6, 34.0, 33.2, 32.0, 31.7, 31.5, 28.1, 24.4, 22.7, 21.8, 15.2.

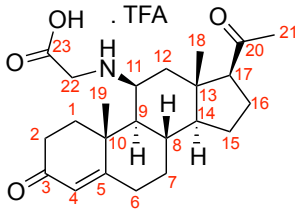
HRMS (ESI) *(m/z)*: Calc'd for $C_{27}H_{42}NO_4$ $[M+H]^+$: 444.3108, found 444.3115

FTIR (thin film) cm^{-1}: 3032, 2934, 1731, 1703, 1671, 1367, 1234, 1158.

$[\alpha]_D$: 137 ($_C$ 2.00, CH_2Cl_2).

TLC (40% hexane, 60% ethyl acetate), **R_f**: 0.5 (UV, CAM).

***N*-[*11β*-Aminopregn-4-enyl-3,20-dione]glycine TFA salt (77):**



A solution of ester **86** (0.100 g, 0.23 mmol) in CH_2Cl_2 (2 mL) was treated with excess trifluoroacetic acid (4 mL), and allowed to stir at room temperature for 6 h. After completion of the reaction, the organic volatiles were evaporated under reduced pressure and the obtained residue was dried under high vacuum to yield the title amino acid **77** (0.101 g, 89 %) as a fluffy TFA salt. The obtained compound appeared to be pure by NMR, and used without any further purification.

^{1}H NMR (600 MHz, CD_3OD): δ 5.73 (d, *J* = 1.5 Hz, 1H, H-4), 4.08 – 4.01 (m, 3H, H-11, CH_2-22), 2.64 – 2.50 (m, 4H, H-6β, H-12β, H-2β, H-17), 2.42 (dt, *J* =

16.7, 4.8 Hz, 1H, H-2α), 2.39-2.32 (m, 1H, H-6α), 2.28 (dt, *J* = 13.5, 5.0 Hz, 1H, H-1β), 2.16 (s, 3H, CH$_3$-21), 2.16 – 1.94 (m, 3H, H-16β, H-7β, H-8), 1.95 – 1.82 (m, 2H, H-1α, H-16α), 1.81 (dd, *J* = 15.5, 5.0 Hz, 1H, H-12α), 1.72 (dd, *J* = 11.8, 4.2 Hz, 1H, H-15α), 1.58 (s, 3H, CH$_3$-19), 1.43 – 1. 26 (m, 3H, H-15β, H-9, H-14), 1.23 – 1.14 (m, 1H, H-7α), 0.89 (s, 3H, CH$_3$-18).

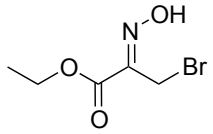
^{13}C NMR (125 MHz, CD$_3$OD): δ 211.3, 200.9, 172.8, 170.0, 123.0, 64.2, 58.4, 57.5, 55.5, 42.3, 39.4, 39.1, 35.2, 34.2, 33.6, 32.4, 32.3, 31.0, 27.7, 24.8, 23.9, 22.0, 14.6.

HRMS (ESI) *(m/z)*: Calc'd for $C_{23}H_{34}NO_4[M+H]^+$: 388.2482, found 388.2483.

FTIR (thin film) cm^{-1}: 3197, 2962, 1699, 1678, 1422, 1199.

[α]$_D$: 112 ($_C$ 1.44, H_2O).

Ethyl 2-(hydroxyimino)-3-bromopropanoate (87):[138]



The title compound was synthesized according to a literature procedure.[138] To a stirred solution of ethyl bromopyruvate (3.6 mL, 28.5 mmol, 1 equiv) in $CHC1_3$ (85 mL) and CH_3OH (57 mL), hydroxylamine hydrochloride (2.00 g, 28.5 mmol, 1 equiv) was added at room temperature. The resulting mixture was left stirring overnight at room temperature and then concentrated to dryness. The residue was dissolved in CH_2Cl_2 (40 mL), washed with 0.1 N HCl (20 mL) and with brine (20 mL), and dried over Na_2SO_4. Evaporation of the solvent in vacuo

gave quantitatively crystalline material, which was recrystallized (CH_2Cl_2-hexane) to yield bromo oxime **87** (3.96 g, 68%) as white needles.

^{1}H NMR (500 MHz, $CDCl_3$): δ 9.33 (s, 1H, =N-OH), 4.38 (q, *J* = 7.2 Hz, 2H, -OCH_2), 4.27 (s, 2H, -CH_2Br), 1.38 (t, *J* = 7.2 Hz, 3H, CH_3CH_2).

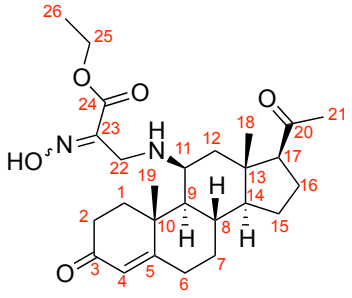
^{13}C NMR (125 MHz, $CDCl_3$): δ 161.7, 147.9, 62.5, 30.1, 14.3.

HRMS (ESI) *(m/z)*: Calc'd for $C_5H_8BrNNaO_3$ $[M+Na]^+$: 231.9580, found 231.9579.

FTIR (thin film) cm^{-1}: 3445 (br), 3275, 3057, 2987, 1724, 1472, 1326, 1225, 1184, 1035.

M.P. °C: 77-78 °C (lit. M.P. °C: 75-76 °C).[138]

11β-*N*-[3-*O*-Ethyl-2-hydroxyimino-propanoyl]-aminopregn-4-ene-3,20-dione (88):



To a mixture of amine **76** (0.05 g, 0.15 mmol, 1equiv), bromo oxime **87** (0.065 g, 0.3 mmol, 2 equiv), tetrabutyl ammonium iodide (0.110 g, 0.3 mmol, 2 equiv) and 4 Å MS in acetonitrile (1 mL) was added K_2CO_3 (0.085 g, 0.6 mmol, 4 equiv) under argon at room temperature. The mixture was then stirred at reflux for 12 h. The resulting mixture was filtered through Celite and the filtrate was evaporated under reduced pressure. The obtained residue was dissolved in CH_2Cl_2

(2 mL) and extracted several times with water (2 x 2 mL) followed by brine (1 x 2 mL). The combined organic phase was dried (Na_2SO_4), concentrated under reduced pressure, and further purified by silica gel flash column chromatography (eluents: ethyl acetate-CH_2Cl_2, 1:1) to give the pure title compound **88** (0.025 g, 33%) as a light yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.97 (s, 1H, =N-OH), 5.66 (d, *J* = 1.6 Hz, 1H, H-4), 4.31 (q, *J* = 7.1 Hz, 2H, CH_2-25), 3.81 (d, *J* = 12.9 Hz, 1H, H-22a), 3.63 (d, *J* = 13.0 Hz, 1H, H-22b), 3.19 (s, 1H, H-11), 2.54 (dd, *J* = 13.8, 2.2 Hz, 1H, H-12β), 2.50 – 2.29 (m, 5H, CH_2-2, H-6β, H-17), 2.24 – 2.18 (m, 1H, H-6α), 2.19 (td, *J* = 4.6, 3.7, 2.1 Hz, 1H, H-16β), 2.15 (s, 3H, CH_3-21), 2.15 – 2.05 (m, 1H, H-1β), 2.06 – 1.97 (m, 1H, H-7β), 1.91 – 1.79 (m, 2H, H-1α, H-8), 1.79 – 1.61 (m, 2H, H-15α, H-16α), 1.42 (s, 3H, CH_3-19), 1.35 (t, *J* = 7.1 Hz, 3H, 26), 1.33 – 1.22 (m, 2H, H-12α, H-15β), 1.15 (dd, *J* = 11.5, 4.7 Hz, 1H, H-9), 1.14 – 1.00 (m, 2H, H-7α, H-14), 0.89 (s, 3H, CH_3-18).

^{13}C NMR (125 MHz, $CDCl_3$): δ 209.2, 199.8, 172.6, 163.6, 152.5, 121.9, 64.3, 62.2, 58.4, 56.1, 55.4, 43.2, 40.9, 39.4, 34.5, 34.1, 33.2, 32.0, 31.8, 31.5, 29.9, 24.5, 22.6, 21.6, 15.4, 14.4.

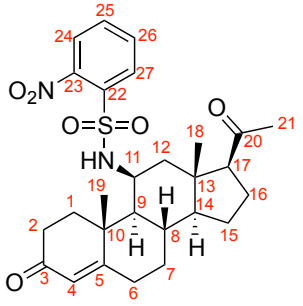
HRMS (ESI) *(m/z)*: Calc'd for $C_{26}H_{39}N_2O_5[M+H]^+$: 459.2853, found 459.2853; and Calc'd for $C_{26}H_{38}N_2NaO_5[M+Na]^+$: 481.2673, found 481.2676.

FTIR (thin film) cm^{-1}: 3300, 3058, 2934, 1703, 1668, 1449, 1357, 1189, 1015.

$[\alpha]_D$: 152 ($_C$ 0.75, CH_2Cl_2).

TLC (50% CH_2Cl_2, 50% ethyl acetate), **R_f**: 0.5 (UV, CAM).

11β-*N*-[*o*-Nitrobenzenesulfonyl]-aminopregn-4-ene-3,20-dione (93):



A mixture of amine **76** (0.2 g, 0.6 mmol, 1 equiv), *o*-nitrobenzenesulfonyl chloride (1.33 g, 6 mmol, 10 equiv) and pyridine (0.48 mL, 6.0 mmol, 10 equiv) was dissolved in CH_2Cl_2 (5 mL) at room temperature and then stirred at reflux for 12 h. The resulting mixture was diluted further with additional CH_2Cl_2 (5 mL) and then extracted several times with water (2 x 5 mL) followed by brine (1 x 5 mL). The combined organic phase was dried (Na_2SO_4), concentrated under reduced pressure, and further purified by silica gel flash column chromatography (eluents; CH_2Cl_2 followed by ethyl acetate:CH_2Cl_2, 1:4) to give the pure title compound **93** (0.19 g, 61%) as a light yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.19 – 8.13 (m, 1H, H-24), 7.93 – 7.87 (m, 1H, H-27), 7.83 – 7.73 (m, 2H, H-25, H-26), 5.68 (d, *J* = 1.6 Hz, 1H, H-4), 5.48 (d, *J* = 10.4 Hz, 1H, NH-SO_2-), 4.46 (app. dtd, *J* = 10.0, 4.7, 2.3 Hz, 1H, H-11), 2.51 – 2.35 (m, 4H, 2, H-1β, H-6β), 2.30 – 2.20 (m, 2H, H-6α, H-17), 2.09 – 1.96 (m, 3H, H-7β, H-12β, H-16β), 1.96 – 1.80 (m, 2H, H-1α, H-8), 1.77 (s, 3H, CH_3-21), 1.76 – 1.63 (m, 2H, H-15α, H-16α), 1.49 (s, 3H, CH_3-19), 1.48 – 1.43 (m, 1H, H-12α), 1.31 – 1.19 (m, 2H, H-9, H-15β), 1.15 – 0.99 (m, 2H, H-7α, H-14), 0.53 (s, 3H, CH_3-18).

^{13}C NMR (125 MHz, $CDCl_3$): δ 207.8, 199.2, 170.4, 147.8, 136.2, 133.7, 133.4, 129.6, 125.7, 122.7, 63.8, 57.3, 55.2, 51.4, 44.4, 41.9, 39.1, 34.8, 33.9, 32.7, 31.8, 31.7, 30.7, 24.3, 22.6, 21.0, 16.1.

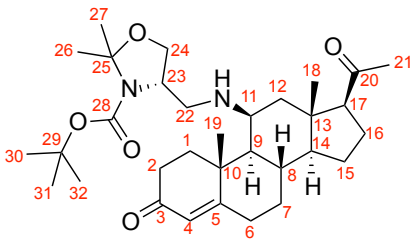
HRMS (ESI) *(m/z)*: Calc'd for $C_{27}H_{34}N_2NaO_6S[M+Na]^+$: 537.2030, found 537.2028.

FTIR (thin film) cm^{-1}: 3388, 3092, 2942, 2881, 1700, 1667, 1542, 1450, 1355, 1166.

$[\alpha]_D$: 275.6 ($_C$ 1.13, CH_2Cl_2).

TLC (80% CH_2Cl_2, 20% ethyl acetate), **R_f:** 0.5 (UV, CAM).

***N*-[(*S*)-3- *tert*-Butoxycarbonyl -2,2-dimethyloxazolidine-4-methyl]-*N*-11β-aminopregn-4-ene-3,20-dione (97):**



The diastereomer **97** was prepared by an adaptation of a known protocol.[155] To a solution of **76** (330 mg, 1 mmol) in dry MeOH (11 mL), containing acetic acid (1% v/v), was added (*S*)-Garner's aldehyde (230 mg, 1 mmol), and the mixture was stirred at room temperature under an atmosphere of argon for 45 min. The resulting imine was reduced by the addition of $NaCNBH_3$ (80 mg, 1.25 mmol) in two portions over 30 min. This was stirred at room temperature for another 2 h, at which point the reaction was quenched with water (5 mL). The organic solvent was evaporated, more water was added (5 mL), and

the aqueous layer was extracted with CH_2Cl_2 (3 x 15 mL). The combined organic extracts were dried over Na_2SO_4, evaporated, and the residue purified by silica gel chromatography (eluents; CH_2Cl_2:MeOH, 96:4). The title product was obtained as a white foam (325 mg, 60%).

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.66 (s, 1H, H-4), 4.08 – 3.92 (m, 2H, H-23, H-24β), 3.91 – 3.82 (m, 1H, H-24α), 3.22 – 3.11 (m, 1H, H-11), 3.09 – 2.91 (m, 2H, CH_2-22), 2.66 – 2.41 (m, 5H, H-6β, H-2β, H-17, H-2α, H-12β), 2.27-2.09 (m, 5H, H-6α, H-1β, CH_3-21), 2.08 – 2.02 (m, 2H, H-16β, H-7β), 1.93 – 1.80 (m, 2H, H-8, H-1α), 1.79 – 1.64 (m, 2H, H-15α, H-16α), 1.60 (s, 3H, CH_3-19), 1.51 (br s, 15H, $C(CH_3)_2$-26, 27, $C(CH_3)_3$-30, 31, 32), 1.35 – 1.24 (m, 2H, H-15β, H-12α), 1.19 (dd, J = 11.5, 4.5 Hz, 1H, H-9), 1.71 – 1.01 (m, 2H, H-14, H-7α), 0.84 (s, 3H, CH_3-18).

^{13}C NMR (125 MHz, $CDCl_3$): δ 208.9, 199.5, 172.4, 152.6, 121.8, 93.9, 80.8, 66.4, 64.2, 58.1, 57.2, 55.1, 55.0, 49.5, 48.9, 43.2, 39.9, 39.3, 34.5, 33.9, 33.1, 31.8, 31.7, 31.5, 28.3, 24.3, 22.6, 21.6, 15.5.

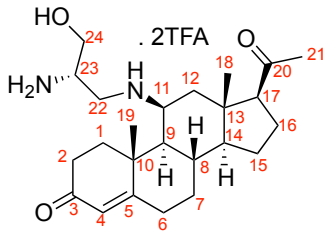
HRMS (ESI) *(m/z)*: Calc'd for $C_{32}H_{50}N_2O_5Na$ $[M+Na]^+$: 565.3612, found 565.3605.

FTIR (thin film) cm^{-1}: 3053, 2974, 2934, 2878, 1699, 1671, 1617, 1389, 1174, 1086.

$[\alpha]_D$: 138 ($_C$ 0.74, CH_2Cl_2).

TLC (70% hexane, 30% ethyl acetate), **R_f:** 0.5 (UV, CAM).

3- [*N*-11β-Aminopregn-4-enyl-3,20-dione]-*2*-(*S*)-amino propyl alcohol•2TFA salt (98):



The diastereomer **98** was generated following the same protocol.[155] The fully protected amino alcohol-appended aminoprogesterone **97** (250 mg, 0.46 mmol) was stirred in a mixture of trifluoroacetic acid and water (6 mL, 5:1 mixture) at 50 °C for 16 h. The solvents were evaporated on a high-vacuum rotary evaporator, and then co-evaporated with water (3 x 10 mL), and dried under high vacuum to afford the desired product **98** (185 mg) in a quantitative yield as a white trifluoroacetate salt. This product was used without further purification. Analytical samples and those used for assays were further purified by HPLC.

^{1}H NMR (600 MHz, D_2O): δ 5.83 (d, *J* = 1.6 Hz, 1H, H-4), 4.18-4.15 (m, 2H, H-24b, H-11), 4.07-4.00 (m, 1H, H-24a), 3.98 (m, 1H, H-23), 3.89 (dd, *J* = 13.9, 6.2 Hz, 1H, H-22a), 3.81-3.75 (m, 1H, H-22b), 2.77-2.64 (m, 2H, H-12β, H-17), 2.62-2.51 (m, 2H, H-2β, H-6β), 2.46 (dt, *J* = 17.2, 5.1 Hz, 1H, H-2α), 2.39-2.34 (m, 1H, H-6α), 2.24 (s, 3H, CH_3-21), 2.22-2.12 (m, 2H, 1β, H-7β), 2.10-1.98 (m, 3H, H-1α, H-16β, H-8), 1.97-1.82 (m, 4H, H-12α, H-9, H-16α, H-15α), 1.45 (s, 3H, CH_3-19), 1.44-1.31 (m, 2H, H-14, H-15β), 1.28-1.16 (m, 1H, H-7α), 0.88 (s, 3H, CH_3-18).

^{13}C NMR (125 MHz, D_2O): δ 216.1, 203.7, 175.4, 129.2, 121.2, 62.9, 62.1, 57.5, 56.7, 53.7, 48.5, 46.3, 41.4, 37.9, 36.5, 33.1, 32.5, 31.8, 31.1, 30.7, 23.3, 22.5, 20.3, 13.1.

HRMS (ESI) *(m/z)*: Calc'd for $C_{24}H_{39}N_2O_3$ $[M+H]^+$: 403.2955, found 403.2946

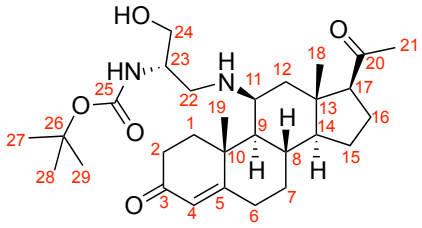
FTIR (thin film) cm^{-1}: 3087 (br), 2960, 2570, 1703, 1435, 1210, 1168.

$[\alpha]_D$: 89 ($_C$ 0.62, CH_2Cl_2).

HPLC: t_R = 32.2 min (System A).

TLC (90% THF, 5% MeOH, 5% NH_4OH), **R_f:** 0.5 (UV, $KMnO_4$).

3- [*N*-11β-Aminopregn-4-enyl-3,20-dione]-*2*-(*S*)-amino-*tert*-butoxycarbonyl-propyl alcohol (99):



The deprotected amino alcohol **98** (0.46 mmol) was dissolved in a water-acetonitrile mixture (6 mL, 1:2 mixture), to which was added $NaHCO_3$ (116 mg, 1.4 mmol) and di-*t*-butyl-dicarbonate (105 mg, 0.48 mmol). The mixture was stirred at room temperature for 2 h, at which point the starting material was completely consumed as determined by TLC analysis (CH_2Cl_2-MeOH, 95:5). The solution was basified with NaOH (3 drops of a 3 M solution), and extracted with ethyl acetate (3 x 30 mL). The combined organic extracts were dried over Na_2SO_4, concentrated and purified by silica gel chromatography (CH_2Cl_2-MeOH, 95:5), to yield the desired product **99** (181 mg, 78 %).

^{1}H NMR (600 MHz, $CDCl_3$): δ 5.66 (s, 1H), 4.99 (br s, 1H), 3.72 (dd, 2H, *J*=4.1, 1.9 Hz), 3.65-3.49 (m, 1H), 3.17-3.13 (m, 1H), 3.10-3.04 (m, 1H), 2.52-2.39 (m, 4H), 2.38-2.32 (m, 1H), 2.24-2.19 (m, 1H), 2.16-2.11 (m, 4H), 2.11-2.06 (m, 1H), 2.05-1.99 (m, 1H), 1.91-1.88 (m, 1H), 1.87-1.80 (m, 1H), 1.79-1.65 (m, 2H), 1.59-1.53 (m, 3H), 1.46 (s, 3H), 1.44-1.40 (m, 9H), 1.30-1.23 (m, 2H), 1.22-1.18 (m, 1H), 1.11-1.03 (m, 2H), 0.84 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 209.0, 199.4, 172.1, 156.5, 122.1, 80.0, 64.2, 58.4, 56.0, 55.8, 52.9, 49.7, 43.2, 40.4, 39.3, 34.8, 34.0, 33.2, 31.9, 31.8, 31.6, 29.9, 28.5, 24.4, 22.8, 21.8, 15.5.

HRMS (ESI) *(m/z)*: Calc'd for $C_{29}H_{46}N_2O_5$ $[M+H]^+$: 503.3479, found 503.3471.

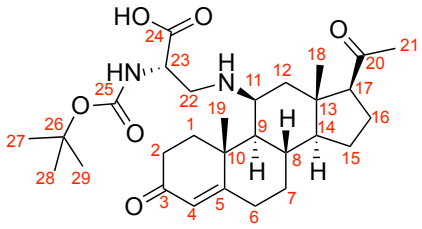
FTIR (thin film) cm^{-1}: 3375.4, 2931.5, 1701.8, 1668.4, 1616.0, 1522.7, 1452.3, 1390.5, 1364.9, 1171.3, 1030.3, 703.2.

$[\alpha]_D$: 120.9 (*c* 0.86, MeOH).

HPLC: t_R = 43.3 min (System A).

TLC (90% THF, 5% MeOH, 5% NH_4OH), **R_f:** 0.7 (UV, $KMnO_4$).

***N-tert*-Butoxycarbonyl-*(N*-11β-aminopregn-4-enyl-3,20-dione)-(*S*)-alanine (100):**



The diastereomer **100** was generated following the Jones oxidation as described herein. The Jones reagent (2 M final concentration) was prepared freshly by dissolution of CrO_3 (100 mg, 1 mmol) in water (0.5 mL) followed by the addition of concentrated H_2SO_4 (0.085 mL, 1.5 mmol). The Boc-protected amino alcohol **99** (127 mg, 0.25 mmol) was stirred at room temperature in HPLC-grade acetone (2.5 mL). Oxidation was achieved by the slow addition of freshly prepared Jones reagent (0.26 mL of a 2 M solution, 0.53 mmol), over 5 min, followed by stirring at room temperature for a further 1.5 h. The progress of the reaction was followed by TLC analysis (CH_2Cl_2-MeOH, 8:2). Once the reaction was deemed complete, excess Jones reagent was quenched with *i*PrOH (1 mL) and the entire mixture was evaporated. The residue was suspended in water (20 mL) and extracted with CH_2Cl_2 (2 x 15 mL) followed by ethyl acetate (2 x 15 mL). The combined organic extracts were dried over Na_2SO_4, evaporated and purified by silica gel chromatography (eluents; CH_2Cl_2:MeOH, 4:1), to yield the product as a pure white solid (50 mg, 39 %).

^{1}H NMR (600 MHz, CD_3OD): δ 5.66 (s, 1H), 4.18-4.10 (m, 1H), 3.76-3.68 (m, 1H), 3.26 (d, 1H, *J*=6.2 Hz), 2.81 (app d, 1H, *J*=13.9 Hz), 2.62 (app t, 1H, *J*=9.1 Hz), 2.60-2.41 (m, 2H), 2.39-2.23 (m, 1H), 2.19 (s, 3H), 2.15-2.04 (m, 3H), 1.98-1.86 (m, 3H), 1.86-1.76 (m, 1H), 1.76-1.64 (m, 1H), 1.65-1.59 (m, 1H), 1.51 (s, 3H), 1.45-1.40 (m, 9H), 1.38-1.22 (m, 3H), 1.20-1.08 (m, 1H), 0.80 (s, 3H).

^{13}C NMR (125 MHz, CD_3OD): δ 210.0, 200.5, 178.1, 173.3, 156.6, 121.0, 79.5, 63.3, 57.8, 54.9, 53.8, 48.2, 42.1, 38.6, 37.9, 33.7, 33.1, 33.0, 31.2, 30.1, 29.0, 27.3, 23.6, 21.9, 20.4, 13.4.

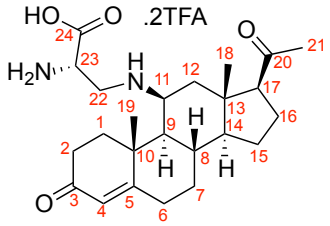
HRMS (ESI) *(m/z)*: Calc'd for $C_{29}H_{44}N_2O_6$ $[M+H]^+$: 517.3272, found 517.3268.

FTIR (thin film) cm^{-1}: 3413.3, 2935.2, 1702.6, 1671.2, 1482.2, 1392.7, 1366.4, 1244.6, 1165.5, 1029.1, 867.8.

$[\alpha]_D$: 131.0 ($_C$ 1.10, MeOH).

HPLC: t_R = 45.1 min (System A).

***N*-11β-Aminopregn-4-enyl-3,20-dione-(*S*)-alanine •2TFA salt (78):**



The Boc-protected amino acid derivative **100** (11 mg, 0.02 mmol) was stirred at room temperature in a CH_2Cl_2:TFA mixture (0.5 mL, 3:1 mixture) for 30 min. The solvent was evaporated, followed by co-evaporation with water (3 x 1 mL), to yield the amino acid **78** as a TFA salt (8.8 mg) in a quantitative yield.

^{1}H NMR (600 MHz, D_2O): δ 5.80 (app s, 1H), 4.18 (dd, 1H, *J*=12.1, 4.0 Hz), 4.17 (m, 1H), 3.78 (dd, 1H, *J*=12.8. 4.0 Hz), 3.62 (app t, 1H, *J*=12.5 Hz), 2.69 (app t, 1H, *J*=9.0 Hz), 2.67-2.60 (m, 1H), 2.58-2.47 (m, 2H), 2.40 (dt, 1H, *J*=17.3, 5.0 Hz), 2.35-2.29 (m, 1H), 2.30-2.16 (m, 4H), 2.15-2.08 (m, 1H), 2.03-1.91 (m, 3H), 1.91-1.77 (m, 4H), 1.45 (app s, 3H), 1.40-1.30 (m, 2H), 1.25-1.11 (m, 1H) 0.81 (app s, 3H).

^{13}C NMR (125 MHz, D_2O): δ 216.1, 203.9, 175.4, 171.5, 121.2, 62.9, 57.0, 53.9, 53.7, 46.0, 43.7, 41.4, 38.2, 37.0, 33.1, 32.6, 31.8, 31.2, 30.8, 30.6, 23.2, 22.5, 20.4,13.3.

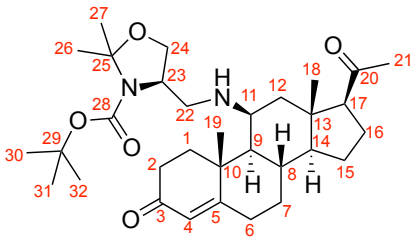
HRMS (ESI) *(m/z)*: Calc'd for $C_{24}H_{36}N_2O_4$ $[M+H]^+$: 417.2748, found 417.2741.

FTIR (thin film) cm^{-1}: 2957.8, 2881.1, 1676.5, 1431.0, 1360.6, 1203.7, 1137.5, 835.8, 799.2, 721.8.

$[\alpha]_D$: 114.1 (*c* 0.80, MeOH).

HPLC: t_R = 28.3 min (System A).

***N*-[(*R*)-3- *tert*-butoxycarbonyl -2,2-dimethyloxazolidine-4-methyl]-*N*-11β-aminopregn-4-ene-3,20-dione (102):**



The title product **102** was obtained as a white foam (303 mg, 56%) by following the procedure used for the compound **97**, but using (*R*)-Garner's aldehyde.

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.67 (s, 1H, H-4), 4.01 – 3.86 (m, 2H, H-23, H-24β), 3.91 – 3.82 (m, 1H, H-24α), 3.15 (br s, 1H, H-11), 3.11 – 3.02 (m, 2H, CH_2-22), 2.58 – 2.30 (m, 5H, H-6β, H-2β, H-17, H-2α, H-12β), 2.30 – 2.19 (m, 2H, H-6α, H-1β), 2.15 (s, 3H, CH_3-21), 2.10 – 1.98 (m, 2H, H-16β, H-7β), 1.96 – 1.80 (m, 4H, H-8, H-1α, H-15α, H-16α), 1.75 (s, 3H, CH_3-19), 1.60 (s, 6H, $C(CH_3)_2$-26, 27), 1.50 (br s, 9H, $C(CH_3)_3$-30, 31, 32), 1.40 – 1.21 (m, 2H, H-15β, H-12α), 1.20 – 1.17 (m, 1H, H-9), 1.17 – 1.00 (m, 2H, H-14, H-7α), 0.87 (s, 3H, CH_3-18).

^{13}C NMR (125 MHz, $CDCl_3$): δ 208.8, 199.7, 169.4, 155.9, 122.7, 95.4, 83.0, 66.8, 64.1, 63.0, 58.2, 56.7, 55.9, 50.9, 43.1, 41.4, 40.6, 39.4, 34.5, 33.6, 33.2, 31.8, 31.4, 30.9, 28.2, 24.3, 22.6, 21.6, 15.5.

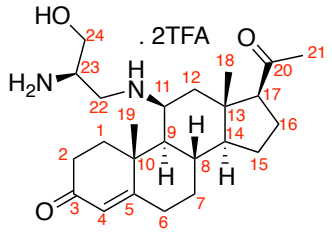
HRMS (ESI) *(m/z)*: Calc'd for $C_{32}H_{51}N_2O_5$ $[M+H]^+$: 543.3792, found 543.3791.

FTIR (thin film) cm^{-1}: 3056, 2972, 2935, 2879, 1699, 1671, 1389, 1365, 1207, 1172.

$[\alpha]_D$: 90 ($_C$ 0.6, CH_2Cl_2).

TLC (70% hexane, 30% ethyl acetate), **R_f:** 0.5 (UV, CAM).

3- [*N*-11β-Aminopregn-4-enyl-3,20-dione]-*2*-(*R*)-amino propyl alcohol •2TFA salt (103):



The title product **103** was obtained as a white foam (185 mg) in a quantitative yield by following the procedure as described for compound **98**.

^{1}H NMR (600 MHz, D_2O): δ 5.83 (s, 1H, H-4), 4.22 – 4.16 (m, 1H, H-24b), 4.16 – 4.11 (m, 1H, H-11), 3.99 – 3.93 (m, 2H, H-23, H-24a), 3.93 – 3.88 (m, 1H, H-22b), 3.64 – 3.56 (m, 1H, H-22a), 2.73 (t, *J* = 9.2 Hz, 1H, H-17), 2.70 – 2.64 (m, 1H, H-12β), 2.61 – 2.51 (m, 2H, H-2β, H-6β), 2.46 (dt, *J* = 17.0, 5.0 Hz, 1H, H-2α), 2.37 (ddd, *J* = 14.8, 4.8, 2.6 Hz, 1H, H-6α), 2.24 (s, 3H, CH_3-21), 2.19 – 2.09 (m, 2H, H-1β, H-7β), 2.09 – 1.98 (m, 3H, H-1α, H-8, H-16β), 1.95 – 1.81 (m, 4H,

H-12α, H-9, H-15α, H-16α), 1.43 (s, 3H, CH_3-19), 1.42 – 1.32 (m, 2H, H-14, H-15β), 1.27 – 1.16 (m, 1H, H-7α), 0.89 (s, 3H, CH_3-18).

^{13}C NMR (125 MHz, D_2O): δ 216.0, 203.4, 175.2, 129.1, 121.2, 62.8, 61.5, 57.6, 56.8, 53.7, 48.3, 46.8, 41.3, 38.0, 36.9, 33.1, 32.5, 31.8, 31.1, 30.7, 23.2, 22.6, 20.2, 13.3.

HRMS (ESI) *(m/z)*: Calc'd for $C_{24}H_{39}N_2O_3$ $[M+H]^+$: 403.2955, found 403.2948.

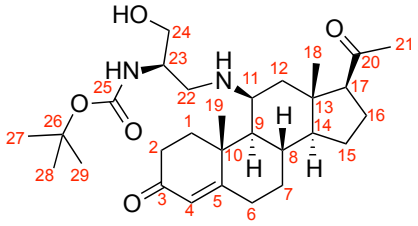
FTIR (thin film) cm^{-1}: 3101 (br), 2949, 2564, 1715, 1426, 1212, 1161.

$[\alpha]_D$: 91.5 ($_C$ 1.8, H_2O).

HPLC: t_R = 31.1 min (System A).

TLC (90% THF, 5% MeOH, 5% NH_4OH), **R_f:** 0.5 (UV, $KMnO_4$).

3- [*N*-11β-Aminopregn-4-ene-3,20-dione]-*2*-(*R*)-amino-*tert*-butoxycarbonyl-propyl alcohol (104):



The title product **104** was obtained as a white foam (174 mg, 75%) by following the procedure used for the preparation of compound of **99**.

^{1}H NMR (600 MHz, $CDCl_3$): δ 5.69 (s, 3H), 5.14-5.02 (br s, 1H), 3.78-3.64 (m, 3H), 3.18-3.11 (br s, 1H), 3.00-2.91 (m, 1H), 2.96 (dd, 1H, *J*=11.0, 3.7 Hz), 2.75-2.65 (m, 1H), 2.52-2.43 (m, 3H), 2.40-2.30 (m, 1H), 2.26-2.21 (m, 1H), 2.19-2.12 (m, 4H), 2.12-2.02 (m, 3H), 1.94-1.82 (m, 3H), 1.81-1.69 (m, 2H), 1.61-1.56 (m,

1H), 1.52 (s, 3H), 1.48-1.43 (m, 9H), 1.36-1.25 (m, 3H), 1.26-1.20 (m, 2H), 0.88 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 209.1, 199.4, 172.1, 156.2, 122.1, 79.9, 65.7, 64.2, 58.3, 55.7, 52.1, 49.9, 43.2, 40.3, 39.3, 34.7, 34.0, 33.2, 31.9, 31.8, 31.6, 29.9, 28.5, 24.5, 22.8, 22.0, 15.5.

HRMS (ESI) *(m/z)*: Calc'd for $C_{29}H_{46}N_2O_5$ $[M+H]^+$: 503.3479, found 503.3470.

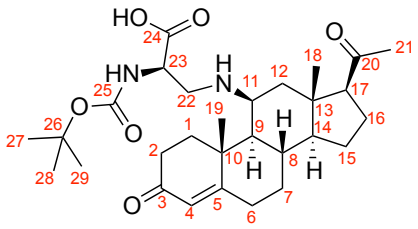
FTIR (thin film) cm^{-1}: 3385.4, 2933.3, 1702.2, 1667.7, 1616.1, 1523.0, 1452.8, 1365.1, 1171.4, 1031.8, 700.4.

$[\alpha]_D$: 135.6 ($_C$ 0.90, MeOH).

HPLC: t_R = 43.3 min (System A).

TLC (90% THF, 5% MeOH, 5% NH_4OH), **R_f:** 0.7 (UV, $KMnO_4$).

***N-tert*-Butoxycarbonyl-(*N*-11β-aminopregn-4-enyl-3,20-dione)-(*R*)-alanine (105):**



The title product **105** was obtained as a white foam (48.7 mg, 38%) by following the procedure detailed above for compound **100**.

^{1}H NMR (600 MHz, CD_3OD): δ 5.64 (s, 1H), 4.19-4.09 (m, 1H), 3.75-3.64 (m, 1H), 3.49-3.41 (m, 1H), 3.03-2.94 (m, 1H), 2.64 (dd, 1H, *J*=15.0, 1.9 Hz), 2.60-2.45 (m, 3H), 2.40-2.24 (m, 3H), 2.17-2.06 (m, 3H), 2.00-1.89 (m, 2H), 1.89-1.76

(m, 2H), 1.63-1.58 (m, 1H), 1.60-1.49 (m, 4H), 1.49-1.40 (m, 9H), 1.40-1.31 (m, 1H), 1.31-1.22 (m, 2H), 1.19-1.09 (m, 1H), 0.90 (s, 3H).

^{13}C NMR (125 MHz, CD_3OD): δ 210.0, 200.2, 174.2, 172.7, 156.4, 121.2, 79.5, 63.0, 57.3, 55.3, 54.6, 48.5, 41.5, 38.4, 37.6, 33.4, 33.0, 32.5, 31.1, 31.0, 29.7, 27.3, 23.5, 22.3, 19.8, 13.0.

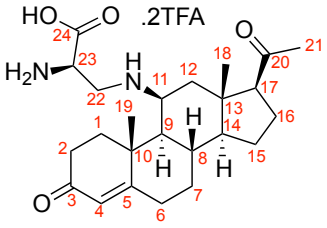
HRMS (ESI) *(m/z)*: Calc'd for $C_{29}H_{44}N_2O_6$ $[M+H]^+$: 517.3272, found 517.3264.

FTIR (thin film) cm^{-1}: 3390.1, 2936.2, 1705.0, 1671.5, 1484.2, 1454.5, 1365.7, 1167.2, 1058.5, 1029.2, 871.6.

$[\alpha]_D$: 80.0 (*c* 1.10, MeOH).

HPLC: t_R = 43.6 min (System A).

***N*-11β-Aminopregn-4-enyl-3,20-dione-(*R*)-alanine •2TFA salt (79):**



The title product **79** was obtained as a white foam (8.8 mg) in a quantitative yield by following the procedure detailed above for compound **78**.

^{1}H NMR (600 MHz, D_2O): δ 5.79 (br s, 1H), 4.08 (dd, 1H, *J*=12.5, 4.0 Hz), 4.05-4.01 (m, 1H), 3.79 (dd, 1H, *J*=11.7, 4.0 Hz), 3.41 (app t, 1H, *J*=12.2 Hz), 2.73-2.66 (m, 1H), 2.62-2.49 (m, 3H), 2.43 (dt, 1H, *J*=17.3, 5.0 Hz), 2.36-2.30 (m, 1H), 2.21-2.16 (m, 4H), 2.14-2.09 (m, 1H), 2.05-1.96 (m, 3H), 1.91-1.77 (m, 4H), 1.48 (app s, 3H), 1.40-1.30 (m, 2H), 1.24-1.13 (m, 1H), 0.85 (app s, 3H).

^{13}C NMR (125 MHz, D_2O): δ 216.2, 203.9, 175.7. 171.7, 120.9, 62.9, 56.7, 56.6, 53.3, 47.5, 45.5, 41.4, 38.1, 36.5, 32.8, 32.7, 32.0, 31.1, 30.7, 30.7, 23.3, 22.5, 20.1, 13.1.

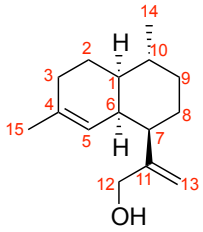
HRMS (ESI) *(m/z)*: Calc'd for $C_{24}H_{36}N_2O_4$ $[M+H]^+$: 417.2748, found 417.2741.

FTIR (thin film) cm^{-1}: 2945.7, 1676.9, 1422.8, 1362.3, 1294.5, 1202.9, 1134.6, 1027.8, 836.7, 721.4.

$[\alpha]_D$: 56.6 (*c* 0.8, MeOH).

HPLC: t_R = 28.7 min (System A).

Amorpha-4,11-diene-12-ol (109):[157]



The title compound was synthesized in two steps from artemisinic acid (**72**) by adapting an existing literature procedure.[157] To an ice-cooled stirred solution of artemisinic acid (0.5 g, 2.14 mmol, 1 equiv) in dry toluene-methanol (6 mL, 2:1 mixture) was added trimethylsilyldiazomethane (1.3 mL of 2 M in hexane, 2.6 mmol, 1.2 equiv) dropwise over a period of 5 minutes. The reaction mixture was allowed to warm to room temperature and stirred for 35 minutes. The reaction progress was monitored by TLC (eluents; EtOAc:hexane, 20:80, CAM). After the disappearance of the acid, the solvent was removed and the residue was diluted further with additional CH_2Cl_2 (10 mL) and then extracted twice with $NaHCO_3$ (2 x 10 mL) followed by brine (1 x 5 mL). The combined organic phase was dried (Na_2SO_4), concentrated under reduced pressure to give the artemisinic acid methyl

ester **108** (0.55 g, 98%) as colorless oil, which was directly used for the next step without isolation and further purification.

To a solution of the artemisinic methyl ester (**108**) (0.5 g, 2 mmol, 1 equiv) in dry CH_2Cl_2 (4 mL) was added DIBAL (6 mL of 1 M in CH_2Cl_2, 6 mmol, 3 equiv) dropwise over a period of 10 minutes at -78 ^oC. After 2.5 h of stirring, the reaction mixture was allowed to warm from -78 ^oC to 0 ^oC. Subsequently, the reaction was quenched by adding 1 N HCl (7 mL) to the mixture and allowed to stir vigorously at room temperature for additional 1 h till two layers of liquids were clearly separated. The resulting organic layer was separated and the aqueous layer was washed with CH_2Cl_2 (2 x 10 ml). The combined organic phase was dried (Na_2SO_4), concentrated under reduced pressure, and further purified by silica gel flash column chromatography (eluents; from ethyl acetate:hexane, 5:95 to 1:4) to give the pure title compound **109** (0.41g, 92%) as a light yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.19 (br s, 1H, H-13b), 5.06 (br s, 1H, H-5), 4.84 (br s, 1H, H-13a), 4.11 (s, 2H, CH_2-12), 2.53 – 2.46 (m, 1H, H-6), 2.21 (dddd, *J* = 13.6, 4.3, 2.7, 1.2 Hz, 1H, H-7), 1.97 – 1.84 (m, 2H, H-2b, H-3b), 1.81 – 1.73 (m, 1H, H-3a), 1.69 (dq, *J* = 13.0, 3.5 Hz, 1H, H-9b), 1.61 – 1.58 (br s, 3H, CH_3-15), 1.56 – 1.39 (m, 3H, H-8b, H-10, H-2a), 1.38 – 1.29 (m, 2H, H-8a, H-1), 1.01 (tdd, *J* = 13.0, 11.6, 3.5 Hz, 1H, H-9a), 0.89 (d, *J* = 6.4 Hz, 3H, CH_3-14).

^{13}C NMR (125 MHz, $CDCl_3$): δ 151.3, 135.1, 120.6, 110.0, 65.8, 43.6, 41.8, 37.9, 35.5, 27.9, 26.6, 25.9, 25.8, 23.8, 19.9.

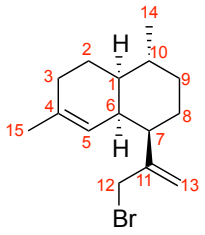
HRMS (EI) *(m/z)*: Calc'd for $C_{15}H_{24}O$ [$M^{+\bullet}$]: 220.18271, found 220.18223.

FTIR (thin film) cm^{-1}: 3305, 3084, 2921, 2867, 1718, 1448, 1435, 1048, 900.

$[\alpha]_D$: -23 ($_C$ 1.2, CH_2Cl_2).

TLC (20% ethyl acetate, 80% hexane), **R_f:** 0.5 (UV, CAM).

Amorpha-4,11-diene-12-bromide (106):[157]



To the stirred solution of triphenylphosphine (0.032 g, 0.12 mmol, 1.2 equiv) and DDQ (0.027 g, 0.12 mmol, 1.2 equiv) in dry CH_2Cl_2 (2 mL) was added tetrabutylammonium bromide (0.039 g, 0.12 mmol, 1.2 equiv), and the reaction was stirred for 2 minutes at room temperature. To that resulting stirred mixture, artemisinic alcohol **109** (0.022 g, 0.1 mmol, 1 equiv) was added. TLC analysis of the mixture showed that the reaction was completed immediately. To avoid the formation of any side products, the organic volatiles were quickly removed under reduced pressure, and the resulting residue was purified by silica gel flash column chromatography (eluents: CH_2Cl_2-hexane, 5:95) to give the pure bromo compound **106** (0.026g, 93%) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.33 (br s, 1H, H-5), 4.95 (br s, 2H, CH_2-12), 4.07 (d, J = 10.2 Hz, 1H, H-13b), 3.98 (d, J = 10.2 Hz, 1H, H-13a), 2.56 – 2.52 (m, 1H, H-6), 2.49 (dddd, J = 13.7, 4.4, 2.8, 1.3 Hz, 1H, H-7), 2.00 – 1.92 (m, 1H, H-2b), 1.79 (ddd, J = 17.9, 6.4, 1.9 Hz, 1H, H-3b), 1.70 (dq, J = 13.0, 3.3 Hz, 1H, H-9b), 1.60 (br s, 3H, CH_3-15), 1.59 – 1.53 (m, 1H, H-3a), 1.53 – 1.50 (m, 1H, H-

8b), 1.45 – 1.37 (m, 3H, H-10, H-2a, H-1), 1.31 (qd, J = 12.6, 3.4 Hz, 1H, H-8a), 1.06 (tdd, J = 13.0, 11.1, 3.3 Hz, 1H, H-9a), 0.90 (d, J = 6.0 Hz, 3H, CH_3-14).

^{13}C NMR (125 MHz, $CDCl_3$): δ 147.7, 135.6, 120.3, 115.6, 43.1, 41.7, 37.4, 36.7, 35.3, 27.9, 26.6, 26.0, 25.9, 23.8, 19.9.

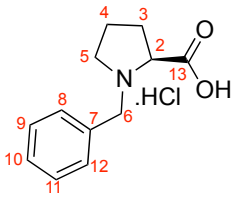
HRMS (EI) *(m/z)*: Calc'd for $C_{15}H_{23}Br$ [$M^{+\bullet}$]: 282.09830, found 282.09758.

FTIR (thin film) cm^{-1}: 3040, 2922, 2867, 1718, 1447, 1209, 910, 675.

$[\alpha]_D$: -3 ($_C$ 0.78, CH_2Cl_2).

TLC (20% ethyl acetate, 80% hexane), **R_f:** 0.8 (UV, CAM).

(*S*)-1-Benzylpyrrolidine-2-carboxylic acid (BP) hydrochloride (111):[159]



BP hydrochloride

A mixture of L-proline (10.09 g, 87.68 mmol, 1 equiv) and potassium hydroxide (18.69 g, 0.33 mol, 3.8 equiv) in isopropanol (85 mL) was heated at 40 °C for 0.5 h. Once the suspended solids were dissolved, benzyl chloride (15.14 mL, 0.13 mol, 1.5 equiv) was added dropwise at 0 °C. The reaction mixture was stirred at 40 °C for 6 h. After cooling to room temperature, the suspension was neutralized with concentrated hydrochloric acid (13 mL) until pH 3 was obtained. To the mixture, CH_2Cl_2 (140 mL) was added to get an emulsified bottom layer and a clear top layer. The biphasic solution was placed at 4 °C overnight. The solid was removed by filtration and the filtrate was concentrated under reduced pressure to give a solid residue. The residue was suspended in acetone (45 mL)

with stirring, filtered and then air dried to give the title compound **111** (14.27 g, 68%) as a white solid.

^{1}H NMR (500 MHz, D_2O): δ 7.69 – 7.39 (m, 5H, H-7, H-9, H-10, H-11, H-12), 4.52 – 4.31 (m, 2H, CH_2-6), 4.07 (dd, *J* = 9.6, 7.1 Hz, 1H, H-2), 3.66 (ddd, *J* = 11.5, 7.3, 4.2 Hz, 1H, H-5b), 3.32 (ddd, *J* = 11.5, 9.1, 7.5 Hz, 1H, H-5a), 2.52 (ddt, *J* = 13.1, 9.5, 7.5 Hz, 1H, H-3b), 2.30 – 1.87 (m, 3H, H-3a, CH_2-4).

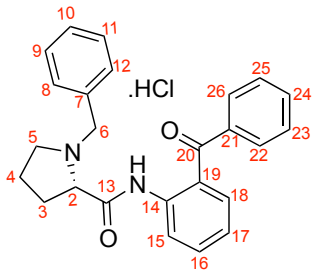
^{13}C NMR (125 MHz, D_2O): δ 173.2, 130.6, 130.1, 130.0, 129.2, 68.4, 58.5, 54.7, 28.7, 22.7.

HRMS (ESI) *(m/z)*: Calc'd for $C_{12}H_{16}NO_2$ $[M+H]^+$: 206.1176, found 206.1174.

FTIR (thin film) cm^{-1}: 3033, 2992, 2969, 1638, 1378, 1314, 1191.

$[\alpha]_D$: -27 (*c* 1.14, H_2O).

(*S*)-*N*-(2-Benzoylphenyl)-1-benzylpyrrolidine-2-carboxamide (BPB) hydrochloride (112):[159]



BPB hydrochloride

To an ice-cooled solution of *N*-methylimidazole (18.4 mL, 0.23 mol, 4 equiv) and methanesulfonyl chloride (4.49 mL, 58.09 mmol, 1 equiv) in dry CH_2Cl_2 (150 mL) was added BP hydrochloride **111** (14 g, 58.09 mmol, 1 equiv) at 0 °C. After stirring for 5 min the reaction was allowed to warm to room temperature. To that mixture, 2-aminobenzophenone (10.29 g, 52.28 mmol, 0.9

equiv) was added and the reaction mixture was left to stir at 50 °C for 24 h. Subsequently, the mixture was quenched with saturated ammonium chloride (95 mL) and the aqueous layer was extracted with CH_2Cl_2 (3 × 265 mL). The combined organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was diluted with acetone (20 mL), acidified with a concentrated hydrochloric acid solution (11 mL) to pH 2, and stirred at room temperature for 3 h. The resulting solid was filtered, washed with ice-cold acetone, and air-dried to yield compound **112** (17.43 g, 71 %) as an off-white solid.

^{1}H NMR (500 MHz, CD_3OD): δ 7.80 – 7.74 (m, 2H, Ar-H), 7.67 – 7.54 (m, 2H, Ar-H), 7.53 – 7.42 (m, 4H, Ar-H), 7.43 – 7.32 (m, 6H, Ar-H), 4.30 (dd, *J* = 19.1, 6.6 Hz, 3H, H-2, CH_2-6), 3.55 (ddd, *J* = 11.4, 7.5, 3.9 Hz, 1H, H-5b), 3.34 – 3.28 (m, 1H, H-5a), 2.45 – 2.33 (m, 1H, H-3b), 2.19 – 2.07 (m, 1H, H-3a), 1.94 – 1.80 (m, 1H, H-4b), 1.64 (dtd, *J* = 13.6, 8.3, 5.5 Hz, 1H, H-4a).

^{13}C NMR (125 MHz, CD_3OD): δ 196.4, 166.0, 137.3, 134.7, 133.0, 132.0, 131.8, 130.7, 130.3, 130.0, 129.8, 129.0, 128.3, 125.7, 124.3, 66.8, 58.1, 54.5, 47.7, 28.3, 22.5.

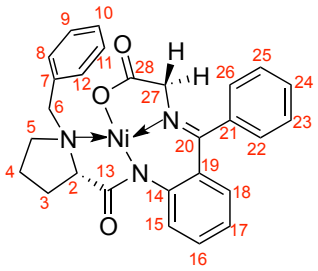
HRMS (ESI) *(m/z)*: Calc'd for $C_{25}H_{24}N_2NaO_2$ $[M+Na]^+$: 407.1730, found 407.1734.

FTIR (thin film) cm^{-1}: 3165, 2960 (br), 1690, 1683, 1606, 1544, 1485, 1449, 1352, 1291, 1266, 754, 699.

$[\alpha]_D$: -49 (*C* 1.0, MeOH).

TLC (25% ethyl acetate, 75% hexane), **R_f:** 0.38 (UV).

Glycine-nickel- (*S*)-*N*-(2-benzoylphenyl)-1-benzylpyrrolidine-2-carboxamide (Gly-Ni-BPB) (113): [159]



Gly-Ni-BPB

To a solution of BPB hydrochloride **112** (10.4 g, 24.6 mmol, 1 equiv) in methanol (100 mL) was added potassium hydroxide (11.7 g, 0.21 mol, 8.5 equiv), glycine (9.2 g, 0.12 mol, 4.8 equiv) and $Ni(NO_3)_2 \cdot 6H_2O$ (14.3 g, 49.3 mmol, 2 equiv) at 50 °C under nitrogen. The suspension was stirred at reflux for 2 h, neutralized with acetic acid (11.9 mL, 0.21 mol, 8.5 equiv) at room temperature and left stirring at that temperature for 15 h. Water (350 mL) was added and the suspension was allowed to stand at room temperature for 2 h. The resulting mixture was extracted with CH_2Cl_2 (2 x 550 mL). The combined organic phases were dried (Na_2SO_4), concentrated under reduced pressure, and further purified by silica gel flash column chromatography (eluents; EtOAc followed by DCM:acetone, 6:4) to give the pure title compound **113** (11.92 g, 97%) as a blood-red solid.

^{1}H NMR (500 MHz, CD_3OD): δ 8.30 (dd, *J* = 8.8, 1.1 Hz, 1H, Ar-H), 8.09 – 8.04 (m, 2H, Ar-H), 7.58 – 7.46 (m, 3H, Ar-H), 7.46 – 7.40 (m, 2H, Ar-H), 7.34 – 7.28 (m, 1H, Ar-H), 7.21 (ddd, *J* = 8.7, 7.0, 1.7 Hz, 1H, Ar-H), 7.12 – 7.07 (m, 1H, Ar-H), 7.01 – 6.95 (m, 1H, Ar-H), 6.80 (dd, *J* = 8.2, 1.7 Hz, 1H, Ar-H), 6.70

(ddd, *J* = 8.2, 6.9, 1.2 Hz, 1H, Ar-H), 4.49 (d, *J* = 12.7 Hz, 1H, H-6b), 3.78 (d, *J* = 20.1 Hz, 1H, H-6a), 3.74 – 3.65 (m, 3H, CH_2-26, H-5b), 3.47 (dd, *J* = 10.8, 5.4 Hz, 1H, H-2), 3.40 – 3.29 (m, 1H, H-4b), 2.62 – 2.53 (m, 1H, H-3b), 2.42 (dddd, *J* = 13.5, 10.7, 9.3, 8.3 Hz, 1H, H-3a), 2.15 (td, *J* = 11.0, 6.1 Hz, 1H, H-4a), 2.07 (dddd, *J* = 16.4, 10.4, 4.9, 2.1 Hz, 1H, H-5a).

^{13}C NMR (125 MHz, CD_3OD): δ 181.3, 177.2, 171.6, 142.5, 134.6, 133.3, 133.1, 132.2, 131.7, 129.7, 129.6, 129.3, 129.1, 128.9, 126.2, 125.7, 125.1, 124.2, 120.8, 69.9, 63.1, 61.2, 57.5, 30.7, 23.6.

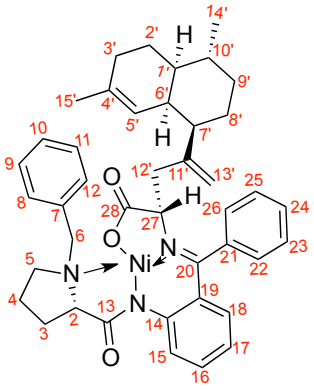
HRMS (ESI) ***(m/z)*:** Calc'd for $C_{27}H_{26}N_3NiO_3$ $[M+H]^+$: 498.1322, found 498.1324.

FTIR (thin film) cm^{-1}: 3051, 2976, 1674, 1638, 1441, 1363, 1337, 1261, 755, 730, 704.

$[\alpha]_D$: 2 ($_C$ 1.08, CH_2Cl_2).

TLC (40% acetone, 60% CH_2Cl_2), **R_f:** 0.5 (UV).

12-Amorpha-4,11-dienyl-(*S*)-glycine-nickel-(*S*)-*N*-(2-benzoylphenyl)-1-benzylpyrrolidine-2-carboxamide (12-Amorpha-4,11-dienyl-(*S*)-glycine-Ni-(*S*)-BPB) (114):



To a stirred suspension of compound **113** (4.85 g, 9.76 mmol, 1 equiv) and finely powdered sodium hydroxide (0.98 g, 24.4 mmol, 2.5 equiv) in acetonitrile (25 mL) was added a CH_2Cl_2 solution (25 mL) of bromide **106** (4.14 g, 14.6 mmol, 1.5 equiv) under an atmosphere of argon. After stirring at reflux for 3 h, the organic volatiles were removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (40 mL). The resulting organic solution was extracted with 0.1 M HCl (2 x 15 mL), dried (Na_2SO_4), concentrated and further purified by silica gel flash column chromatography (eluents; hexane followed by CH_2Cl_2:acetone, 95:5) to give the pure title compound **114** (6.14 g, 89%) as a blood-red solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.08 – 8.02 (m, 2H, Ar-H), 7.56 – 7.46 (m, 2H, Ar-H), 7.47 – 7.39 (m, 1H, Ar-H), 7.34 (t, *J* = 7.7 Hz, 2H, Ar-H), 7.30 (dt, *J* = 6.9, 1.7 Hz, 1H, Ar-H), 7.23 – 7.16 (m, 1H, Ar-H), 7.13 (ddd, *J* = 8.7, 6.9, 1.8 Hz, 1H, Ar-H), 6.95 (dt, *J* = 8.2, 1.4 Hz, 1H, Ar-H), 6.66 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H,

Ar-H), 6.60 (dd, *J* = 8.2, 1.8 Hz, 1H, Ar-H), 5.10 (s, 1H, H-13'b), 5.03 – 4.80 (m, 2H, H-5', H-13'a), 4.44 (d, *J* = 12.7 Hz, 1H, H-6b), 3.98 (dd, *J* = 10.4, 5.2 Hz, 1H, H-27), 3.76 – 3.61 (m, 1H, H-4b), 3.59 (d, *J* = 12.7 Hz, 1H, H-6a), 3.56 – 3.49 (m, 1H, H-5b), 3.46 (dd, *J* = 10.9, 5.9 Hz, 1H, H-2), 3.27 (ddd, *J* = 13.3, 10.4, 7.2 Hz, 1H, H-12'b), 2.86 – 2.75 (m, 1H, H-3b), 2.61 – 2.44 (m, 2H, H-3a, H-12'a), 2.25 – 2.15 (m, 1H, H-4a), 2.12 – 2.02 (m, 1H, H-5a), 1.90 – 1.77 (m, 2H, H-3'b, H-2'b), 1.78 – 1.60 (m, 3H, H-7', H-6', H-3'a), 1.63 – 1.50 (m, 4H, CH_3-15', H-9'b), 1.50 – 1.32 (m, 2H, H-8'b, H-2'a), 1.36 – 1.19 (m, 1H, H-10'), 1.20 – 1.04 (m, 1H, H-8'a), 0.99 – 0.85 (m, 2H, H-9'a, H-1'), 0.81 (d, *J* = 6.5 Hz, 3H, CH_3-14').

^{13}C NMR (125 MHz, $CDCl_3$): δ 180.4, 177.8, 170.0, 146.0, 142.4, 134.7, 133.6, 133.3, 133.2, 132.2, 131.8, 130.0, 129.0, 128.9, 128.8, 127.9, 127.8, 126.7, 124.0, 120.9, 120.6, 118.6, 114.8, 70.4, 69.9, 63.1, 57.1, 44.3, 42.7, 41.5, 37.3, 35.2, 31.0, 27.7, 26.4, 26.0, 25.6, 24.1, 23.8, 19.9.

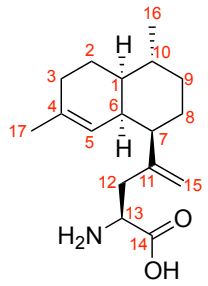
HRMS (ESI) *(m/z)*: Calc'd for $C_{42}H_{48}N_3NiO_3$ $[M+H]^+$: 700.3044, found 700.3032; and Calc'd for $C_{42}H_{47}N_3NaNiO_3$ $[M+Na]^+$: 722.2863, found 722.2858.

FTIR (thin film) cm^{-1}: 3052, 2921, 2867, 1678, 1640, 1441, 1361, 1334, 1260, 1164, 753, 730.

$[\alpha]_D$: 1945 ($_C$ 1.03, CH_2Cl_2).

TLC (5% acetone, 95% CH_2Cl_2), **R_f:** 0.5 (UV).

12-Amorpha-4,11-dienyl-(*S*)-glycine (80):



The nickel complex **114** (0.5 g, 0.7 mmol) was dissolved in a MeOH-CH_2Cl_2 mixture (7 mL, 2:1) to which was added 3 M HCl (4.5 mL) and MeOH (3 mL). The red mixture was heated at 60 °C for 30 min, at which point it turned green. The solvent was evaporated, and water (9 mL) was added to the residue, followed by neutralization with 3 M NaOH (4.5 mL). This was extracted with CH_2Cl_2 (3 x 7 mL). The aqueous layer was basified with 3 M NaOH (1 mL) and re-extracted with CH_2Cl_2 (2 x 6 mL). The combined organic layers (gelatinous at low volumes) were dried over sodium sulfate and the solvent was removed. The residue was dissolved in MeOH (2 mL) to which was added diethyl ether (10 mL). This was stored at 4 °C overnight, at which point, a fine precipitate appeared. This precipitate was collected by filtration, and dried to provide the desired amino acid **80** (45 mg, 23%).

^{1}H NMR (600 MHz, D_2O): δ 5.10-5.07 (m, 2H), 4.95-4.93 (m, 1H), 3.72 (dd, 1H, *J*=7.9, 5.1 Hz), 2.72 (dd, 1H, *J*=15.3, 5.1 Hz), 2.64-2.60 (m, 1H), 2.54 (dd, 1H, *J*=15.3, 7.9 Hz), 2.25-2.14 (m, 1H), 2.08-1.94 (m, 1H), 1.96-1.85 (m, 1H), 1.80 (dd, 1H, *J*=17.8, 5.7 Hz), 1.69 (qd, 1H, *J*=12.8, 3.4 Hz), 1.64-1.53 (m, 4H), 1.48-1.37 (m, 2H), 1.31 (qd, 1H, *J*=12.6, 3.2 Hz), 1.05 (qd, 1H, *J*=12.2, 3.2 Hz), 0.91 (d, 3H, *J*=6.24 Hz).

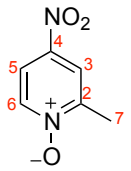
^{13}C NMR (125 MHz, D_2O): δ 172.8, 146.4, 134.7, 120.1, 112.1, 46.4, 41.7, 37.4, 36.2, 35.0, 27.7, 27.6, 25.9, 25.6, 25.4, 22., 18.7.

HRMS (ESI) *(m/z)*: Calc'd for $C_{17}H_{27}NO_2Na$ $[M+Na]^+$: 300.1934, found 300.1931

FTIR (thin film) cm^{-1}: 3085.4, 2922.3, 2868.1, 1635.3, 1505.8, 1395.0, 1323.6, 1109.2, 898.6, 675.7.

$[\alpha]_D$: 22.0 (*c* 1.10, MeOH).

4-Nitro-(2-picoline)-*N*-oxide (148):[212]



To a solution of (2-methyl)pyridine-*N*-oxide (25 g, 0.26 mol, 1 equiv) in H_2SO_4 (88 mL, 1.64 mol, 6.3 equiv) was added 90% fuming HNO_3 (77 mL, 1.52 mol, 5.9 equiv) slowly at 0 °C, which was then stirred at 100 °C for 2 h. After cooling at 10 °C, the resulting mixture was poured into crushed ice (~ 0.3 g), neutralized with Na_2CO_3, and extracted with $CHCl_3$ (3 x 100 mL). The combined organic layers were dried (Na_2SO_4) and evaporated under reduced pressure to yield title compound **148** (36.4 g, 92%) as a pale yellow solid.

1H NMR (500 MHz, $CDCl_3$): δ 8.29 (d, *J* = 7.2 Hz, 1H, H-6), 8.11 (d, *J* = 3.2 Hz, 1H, H-3), 7.97 (dd, *J* = 7.1, 3.2 Hz, 1H, H-5), 2.53 (s, 3H, CH_3-7).

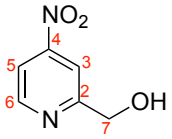
13C NMR (125 MHz, $CDCl_3$): 150.8, 141.9, 140.2, 120.8, 118.3, 18.2.

HRMS (EI) (*m/z*): Calc'd for $C_6H_7N_2O_3$ $[M+H]^+$: 155.0451, found 155.0447.

FTIR (thin film) cm^{-1}: 3117, 3083, 3053, 3002, 1613, 1582, 1501, 1464, 1324, 1096.

TLC (90% CH_2Cl_2, 10% MeOH), **R_f:** 0.6 (UV).

4-Nitro-2-pyridinemethanol (150): [210, 212]



To a yellow solution of 4-nitro-2-picoline-*N*-oxide (**148**) (36 g, 0.23 mol, 1 equiv) in dry dichloromethane (550 mL) was added a solution of trifluoroacetic anhydride (99.5 mL, 0.7 mol, 3 equiv) in dichloromethane (140 mL) dropwise at room temperature. The resulting red solution was stirred at room temperature for 3 days under argon. Organic volatiles were removed and methanol (780 mL) and a saturated $K_2CO_{3(aq)}$ solution (390 mL) were added, and the mixture was stirred at room temperature for 4 h. Methanol was evaporated, and the compound was extracted with dichloromethane (3 x 900 mL). Combined organic layers were washed with brine solution (3 x 900 mL) and dried over Na_2SO_4. The organic volatiles were removed under reduced pressure and the residue was purified on silica gel by flash column chromatography (eluents; EtOAc:diethyl ether, 50:50) to give yellow solid **150** (17.5 g, 55%).

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.83 (d, *J* = 5.4 Hz, 1H, H-6), 8.09 (d, *J* = 2.0 Hz, 1H, H-3), 7.92 (dd, *J* = 5.5, 2.1 Hz, 1H, H-5), 4.91 (s, 2H, CH_2-6), 3.67 (s, 1H, -OH).

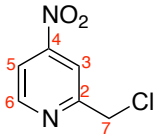
^{13}C NMR (125 MHz, $CDCl_3$): δ 163.8, 154.7, 151.3, 115.2, 113.5, 64.7.

HRMS (EI) *(m/z)*: Calc'd for $C_6H_7N_2O_3$ $[M+H]^+$: 155.0451, found 155.0449.

FTIR (thin film) cm^{-1}: 3266, 3058, 3030, 2962, 2928, 1582, 1539, 1353, 1046.

TLC (50% ethyl acetate, 50% ether), **Rf:** 0.4 (UV).

4-Nitro-2-(chloromethyl)pyridine (145): [212]



To a solution of pyridine-methanol **150** (2 g, 13 mmol, 1 equiv) in CH_2Cl_2 (20 mL) was added thionyl chloride (2.84 mL, 39 mmol, 3 equiv) slowly at room temperature, which was then stirred at reflux for 3 h. The solvent was then evaporated, and the residue was basified with a 2 N Na_2CO_3 aqueous solution. The product was extracted with CH_2Cl_2 (3 x 30 mL). The combined organic layers were dried (Na_2SO_4) and evaporated under reduced pressure to yield the title compound **145** (2.05 g, 92%) as a yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.88 (dd, *J* = 5.4, 0.7 Hz, 1H, H-6), 8.32 – 8.21 (m, 1H, H-3), 7.99 (dd, *J* = 5.4, 2.1 Hz, 1H, H-5), 4.81 (s, 2H, CH_2-7).

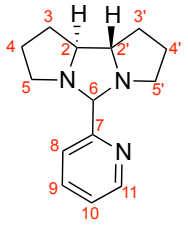
^{13}C NMR (125 MHz, $CDCl_3$): δ 160.3, 154.9, 151.9, 115.9, 115.7, 45.9.

TLC (50% **HRMS (EI) *(m/z)*:** Calc'd for $C_6H_5N_2O_2Cl[M+H]^+$: 174.0010, found 173.9998.

FTIR (thin film) cm^{-1}: 3094, 2964, 1576, 1534, 1357, 1242, 763.

ethyl acetate, 50% ether), **R_f:** 0.7 (UV).

(*S*,*S*)-2, 2'-Bispyrrolidine 2-pyridine carboxaldehyde aminal (151):



To a stirred solution of (*S*,*S*)-2, 2'-bispyrrolidine (1.66 g, 11.85 mmol, 1 equiv) in dry diethyl ether (25 mL) was added 4 Å molecular sieves (~1.5 g) and 2-pyridine carboxaldehyde (1.13 mL, 11.85 mmol, 1 equiv). The resulting mixture was stirred overnight at room temperature under argon. K_2CO_3 (~2 g) was added and the mixture was filtered through Celite. Volatiles were removed to give the crude aminal **151** as pale yellow oil (2.47 g, 91%), which was reasonably pure and used further for the next step without any purification.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.62 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 1H, H-11), 7.71 – 7.59 (m, 2H, H-8, H-9), 7.21 – 7.14 (m, 1H, H-10), 4.86 (s, 1H, H-6), 3.46 – 3.31 (m, 2H, H-2, H-2'), 3.01 – 2.92 (m, 1H, H-5b), 2.63 – 2.54 (m, 2H, H-5a, H-5'b), 2.31 (ddd, *J* = 9.4, 7.2, 2.9 Hz, 1H, H-5'a), 2.17 – 2.07 (m, 1H, H-3'b), 1.98 – 1.69 (m, 6H, CH_2-4, H-3b, CH_2-4', H-3'a), 1.66 – 1.56 (m, 1H, H-3a).

^{13}C NMR (100 MHz, $CDCl_3$): δ 159.7, 149.8, 136.5, 122.7, 122.5, 88.6, 71.5, 71.2, 52.2, 47.7, 30.9, 29.2, 25.9, 25.1.

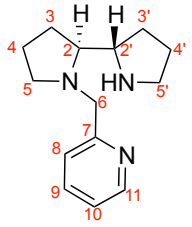
HRMS (ESI) *(m/z)*: Calc'd for $C_{14}H_{20}N_3$ $[M+H]^+$: 230.1652, found 230.1655.

FTIR (thin film) cm^{-1}: 3053, 2956, 2870, 1584, 1468, 1434, 1372.

$[\alpha]_D$: 182 ($_C$ 0.83, CH_2Cl_2).

TLC (4% MeOH, 4% NH_4OH, 92% DCM), **R_f:** 0.5 (UV, CAM).

(*S,S*)-*N*- (2-Picolyl)-2, 2'-bispyrrolidine (152):



To a stirred solution of aminal **151** (0.65 g, 2.84 mmol, 1 equiv) in methanol (10 mL) was added $NaBH_4$ (0.32 g, 8.52 mmol, 3 equiv), in portions at 0 ^{o}C, and acetic acid (1.3 mL, 22.7 mmol, 8 equiv). The solution was stirred at that temperature for 3 h and diluted with diethyl ether (10 mL), 30% $NaOH_{(aq)}$ (5 mL) and water (5 mL). The solution was decanted and the aqueous phase was extracted with EtOAc (2 x 20 mL). The combined organic phases were dried (Na_2SO_4), concentrated under reduced pressure, and further purified by silica gel by flash column chromatography (eluents; THF:MeOH:NH_4OH, 90:5:5) to give colorless oil **152** (0.52 g, 79%).

^{1}H NMR (600 MHz, $CDCl_3$): δ 8.55 – 8.49 (m, 1H, H-11), 7.63 (td, *J* = 7.7, 1.8 Hz, 1H, H-9), 7.32 (d, *J* = 7.8 Hz, 1H, H-8), 7.18 – 7.11 (m, 1H, H-10), 4.19 (d, *J* = 14.6 Hz, 1H, H-6b), 3.71 (d, *J* = 14.6 Hz, 1H, H-6a), 3.23 (q, *J* = 7.5 Hz, 1H, H-2), 3.04 (dt, *J* = 10.2, 5.9 Hz, 2H, H-5b, H-5'b), 2.98 (ddd, *J* = 10.5, 7.7, 5.8 Hz, 1H, H-5a), 2.81 (td, *J* = 8.2, 4.5 Hz, 1H, H-2'), 2.47 (dt, *J* = 10.2, 7.6 Hz, 1H, H-5'a), 1.96 – 1.69 (m, 6H, CH_2-4, 3b, CH_2-4', H-3'b), 1.60 – 1.51 (m, 1H, H-3'a), 1.46 (ddt, *J* = 12.3, 8.7, 7.1 Hz, 1H, H-3a).

^{13}C NMR (100 MHz, $CDCl_3$): δ 160.6, 149.2, 136.4, 122.9, 121.8, 68.2, 64.1, 62.7, 55.2, 46.6, 28.4, 28.3, 25.0, 24.0.

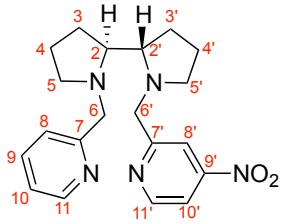
HRMS (ESI) *(m/z)*: Calc'd for $C_{14}H_{22}N_3$ $[M+H]^+$: 232.1808, found 232.1806.

FTIR (thin film) cm^{-1}: 3354, 3177, 2962, 2807, 1677, 1590, 1432, 1391.

[α]$_D$: -17 ($_C$ 1.48, CH_2Cl_2).

TLC (5% MeOH, 5% NH_4OH, 90% THF), **R_f:** 0.4 (UV, CAM).

(*S,S*)-[*N*-(2-Picolyl)-*N*'-(4-nitro-2-picolyl)]-2, 2'-bispyrrolidine (153):



Monoalkylated (*S,S*)-bispyrrolidine **152** (0.89 g, 3.81 mmol, 1 equiv), tetrabutylammonium iodide (1.41 g, 3.81 mmol, 1 equiv), and lithium hydroxide monohydrate (0.16 g, 3.9 mmol, 1.02 equiv) were dissolved in dry CH_2Cl_2 (10 mL). To that, a dry CH_2Cl_2 solution (10 mL) of 4-nitro-2-(chloromethyl)pyridine (**145**) (0.66 g, 3.81 mmol, 1 equiv) was added. After stirring the resulting mixture for 15 h at room temperature, the organic volatiles were removed under reduced pressure and the residue was purified on silica gel by flash column chromatography (eluents; THF:MeOH:NH_4OH, 96:2:2) to give dark yellow oil **153** (1.07 g, 76%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.76 (d, *J* = 5.4 Hz, 1H, H-11'), 8.47 (ddd, *J* = 4.9, 1.9, 0.9 Hz, 1H, H-11), 8.12 (d, *J* = 2.2 Hz, 1H, H-8'), 7.81 (dd, *J* = 5.4, 2.2 Hz, 1H, H-10'), 7.58 (td, *J* = 7.7, 1.8 Hz, 1H, H-9), 7.37 (d, *J* = 7.9 Hz, 1H, H-8), 7.13 – 7.05 (m, 1H, H-10), 4.36 (d, *J* = 15.5 Hz, 1H, H-6'b), 4.17 (d, *J* = 14.4 Hz, 1H, H-6b), 3.67 (d, *J* = 15.5 Hz, 1H, H-6'a), 3.56 (d, *J* = 14.4 Hz, 1H, H-6a), 3.06

– 2.96 (m, 2H, H-5b, H-5'b), 2.82 (q, J = 8.5, 7.9 Hz, 2H, H-2, H-2'), 2.32 – 2.20 (m, 2H, H-5a, H-5'a), 1.91 – 1.65 (m, 8H, CH_2-4, CH_2-3, CH_2-4', CH_2-3').

^{13}C NMR (100 MHz, $CDCl_3$): δ 165.3,160.5, 154.8, 151.2, 149.2, 136.5, 122.8, 121.9, 115.1, 114.3, 66.2, 66.1, 61.6, 61.2, 55.7, 55.6, 28.6, 26.5, 24.0, 23.9.

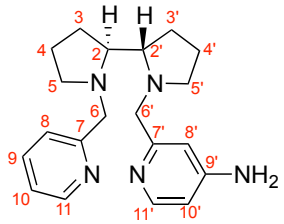
HRMS (ESI) *(m/z)*: Calc'd for $C_{20}H_{26}N_5O_2[M+H]^+$: 368.2081, found 368.2077.

FTIR (thin film) cm^{-1}: 3063, 2962, 2873, 2802, 1588, 1571, 1530, 1354.

$[\alpha]_D$: -5 ($_C$ 0.95, CH_2Cl_2).

TLC (4% MeOH, 4% NH_4OH, 92% THF), **R_f:** 0.6 (UV, CAM).

(*S*,*S*)-[*N*-(2-Picolyl)-*N'*-(4-amino-2-picolyl)]-2, 2'-bispyrrolidine (154):



To a cooled solution of $TiCl_4$ (16.5 mL in 1 M CH_2Cl_2, 16.5 mmol, 3 equiv) in dry THF (50 mL) was added $LiAlH_4$ (6 mL in 2 M THF, 12 mmol, 2.2 equiv) drop by drop at 0 ^oC. The resulting black suspension was stirred at room temperature for 15 minutes. To that stirred Ti(0) slurry, nitroamine **153** (2.0 g, 5.5 mmol, 1 equiv) was added at 0 ^oC, and the reaction mixture was allowed to stir for 20 minutes at room temperature. The reaction mixture was quenched with NH_4OH (10 mL), and the organic volatiles were removed under reduced pressure. The obtained residue was dissolved in CH_2Cl_2 (20 mL) and extracted with water (2 x 10 mL), dried (Na_2SO_4), concentrated and further purified on silica gel by

flash column chromatography (eluents; THF:MeOH:NH_4OH, 98:1:1 to 96:2:2) to give dark yellow oil **154** (1.47 g, 80%).

^{1}H NMR (600 MHz, $CDCl_3$): δ 8.49 – 8.44 (m, 1H, H-11), 8.06 (d, *J* = 5.6 Hz, 1H, H-11'), 7.59 (td, *J* = 7.7, 1.8 Hz, 1H, H-9), 7.38 (d, *J* = 7.8 Hz, 1H, H-8), 7.12 – 7.07 (m, 1H, H-10), 6.64 (d, *J* = 2.3 Hz, 1H, H-8'), 6.35 (dd, *J* = 5.6, 2.4 Hz, 1H, H-10'), 4.23 (s, 2H, -NH_2), 4.18 (d, *J* = 14.3 Hz, 1H, H-6'b), 4.01 (d, *J* = 14.3 Hz, 1H, H-6b), 3.49 (d, *J* = 14.3 Hz, 1H, H-6'a), 3.34 (d, *J* = 14.3 Hz, 1H, H-6a), 3.03 – 2.94 (m, 2H, H-5'b, H-5b), 2.79 – 2.70 (m, 2H, H-2, H-2'), 2.24 – 2.16 (m, 2H, H-5'a, H-5a), 1.84 – 1.63 (m, 8H, CH_2-4, CH_2-3, CH_2-4', CH_2-3').

^{13}C NMR (100 MHz, $CDCl_3$): δ 172.8, 160.2, 154.3, 148.9, 148.0, 136.6, 123.0, 121.9, 108.3, 108.2, 66.9, 65.9, 61.5, 60.7, 55.3, 55.2, 26.6, 26.4, 24.0, 23.8.

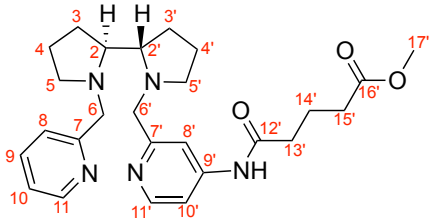
HRMS (ESI) *(m/z)*: Calc'd for $C_{20}H_{28}N_5$ $[M+H]^+$: 338.2339, found 338.2336.

FTIR (thin film) cm^{-1}: 3328, 3182, 2963, 2874, 2805,1670, 1604, 1434, 1120.

$[\alpha]_D$: -58.2 ($_C$ 0.8, CH_2Cl_2).

TLC (4% MeOH, 4% NH_4OH, 92% THF), **R_f:** 0.7 (UV, CAM).

(*S*,*S*)-[*N*-(2-Picolyl) - *N'*-(2-picolyl-4-amidoglutarate)]-2, 2'-bispyrrolidine (141):



To a stirred mixture of amine **154** (0.96 g, 2.84 mmol, 1 equiv), Et_3N (1.18 mL, 8.49 mmol, 3 equiv) and DMAP (0.068 g, 0.56 mmol, 0.2 equiv) in dry

CH_2Cl_2 (10 mL) was added methyl glutaryl chloride (0.59 mL, 4.26 mmol, 1.5 equiv) at room temperature. The mixture was stirred at 45 ^oC for 2 h. The organic volatiles were removed under reduced pressure and the residue was purified by flash column chromatography (eluents; THF:MeOH:NH_4OH, 98:1:1 to 92:4:4) to give dark yellow oil **141** (0.74 g, 56%).

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.47 (dt, *J* = 4.9, 1.2 Hz, 1H, H-11'), 8.35 (d, *J* = 5.6 Hz, 1H, H-11), 7.67 – 7.56 (m, 2H, H-9, H-10), 7.40 (d, *J* = 7.8 Hz, 1H, H-8), 7.30 (s, 1H, H-8'), 7.18 – 7.07 (m, 1H, H-10'), 4.24 (d, *J* = 14.7 Hz, 1H, H-6'b), 4.11 (d, *J* = 14.4 Hz, 1H, H-6b), 3.69 (s, 3H, CH_3-17'), 3.57 (d, *J* = 14.7 Hz, 1H, H-6'a), 3.51 (d, *J* = 14.5 Hz, 1H, H-6a), 3.07 – 2.96 (m, 2H, H-5b, H-5'b), 2.75 (dq, *J* = 10.7, 5.3 Hz, 2H, H-2, H-2'), 2.45 (dt, *J* = 10.7, 7.1 Hz, 4H, CH_2-13', CH_2-15'), 2.38 – 2.20 (m, 2H, H-5a, H-5'a), 2.05 (p, *J* = 7.2 Hz, 2H, H-14'), 1.90 – 1.64 (m, 8H, CH_2-4, CH_2-3, CH_2-4', CH_2-3').

^{13}C NMR (100 MHz, $CDCl_3$): δ 173.70, 171.4, 162.3, 158.5, 149.7, 148.6, 145.8, 136.5, 122.8, 121.8, 112.2, 112.1, 66.1, 61.3, 55.4, 55.2, 51.7, 36.4, 32.9, 26.5, 26.4, 23.7, 20.5.

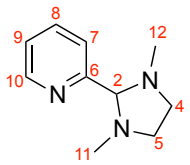
HRMS (ESI) *(m/z)*: Calc'd for $C_{20}H_{28}N_5$ $[M+H]^+$: 338.2339, found 338.2336.

FTIR (thin film) cm^{-1}: 3350, 3167, 2956, 2804, 1736, 1669, 1522, 1434, 1396, 1205, 1152.

$[\alpha]_D$: -34 ($_C$ 1.0, CH_2Cl_2).

TLC (4% MeOH, 4% NH_4OH, 92% THF), **R_f:** 0.8 (UV, CAM).

2-(*N, N'*-Dimethylimidazolidine-2-yl)pyridine (156):



To a stirred solution of *N, N'*-dimethylethylenediamine (2.64 g, 30 mmol, 1 equiv) in dry THF (50 mL) was added 4 Å molecular sieves (~ 3 g) and 2-pyridine carboxaldehyde (2.85 mL, 30 mmol, 1 equiv). The resulting mixture was stirred overnight at room temperature under argon, then K_2CO_3 was added and the mixture was filtered through Celite. Volatiles were removed to give the crude aminal **156** as pale yellow oil (4.94 g, 93%), which was used for the next step without any further purification.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.65 – 8.48 (m, 1H, H-10), 7.82 – 7.60 (m, 2H, H-7, H-8), 7.21 (ddd, *J* = 8.4, 4.2, 1.9 Hz, 1H, H-9), 3.56 – 3.45 (m, 1H, H-2), 3.48 – 3.29 (m, 2H, H-5b, H-4b), 2.71 – 2.52 (m, 2H, H-5a, H-4a), 2.36 – 2.20 (m, 6H, CH_3-12, CH_3-11).

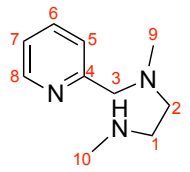
^{13}C NMR (100 MHz, $CDCl_3$): δ 160.8, 148.7, 137.1, 123.5, 122.7, 92.9, 53.9, 40.0.

HRMS (ESI) *(m/z)*: Calc'd for $C_{10}H_{15}N_3$ $[M+H]^+$: 177.12660, found 177.12622.

FTIR (thin film) cm^{-1}: 3054, 3013, 2971, 2943, 2842, 2780, 1590, 1471, 1436, 1361.

TLC (10% NH_4OH, 90% MeCN), **R_f:** 0.5 (UV, CAM, I_2).

***N*, *N'*-Dimethyl-[*N'*-(2-picolyl)]ethane-1,2-diamine (158):**[217]



The title compound was obtained using one of the following protocols. Protocol 1 avoids an excessive use of diamine and bisalkylated product, while protocol 2 uses an excess of diamine, but offers the better yield.

Protocol 1:

To a stirred solution of aminal **156** (0.2 g, 1.12 mmol, 1 equiv) in methanol (5 mL) was first added $NaBH_4$ (0.38 g, 10.1 mmol, 9 equiv) in portions at 0 ^oC, and then acetic acid (1.56 mL, 26.9 mmol, 24 equiv). The solution was stirred at room temperature for 12 h and diluted with diethyl ether (5 mL), 30% $NaOH_{(aq)}$ (5 mL) and water (5 mL). The solution was decanted and the aqueous phase was extracted with EtOAc (2 x 10 mL). The combined organic phases were dried (Na_2SO_4), concentrated under reduced pressure, and further purified on a silica gel by flash column chromatography (eluents; MeCN:NH_4OH, 90:10) to give the title product **158** as a light yellow oil (0.13 g, 44%).

Protocol 2:[218]

To a stirred solution of *N, N'*-dimethylethylenediamine (8.82 g, 0.1 mol, 5 equiv) in acetonitrile (150 mL) was added 2-(chloromethyl)pyridine hydrochloride (3.28 g, 0.02 mol, 1 equiv) and K_2CO_3 (13.8 g, 0.1 mol, 5 equiv) at room temperature. After stirring at 60 °C for 24 h, the organic volatiles were removed under reduced pressure and the residual liquid was dissolved in CH_2Cl_2 (70 mL). The resulting

organic solution was extracted with saturated aqueous Na_2CO_3 (2 x 60 mL), dried (Na_2SO_4), concentrated and further purified on a silica gel by flash column chromatography (eluents; MeCN:NH_4OH ,90:10) to give the title product **158** as light yellow oil (2.45 g, 68%).

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.56 – 8.49 (m, 1H, H-8), 7.68 – 7.59 (m, 1H, H-6), 7.39 (d, *J* = 7.9 Hz, 1H, H-5), 7.17 – 7.10 (m, 1H, H-7), 3.66 (d, *J* = 2.6 Hz, 2H, CH_2-3), 2.71 – 2.64 (m, 2H, H-1b, H-2b), 2.61 – 2.55 (m, 2H, H-1a, H-2a), 2.40 (s, 3H, CH_3-9), 2.26 (d, *J* = 3.7 Hz, 3H, CH_3-10), 2.04 (s, 1H, -NH-).

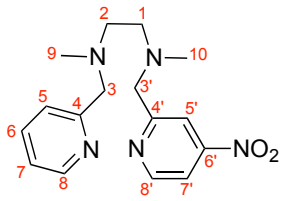
^{13}C NMR (100 MHz, $CDCl_3$): δ 159.4, 149.3, 136.8, 123.3, 122.4, 63.7, 56.1, 49.0, 42.9, 35.8.

HRMS (ESI) *(m/z)*: Calc'd for $C_{10}H_{18}N_3$ $[M+H]^+$: 180.1495, found 180.1494.

FTIR (thin film) cm^{-1}: 3323, 3053, 2971, 2943, 2841, 2779, 1590, 1453, 1361, 1035.

TLC (10% NH_4OH, 90% MeCN), **R_f:** 0.4 (UV, CAM, I_2).

***N*, *N'*-Dimethyl-[*N*-(2-picolyl)- *N'*-(4-nitro-2-picolyl)]ethane-1,2-diamine (159):**



N-2-picolyl-*N,N'*-dimethylethylenediamine (**158**) (1.97 g, 11 mmol, 1 equiv), tetrabutylammonium iodide (4.06 g, 11 mmol, 1 equiv), and lithium hydroxide monohydrate (0.47 g, 11.22 mmol, 1.02 equiv) were dissolved in dry

CH_2Cl_2 (10 mL). To that, a CH_2Cl_2 solution (25 mL) of 4-nitro-2-(chloromethyl)pyridine (**145**) (1.89 g, 11 mmol, 1 equiv) was added. After stirring the resulting mixture for 15 h at room temperature, the organic volatiles were removed under reduced pressure and the residue was purified on silica gel by flash column chromatography (eluents; THF:MeOH:NH_4OH, 96:2:2) to give dark yellow oil **159** (2.45 g, 71%).

^{1}H NMR (600 MHz, $CDCl_3$): δ 8.80 (d, *J* = 5.4 Hz, 1H, H-8'), 8.64 – 8.42 (m, 1H, H-8), 8.21 (d, *J* = 2.1 Hz, 1H, H-5'), 7.86 (dd, *J* = 5.5, 2.1 Hz, 1H, H-7'), 7.63 (td, *J* = 7.7, 1.7 Hz, 1H, H-6), 7.42 (d, *J* = 7.8 Hz, 1H, H-5), 7.26 – 7.04 (m, 1H, H-7), 3.82 (s, 2H, CH_2-3'), 3.70 (s, 2H, CH_2-3), 2.79 – 2.55 (m, 4H, CH_2-2, CH_2-1), 2.29 (s, 6H, CH_3-10, CH_3-9).

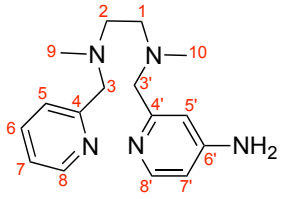
^{13}C NMR (100 MHz, $CDCl_3$): δ 164.1, 159.3, 154.6, 151.2, 149.2, 136.5, 123.2, 122.1, 115.4, 114.5, 64.3, 63.6, 55.7, 55.6, 43.1, 43.0.

HRMS (ESI) *(m/z)*: Calc'd for $C_{16}H_{22}N_5O_2[M+H]^+$: 316.1768, found 316.1765.

FTIR (thin film) cm^{-1}: 3167, 2947, 2841, 2800, 1680, 1533, 1355, 1036.

TLC (4% MeOH, 4% NH_4OH, 92% THF), **R_f:** 0.6 (UV, CAM).

***N*, *N'*-Dimethyl-[*N*-(2-picolyl)- *N'*-(4-amino-2-picolyl)]ethane-1,2-diamine (160):**



To a cooled solution of $TiCl_4$ (8.6 mL in 1 M CH_2Cl_2, 8.6 mmol, 3 equiv) in dry THF (30 mL) was added $LiAlH_4$ (3.15 mL as a 2 M THF solution, 6.3 mmol, 2.2 equiv) drop by drop at 0 ^oC. The resulting black suspension was stirred at room temperature for 15 minutes. To that stirred Ti(0) slurry, nitro amine **159** (0.9 g, 2.86 mmol, 1 equiv) was added at 0 ^oC, and the reaction mixture was allowed to stir for 20 minutes at room temperature. The reaction mixture was quenched with NH_4OH (7 mL), and the organic volatiles were removed under reduced pressure. The obtained residue was dissolved in CH_2Cl_2 (15 mL) and extracted with water (2 x 5 mL), dried (Na_2SO_4), concentrated and further purified by on silica gel by flash column chromatography (eluents; THF:MeOH:NH_4OH, 98:1:1 to 92:4:4) to give dark yellow oil **160** (0.62 g, 76%).

^{1}H NMR (600 MHz, $CDCl_3$): δ 8.53 (dt, *J* = 4.8, 1.2 Hz, 1H, H-8), 8.12 (d, *J* = 5.6 Hz, 1H, H-8'), 7.63 (td, *J* = 7.7, 1.8 Hz, 1H, H-6), 7.41 (d, *J* = 7.8 Hz, 1H, H-5), 7.17 – 7.10 (m, 1H, H-7), 6.71 (d, *J* = 2.3 Hz, 1H, H-5'), 6.39 (dd, *J* = 5.6, 2.4 Hz, 1H, H-7'), 4.13 (s, 2H, -NH_2), 3.68 (s, 2H, CH_2-3'), 3.54 (s, 2H, CH_2-3), 2.63 (s, 4H, CH_2-2, CH_2-1), 2.26 (d, *J* = 3.6 Hz, 6H, CH_3-10, CH_3-9).

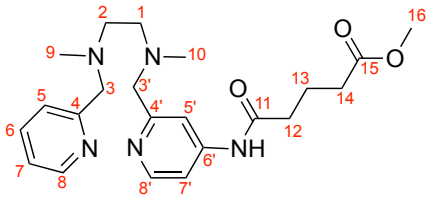
^{13}C NMR (100 MHz, $CDCl_3$): δ 159.4, 154.0, 151.7, 149.2, 148.7, 136.6, 123.4, 122.2, 108.5, 108.4, 64.2, 63.7, 55.7, 55.2, 43.1, 42.9.

HRMS (ESI) *(m/z)*: Calc'd for $C_{16}H_{24}N_5$ $[M+H]^+$: 286.2026, found 286.2019.

FTIR (thin film) cm^{-1}: 3328, 3189, 2948, 2802, 1604, 1454, 1363, 1032.

TLC (4% MeOH, 4% NH_4OH, 92% THF), **R_f:** 0.4 (UV, CAM).

***N*, *N'*-Dimethyl-[*N*-(2-picolyl)- *N'*-(2-picolyl-4-amidoglutarate)]ethane-1,2-diamine (142):**



To a stirred mixture of amine **160** (0.49 g, 1.73 mmol, 1 equiv), Et_3N (0.72 mL, 5.16 mmol, 3 equiv) and DMAP (0.042 g, 0.34 mmol, 0.2 equiv) in dry CH_2Cl_2 (5 mL) was added methyl glutaryl chloride (0.36 mL, 2.6 mmol, 1.5 equiv) at room temperature. The mixture was stirred at 45 ^oC for 2 h. The organic volatiles were removed under reduced pressure and the residue was purified on silica gel by flash column chromatography (eluents; THF:MeOH:NH_4OH, 98:1:1 to 92:4:4) to give dark yellow oil **142** (0.44 g, 62%).

^{1}H NMR (600 MHz, $CDCl_3$): δ 8.64 (s, 1H, -NHCO-), 8.52 (ddt, *J* = 4.9, 1.6, 0.8 Hz, 1H, H-8'), 8.39 (d, *J* = 5.6 Hz, 1H, H-8), 7.71 – 7.66 (m, 1H, H-7), 7.68 – 7.62 (m, 1H, H-6), 7.44 – 7.40 (m, 2H, H-5, H-5'), 7.17 (dddd, *J* = 7.5, 4.9, 1.2, 0.6 Hz, 1H, H-7'), 3.71 (s, 2H, CH_2-3'), 3.67 (s, 3H, CH_3-16), 3.62 (s, 2H, CH_2-3), 2.67 – 2.57 (m, 4H, CH_2-2, CH_2-1), 2.44 (dt, *J* = 11.6, 7.2 Hz, 4H, CH_2-12, CH_2-14), 2.25 (d, *J* = 20.5 Hz, 6H, CH_3-10, CH_3-9), 2.07 – 1.99 (m, 2H, CH_2-13).

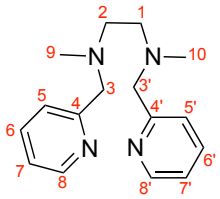
^{13}C NMR (100 MHz, $CDCl_3$): δ 173.8, 172.0, 159.9, 159.0, 149.9, 149.0, 146.4, 136.8, 123.6, 122.3, 112.7, 112.4, 64.0, 63.8, 55.2, 55.1, 51.7, 42.8, 42.6, 36.4, 33.0, 20.6.

HRMS (ESI) *(m/z)*: Calc'd for $C_{22}H_{32}N_5O_3$ $[M+H]^+$: 414.2500, found 414.2503.

FTIR (thin film) cm^{-1}: 3165, 3064, 2957, 2802, 1736, 1702, 1598, 1434, 1212, 766.

TLC (4% MeOH, 4% NH_4OH, 92% THF), **R_f:** 0.7 (UV, CAM).

***N*, *N'*-Dimethyl-[*N, N'*-di(2-picolyl)]ethane-1,2-diamine (picenMe_2) (132):** [222-223]



To a solution of *N,N'*-dimethylethylenediamine (1.01 g, 11.5 mmol, 1 equiv) in acetonitrile (30 mL), 2-(chloromethyl)pyridine hydrochloride (3.77 g, 23.02 mmol, 2 equiv) and anhydrous K_2CO_3 (9.54 g, 69.05 mmol, 6 equiv) were added. The solution was heated to 60 ^oC for 24 h, under argon. The organic volatiles were removed under reduced pressure and the remaining yellow liquid was dissolved in 10 mL of methylene chloride and washed with saturated aqueous Na_2CO_3 (2 x 10 mL). The organic phase was dried (Na_2SO_4), then concentrated under reduced pressure to give the title product **132** as a light yellow oil (2.85 g, 92%). No additional purification was required.

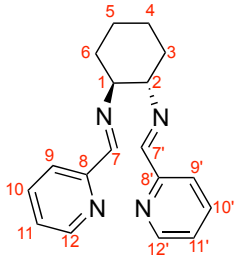
^{1}H NMR (500 MHz, $CDCl_3$): δ 8.52 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 2H, H-8, H-8'), 7.61 (td, *J* = 7.6, 1.8 Hz, 2H, H-6, H-6'), 7.39 (dt, *J* = 7.9, 1.1 Hz, 2H, H-5, H-5'), 7.12 (ddd, *J* = 7.5, 4.9, 1.2 Hz, 2H, H-7, H-7'), 3.66 (s, 4H, CH_2-3, CH_2-3'), 2.63 (s, 4H, CH_2-2, CH_2-1), 2.26 (s, 6H, CH_3-10, CH_3-9).

^{13}C NMR (125 MHz, $CDCl_3$): δ 159.3, 149.0, 136.3, 123.0, 121.9, 64.1, 55.4, 42.8.

HRMS (ESI) *(m/z)*: Calc'd for $C_{16}H_{22}N_4Na$ $[M+Na]^+$: 293.1737, found 293.1738.

FTIR (thin film) cm^{-1}: 3008, 2947, 2839, 2802, 1589, 1473, 1433, 1046, 735.

***(1S,2S)-N,N'*-Di(2-picolyl)-1,2-cyclohexanediimine (169):** [224]



To a solution of 2-pyridine carboxaldehyde (0.94 g, 8.8 mmol, 2 equiv) in MeOH (10 mL) was added (*S,S*)-1,2-cyclohexanediamine (0.457 g, 4.0 mmol) and $CaSO_4$ (1.5 g) at room temperature. After stirring for 3 h at room temperature, the mixture was filtered and the volatiles evaporated *in vacuo* to afford an oily liquid. 10 mL of Et_2O was added to that oily liquid and left at -20 ^oC overnight to produce the title diimine **169** as a yellowish precipitate (1.05 g, 82 %). No further purification was required at this stage.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.53 (ddd, *J* = 4.9, 1.8, 1.0 Hz, 2H, H-12, H-12'), 8.30 (s, 2H, H-7, H-7'), 7.87 (dt, *J* = 7.9, 1.1 Hz, 2H, H-9, H-9'), 7.63 (tdd, *J* = 8.0, 1.7, 0.6 Hz, 2H, H-10, H-10'), 7.20 (ddd, *J* = 7.5, 4.8, 1.2 Hz, 2H, H-11, H-11'), 3.57 – 3.46 (m, 2H, H-1, H-2), 1.93 – 1.75 (m, 6H, H-3b, CH_2-4, CH_2-5, H-6b), 1.57 – 1.43 (m, 2H, H-3a, H-6a).

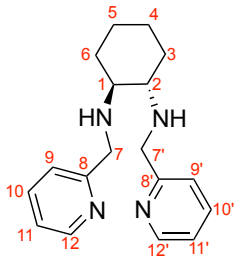
^{13}C NMR (125 MHz, $CDCl_3$): δ 161.6, 154.8, 149.5, 136.5, 124.6, 121.4, 73.7, 32.9, 24.5.

HRMS (ESI) *(m/z)*: Calc'd for $C_{18}H_{21}N_4$ $[M+H]^+$: 293.1761, found 293.1756; and Calc'd for $C_{18}H_{20}N_4Na[M+Na]^+$: 315.1580, found 315.1577.

FTIR (thin film) cm^{-1}: 3053, 3008, 2930, 2858, 1647, 1587, 1469, 993, 773.

$[\alpha]_D$: 199 ($_C$ 0.84, CH_2Cl_2).

(*1S,2S*)-*N,N'*-Di(2-picolyl)-1,2-cyclohexanediamine (piccyhxn) (170): [224]



To the solution of diimine **169** (1.04 g, 3.54 mmol, 1 equiv) in MeOH (5 mL) at 0 °C, $NaBH_4$ (0.703 g, 14.2 mmol, 4 equiv) was added slowly. The mixture was stirred at room temperature for 1 h and then refluxed for 3 h. Once cooled, the reaction was quenched with 3 N HCl until pH ~ 3 and the volatiles were removed *in vacuo*. The residue was basified with 20% $NaOH_{aq}$ to pH > 10, then extracted with CH_2Cl_2 (4 × 20 mL). The combined organic phases were dried (Na_2SO_4) and concentrated under reduced pressure to yield the title diamine **170** as a yellow oil (0.87 g, 83 %).

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.52 (ddd, *J* = 4.9, 1.9, 0.9 Hz, 2H, H-12, H-12'), 7.61 (td, *J* = 7.6, 1.8 Hz, 2H, H-10, H-10'), 7.39 (dt, *J* = 7.9, 1.0 Hz, 2H, H-9, H-9'), 7.13 (ddd, *J* = 7.5, 4.8, 1.2 Hz, 2H, H-11, H-11'), 4.02 (d, *J* = 14.2 Hz, 2H, H-7b, H-7'b), 3.84 (d, *J* = 14.2 Hz, 2H, H-7a, H-7'a), 2.37 – 2.27 (m, 2H, H-1, H-2), 2.18 – 2.08 (m, 2H, H-6b, H-3b), 1.77 – 1.64 (m, 2H, H-6a, H-3a), 1.30 – 1.17 (m, 2H, H-4b, H-5b), 1.14 – 1.01 (m, 2H, H-4a, H-5a).

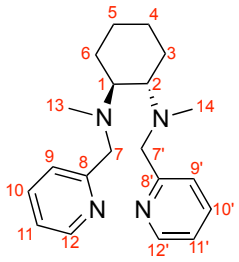
^{13}C NMR (125 MHz, $CDCl_3$): δ 161.0, 149.2, 136.5, 122.4, 121.9, 61.5, 52.7, 31.8, 25.1.

HRMS (ESI) *(m/z)*: Calc'd for $C_{18}H_{25}N_4$ $[M+H]^+$: 297.2074, found 297.2072.

FTIR (thin film) cm^{-1}: 3293, 3062, 3007, 2926, 2853, 1591, 1569, 1432, 1123, 756.

$[\alpha]_D$: 85 ($_C$ 0.93, CH_2Cl_2).

(*1S,2S*)-*N, N'*-Dimethyl-[*N,N'*-di(2-picolyl)]-1,2-cyclohexanediamine (*S,S*-picchxnMe_2) (166): [224]



To the solution of diamine **170** (0.8 g, 2.7 mmol, 1 equiv) in CH_3CN (7.5 mL) and CH_3COOH (0.9 mL) was added 37% aqueous CH_2O (2.02 mL), and the reaction was stirred for 2 h at room temperature. $NaBH_4$ (0.62 g, 16.2 mmol, 6 equiv) was added portionwise and the mixture was left to stir at room temperature for 18 h. The progress of reaction was monitored by TLC. Once completed, the reaction was quenched with 3 N HCl until pH ~ 3 and the volatiles were removed *in vacuo*. The residue was basified with 20% $NaOH_{(aq)}$ to pH > 10 and extracted with CH_2Cl_2 (4 × 15 mL). The combined organic phases were dried (Na_2SO_4) and concentrated under reduced pressure to yield the title dimethylated diamine **166** as a red viscous gum (0.81g, 92 %).

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.49 (dt, *J* = 4.9, 1.5 Hz, 2H, H-12, H-12'), 7.62 – 7.54 (m, 4H, H-10, H-10', H-9', H- 9), 7.17 – 7.07 (m, 2H, H-11, H-11'), 3.92 (d, *J* = 14.6 Hz, 2H, H-7b, H-7'b), 3.80 (d, *J* = 14.6 Hz, 2H, H-7a, H-7'a), 2.71 – 2.61 (m, 2H, H-1, H-2), 2.29 (s, 6H, CH_3-14, CH_3-13), 1.98 (dq, *J* = 12.4, 2.5 Hz, 2H, H-3b, H-6b), 1.81 – 1.70 (m, 2H, H-4b, H-5b), 1.35 – 1.23 (m, 2H, H-3a, H-6a), 1.23 – 1.09 (m, 2H, H-4a, H-5a).

^{13}C NMR (125 MHz, $CDCl_3$): δ 161.5, 148.6, 136.2, 122.8, 121.6, 64.6, 60.5, 36.7, 25.9, 25.8.

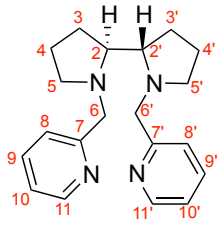
HRMS (ESI) *(m/z)*: Calc'd for $C_{20}H_{29}N_4$ $[M+H]^+$: 325.2387, found 325.2384.

FTIR (thin film) cm^{-1}: 3051, 3006, 2929, 2854, 2788, 1590, 1472, 1433, 1087, 796.

$[\alpha]_D$: 1 ($_C$ 0.74, CH_2Cl_2).

TLC (5% MeOH, 5% NH_4OH, 90% THF), **Rf:** 0.3 (UV, CAM, ninhydrin).

(*S,S*)-[*N, N'*-Di(2-picolyl)]-2, 2'-bispyrrolidine (*S,S*-picbipyrro) (167):[209]



To a solution of (*S,S*)-2,2'-bispyrrolidine (0.93 g, 6.6 mmol, 1 equiv) in acetonitrile (40 mL), 2-(chloromethyl)pyridine hydrochloride (2.16 g, 13.2 mmol, 2 equiv) and anhydrous K_2CO_3 (5.53 g, 40 mmol, 6 equiv) were added. The solution was heated to 60 ^oC for 24 h, under argon. The organic volatiles were removed under reduced pressure and the remaining yellow liquid was dissolved in

10 mL of CH_2Cl_2 and washed with saturated aqueous Na_2CO_3 (2 x 10 mL). The organic phase was dried (Na_2SO_4), concentrated under reduced pressure to give the title product **167** as a light yellow oil (2.08 g, 97%). No additional purification was required.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.49 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 2H, H-11, H-11'), 7.59 (td, *J* = 7.7, 1.8 Hz, 2H, H-9, H-9'), 7.39 (dq, *J* = 7.8, 0.9 Hz, 2H, H-8, H-8'), 7.10 (ddd, *J* = 7.5, 4.9, 1.2 Hz, 2H, H-10, H-10'), 4.19 (d, *J* = 14.3 Hz, 2H, H-6b, H-6'b), 3.50 (d, *J* = 14.3 Hz, 2H, H-6a, H-6'a), 2.99 (ddd, *J* = 9.2, 5.3, 3.2 Hz, 2H, H-5b, H-5'b), 2.84 – 2.75 (m, 2H, H-2, H-2'), 2.28 – 2.19 (m, 2H, H-5a, H-5'a), 1.89 – 1.63 (m, 8H, CH_2-4, CH_2-3, CH_2-4', CH_2-3').

^{13}C NMR (125 MHz, $CDCl_3$): δ 160.5, 149.0, 136.4, 122.9, 121.8, 65.3, 61.3, 55.4, 26.2, 23.7.

HRMS (ESI) *(m/z)*: Calc'd for $C_{20}H_{27}N_4$ $[M+H]^+$: 323.2230, found 323.2230.

FTIR (thin film) cm^{-1}: 3059, 3007, 2963, 2802, 1588, 1569, 1473, 1432, 1120, 757.

$[\alpha]_D$: -116 ($_C$ 0.8, CH_2Cl_2).

Synthesis of $Co[N_4Cl_2]Cl$:

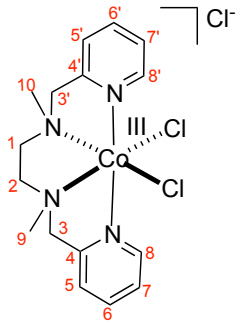
General Procedure:

To a yellow solution of ligand (1 mmol, 1 equiv) in absolute ethanol (16 mL) and concentrated HCl (0.5 mL) was added a blue solution of $CoCl_2 \cdot 6H_2O$ (1 mmol, 1 equiv) dissolved in absolute ethanol (16 mL). The addition rate was controlled so that any precipitated blue-green solid was allowed to dissolve to a maximum extent before any further addition. Once the addition was complete (~ 2

h), the mixture was stirred for 15 min at room temperature, after which 30% wt./vol. H_2O_2 (1.6 mL) was added. The resulting clear blue-green solution was stirred for an additional 45 minutes and subsequently the reaction volume was reduced to 2 mL by removing volatiles under reduced pressure. The solution was then stored at -20 °C overnight. The precipitated blue-green solid was removed by filtration and rinsed several times with cold absolute ethanol followed by cold acetone, and then dried under high vacuum to give the title compound $Co[N_4Cl_2]Cl$ as a blue-green solid. The mother liquor and washings were combined and reduced in volume to 1 mL on a rotary evaporator and then left at -20 °C for 12 h. Any additional product was collected as discussed before. Both products were used further without any additional purification.

Δ,Λ-α-Co[{*N*, *N'*-Dimethyl-(*N*, *N'*-di(2-picolyl))ethane-1,2-diamine}Cl_2]Cl

Δ,Λ-α-Co[(picenMe_2)Cl_2]Cl (171):[201]



Following the general procedure as described above, the N_4 tetradentate **132** (0.21 g, 0.77 mmol, 1 equiv) was complexed with $CoCl_2 \cdot 6H_2O$ (0.18 g, 0.77 mmol, 1 equiv) to yield the title $Co[N_4Cl_2]Cl$ complex **171** (0.21 g, 65%).

^{1}H NMR (500 MHz, DMSO): δ 9.41 – 9.14 (m, 2H, H-8', H-8), 8.19 (td, *J* = 7.6, 1.3 Hz, 2H, H-6', H-6), 7.75 (t, *J* = 6.7 Hz, 4H, H-7', H-5', H-7, H-5), 4.77 (d, *J* = 15.8 Hz, 2H, CH_2-3'), 3.99 (d, *J* = 16.0 Hz, 2H, CH_2-3), 2.87 (d, *J* = 9.1 Hz, 2H, CH_2-1), 2.53 (d, *J* = 9.4 Hz, 2H, CH_2-2), 2.22 (s, 6H, CH_3-10, CH_3-9).

^{13}C NMR (125 MHz, DMSO): δ 162.3, 153.1, 140.4, 125.0, 122.9, 69.0, 60.0, 45.9.

HRMS (ESI) *(m/z)*: Calc'd for $C_{16}H_{22}Cl_2CoN_4$ $[M^*]^+$: 399.0548, found 399.0558.

FTIR (thin film) cm^{-1}: 3490, 3111, 3079, 3028, 2998, 2965, 1610, 1479, 1443, 1296, 1053, 975, 821, 773, 721, 524, 458.

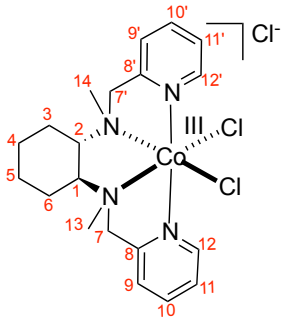
$[\alpha]_D$: 0 ($_C$ 0.042, 12M HCl).

UV-Vis [λ nm (ε x 10^{-3} dm^2 mol^{-1})]: 690 (1.87), 662 (1.87) (12M HCl, 0.5 mg/mL, 1cm cell).

CD [λ nm (Δε dm^2 mol^{-1})]: No CD data was generated, due to a racemic mixture.

Λ-α-Co[{*(1S,2S)-N, N'*-Dimethyl-(*N,N'*-di(2-picolyl)-1,2-cyclohexanediamine}Cl_2]Cl

Λ-α-Co[(*S,S*-picchxnMe$_2$)Cl_2]Cl (172): [219]



Following the general procedure described above, the N_4 tetradentate **166** (0.185 g, 0.57 mmol, 1 equiv) was complexed with $CoCl_2 \cdot 6H_2O$ (0.136 g, 0.57 mmol, 1 equiv) to yield the title $[CoN_4Cl_2]Cl$ complex **172** (0.19 g, 68%).

^{1}H NMR (500 MHz, DMSO): δ 9.25 (dd, *J* = 6.1, 1.3 Hz, 2H, H-12', H-12), 8.17 (td, *J* = 7.6, 1.4 Hz, 2H, H-10, H-10'), 7.74 (t, *J* = 6.9 Hz, 2H, H-11', H-11), 7.68 (d, *J* = 7.5 Hz, 2H, H-9', H-9), 4.64 (d, *J* = 16.1 Hz, 2H, CH_2-7'), 4.16 (d, *J* = 16.2 Hz, 2H, CH_2-7), 3.44 (q, *J* = 7.0 Hz, 2H, H-2, H-1), 2.73 – 2.67 (m, 2H, H-6b, H-3b), 2.27 – 2.19 (m, 2H, H-5b, H-4b), 2.16 (s, 6H, CH_3-13, CH_3-14), 1.49 (d, *J* = 8.6 Hz, 2H, H-6a, H-3a), 1.40 (d, *J* = 11.1 Hz, 2H, H-5a, H-4a).

^{13}C NMR (125 MHz, DMSO): δ 158.6, 149.4, 136.4, 122.3, 121.9, 66.2, 61.4, 55.5, 27.0, 23.9.

HRMS (ESI) *(m/z)*: Calc'd for $C_{20}H_{28}Cl_2CoN_4$ $[M^*]^+$: 453.1018, found 453.1012.

FTIR (thin film) cm^{-1}: 3439, 3080, 2937, 1612, 1481, 1448, 1296, 1094, 772, 623, 527, 455.

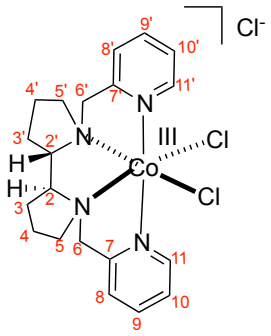
$[\alpha]_D$: 81 ($_C$ 0.06, 12 M HCl).

UV-Vis [λ nm (ε x 10^{-3} dm^2 mol^{-1})]: 690 (1.32), 663 (1.32) (12 M HCl, 0.6 mg/mL, 1 cm cell).

CD [λ nm (Δε dm^2 mol^{-1})]: 653 (-0.33), 575 (0.39), 428 (0.74), 347 (-2.45) (12 M HCl, 0.6 mg/mL, 1 cm cell).

Λ-α-Co[{(*S*,*S*)-(*N*, *N*'-Di(2-picolyl))-2, 2'-bipyrrolidine}Cl_2]Cl

Λ-α-Co[(*S*,*S*-picbipyrro)Cl_2]Cl (173):



Following the general procedure described above, the N_4 tetradentate **167** (0.2 g, 0.62 mmol, 1 equiv) was complexed with $CoCl_2$•$6H_2O$ (0.15 g, 0.62 mmol, 1 equiv) to yield the title Co[N_4Cl_2]Cl complex **173** (0.16 g, 60%).

^{1}H NMR (500 MHz, DMSO): δ 9.42 (d, *J* = 5.9 Hz, 2H, H-11', H-11), 8.18 (t, *J* = 7.5 Hz, 2H, H-9', H-9), 7.77 (t, *J* = 6.8 Hz, 2H, H-10', H-10), 7.66 (d, *J* = 7.6 Hz, 2H, H-8', H-8), 4.66 (d, *J* = 15.8 Hz, 2H, CH_2-6'), 4.02 (d, *J* = 15.8 Hz, 2H, CH_2-6), 3.28 (dd, *J* = 12.1, 6.0 Hz, 2H, H-5'b, H-5b), 3.10 (t, *J* = 3.2 Hz, 2H, H-2', H-2), 2.22 (td, *J* = 11.4, 7.2 Hz, 2H, H-5a, H-5'a), 2.18 – 2.05 (m, 4H, CH_2-4', CH_2-4), 1.88 – 1.78 (m, 2H, H-3'b, H-3b), 1.46 – 1.37 (m, 2H, H-3'a, H-3a).

^{13}C NMR (125 MHz, DMSO): δ 162.3, 152.5, 140.0, 124.8, 122.5, 72.9, 65.6, 55.4, 22.6, 21.6.

HRMS (ESI) *(m/z)*: Calc'd for $C_{20}H_{26}Cl_2CoN_4$ $[M^*]^+$: 451.0861, found 451.0859.

FTIR (thin film) cm^{-1}: 3307,3049, 2965, 2811, 1631, 1592, 1436, 1365, 450, 289, 247.

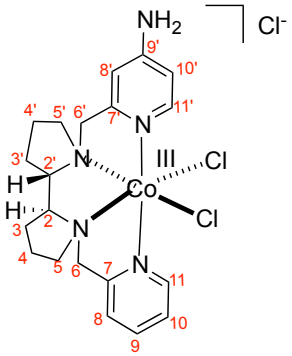
$[\alpha]_D$: 845 ($_C$ 0.046, 12 M HCl).

UV-Vis [λ nm (ε x 10^{-3} dm^2 mol^{-1})]: 690 (2.16), 663 (2.15) (12M HCl, 0.46 mg/mL, 1 cm cell).

CD [λ nm (Δε dm^2 mol^{-1})]: 635 (-0.76), 548 (1.35), 418 (0.67), 341 (-2.70) (12 M HCl, 0.46 mg/mL, 1 cm cell).

Λ-α-Co[{*(S,S)*-(*N*-(2-Picolyl)-*N'*-(4-amino-2-methyl)pyridine)-2, 2'-bipyrrolidine}Cl_2]Cl

Λ-α-Co[(*S,S*-picbipyrro-NH_2) Cl_2]Cl (174):



Following the general procedure described above, the N_4 tetradentate **154** (0.046 g, 0.14 mmol, 1 equiv) was complexed with $CoCl_2 \cdot 6H_2O$ (0.033 g, 0.14 mmol, 1 equiv) to yield the title Co[N_4Cl_2]Cl complex **174** (0.03 g, 46%).

^{1}H NMR (500 MHz, DMSO): δ 9.36 (dd, *J* = 6.1, 1.3 Hz, 1H, H-11), 8.76 (s, 1H, H-11'), 8.17 (td, *J* = 7.6, 1.4 Hz, 1H, H-9), 7.80 – 7.72 (m, 3H, H-8', H-10', H-10), 7.64 (d, *J* = 7.9 Hz, 1H, H-8), 4.61 (d, *J* = 16.0 Hz, 1H, H-6'b), 4.51 (d, *J* = 16.3 Hz, 1H, H-6b), 4.00 (d, *J* = 16.0 Hz, 1H, H-6'a), 3.75 (d, *J* = 16.2 Hz, 1H, H-6a), 3.32 – 3.28 (m, 3H, H-5'b, H-5b, H-2'), 3.13 – 3.05 (m, 1H, H-2), 2.59 (dt, *J*

= 11.1, 5.7 Hz, H-1H, H-5'a), 2.27 – 2.09 (m, 5H, H-1a, CH_2-4', CH_2-4), 1.92 – 1.77 (m, 2H, H-3'b, H-3b), 1.49 – 1.37 (m, 2H, H-3'a, H-3a).

^{13}C NMR (125 MHz, DMSO): δ 162.7, 162.0, 156.9, 152.9, 146.9, 140.3, 125.9, 125.1, 123.5, 122.8, 73.5, 73.1, 65.8, 65.2, 55.9, 55.5, 22.9, 22.7, 21.7, 21.6.

HRMS (ESI) *(m/z)*: Calc'd for $C_{20}H_{27}Cl_2CoN_5[M^*]^+$: 466.0976, found 466.0981.

FTIR (thin film) cm^{-1}: 3301, 3192, 2963, 1624, 1487, 1294, 513, 453, 299, 262.

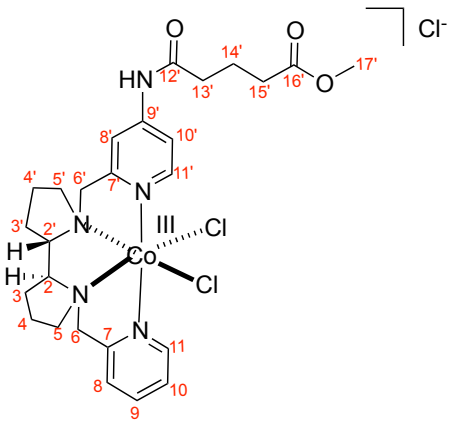
$[\alpha]_D$: 984 ($_C$ 0.048, 12 M HCl).

UV-Vis [λ nm (ε x 10^{-3} dm^2 mol^{-1})]: 690 (1.96), 662 (1.96) (12M HCl, 0.5 mg/mL, 1 cm cell).

CD [λ nm (Δε dm^2 mol^{-1})]: 635 (-0.80), 554 (1.55), 404 (-0.17), 333 (-2.34) (12 M HCl, 0.5 mg/mL, 1 cm cell).

Λ-α-Co[{*(S,S)*-(*N*-(2-Picolyl)- *N'*-(2-picolyl-4-amidoglutarate))-2, 2'-bipyrrolidine}Cl_2]Cl

Λ-α-Co[(*S,S*-picbipyrro-amidoglutarate)Cl_2]Cl (161):



Following the general procedure described above, the N_4 tetradentate **141** (0.087 g, 0.19 mmol, 1 equiv) was complexed with $CoCl_2 \bullet 6H_2O$ (0.044 g, 0.19 mmol, 1 equiv) to yield the title $Co[N_4Cl_2]Cl$ complex **161** (0.068 g, 62%).

^{1}H NMR (500 MHz, DMSO): δ 9.42 (d, *J* = 5.9 Hz, 1H, H-11'), 9.15 (d, *J* = 6.8 Hz, 1H, H-11), 8.16 (t, *J* = 7.4 Hz, 1H, H-9), 8.04 (d, *J* = 2.5 Hz, 1H, H-8'), 7.79 – 7.71 (m, 2H, H-10', H-10), 7.63 (d, *J* = 7.6 Hz, 1H, H-8), 4.59 (dd, *J* = 23.2, 15.7 Hz, 2H, H-6'b, H-6b), 3.95 (d, *J* = 15.8 Hz, 2H, H-6'a, H-6a), 3.60 (s, 3H, CH_3-17'), 3.22 (td, *J* = 13.8, 11.9, 5.4 Hz, 2H, H-5'b, H-5b), 3.06 (q, *J* = 9.6, 7.4 Hz, 2H, H-2', H-2), 2.54 – 2.47 (m, 2H, CH_2-15'), 2.39 (t, *J* = 7.3 Hz, 2H, CH_2-13'), 2.33 – 2.06 (m, 6H, H-5'a, CH_2-4', H-5a, CH_2-4), 1.92 – 1.73 (m, 4H, H-3'b, H-3b, CH_2-14'), 1.44 – 1.34 (m, 2H, H-3'a, H-3a).

^{13}C NMR (125 MHz, DMSO): δ 172.7, 172.6, 162.8, 162.5, 152.6, 152.5, 148.7, 140.2, 124.9, 122.7, 114.2, 110.9, 73.1, 73.0, 65.8, 55.6, 55.5, 51.2, 35.4, 32.2, 30.5, 22.9, 22.8, 21.8, 21.7, 19.8.

HRMS (ESI) *(m/z)*: Calc'd for $C_{26}H_{35}Cl_2CoN_5O_3$ $[M^*]^+$: 594.1443, found 594.1435.

FTIR (thin film) cm^{-1}: 3255, 3088, 2953, 1727, 1658, 1591, 1521, 1446, 1285, 1199, 771, 292, 267.

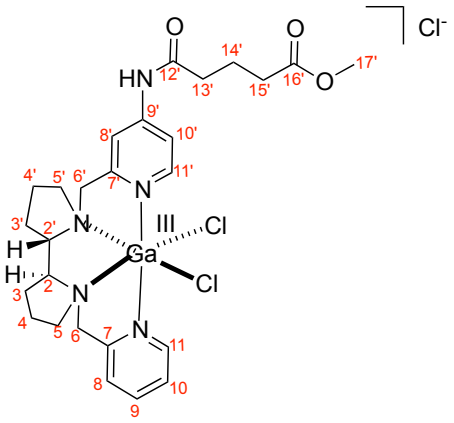
$[\alpha]_D$: 563 ($_C$ 0.048, 12 M HCl).

UV-Vis [λ nm (ε x 10^{-3} dm^2 mol^{-1})]: 690 (2.41), 662 (2.35), 624 (1.81) (12 M HCl, 0.48 mg/mL, 1 cm cell).

CD [λ nm (Δε dm^2 mol^{-1})]: 634 (-0.66), 554 (1.19), 428 (0.40), 347 (-1.53) (12 M HCl, 0.48 mg/mL, 1 cm cell).

Λ-α-Ga[{(*S*,*S*)-(*N*-(2-Picolyl)- *N'*-(2-picolyl-4-amidoglutarate))-2, 2'-bipyrrolidine}Cl_2]Cl

Λ-α-Ga[(*S*,*S*-picbipyrro-4-amidoglutarate)Cl_2]Cl (164):



To a solution of a N_4 tetradentate **141** (0.18 g, 0.39 mmol, 1 equiv) in dry acetonitrile (5 mL) was added anhydrous $GaCl_3$ (0.068 g, 0.39 mmol, 1 equiv) under argon. The reaction mixture was stirred at refluxed for 2 h followed by an overnight stirring at room temperature. The organic volatiles were removed under reduced pressure and the resulting mixture was triturated with dry diethyl ether (2 mL) to give the title Co[N_4Cl_2]Cl complex **164** (0.21 g, 83%) as an off-white solid. **^{1}H NMR (600 MHz, CD_3OD):** δ 9.51 (t, *J* = 6.3 Hz, 1H, H-11'), 9.25 (t, *J* = 6.6 Hz, 1H, H-11), 8.25 (q, *J* = 7.5 Hz, 1H, H-9), 8.07 (d, *J* = 6.1 Hz, 1H, H-8'), 7.77 (q, *J* = 6.8 Hz, 1H, H-10'), 7.71 (q, *J* = 6.8, 6.4 Hz, 2H, H-10, H-8), 4.62 (ddd, *J* = 31.2, 15.8, 6.4 Hz, 2H, H-6'b, H-6b), 4.30 (ddd, *J* = 51.7, 15.6, 6.4 Hz, 2H, H-6'a, H-6a), 3.68 (d, *J* = 6.6 Hz, 3H, CH_3-17'), 3.30 – 3.23 (m, 2H, H-5'b, H-5b), 2.91 (q, *J* = 7.4 Hz, 2H, H-2', H-2), 2.75 – 2.60 (m, 2H, H-5'a, H-5a), 2.56 (q, *J* = 7.3 Hz, 2H, CH_2-15'), 2.45 (q, *J* = 7.1 Hz, 2H, CH_2-13'), 2.27 (td, *J* = 22.1, 18.5,10.8

Hz, 2H, H-4'b, H-4b), 2.14 (tp, *J* = 12.2, 6.3 Hz, 2H, H-4'a, H-4a), 2.00 (h, *J* = 7.2 Hz, 2H, CH_2-14'), 1.96 – 1.84 (m, 2H, H-3'b, H-3b), 1.56 (tq, *J* = 14.3, 7.5 Hz, 2H, H-3'a, H-3a).

^{13}C NMR (125 MHz, CD_3OD): δ 175.2, 174.7, 154.9, 154.0, 152.0, 148.5, 148.2, 143.5, 126.6, 126.2, 114.9, 114.3, 67.0, 66.9, 57.9, 57.7, 54.7, 52.1, 36.9, 33.8, 31.3, 30.9, 28.9, 25.7, 23.1, 21.2.

HRMS (ESI) *(m/z)*: Calc'd for $C_{26}H_{35}Cl_2GaN_5O_3$ $[M^*]^+$: 604.1367, found 604.1366.

FTIR (thin film) cm^{-1}: 3088, 2944, 1725, 1661, 1583, 1526, 1444, 1278, 1206, 377, 298.

$[\alpha]_D$: 69 ($_C$ 0.047, MeOH).

UV-Vis [λ nm (ε x 10^{-3} dm^2 mol^{-1})]: 265 (1.42) (MeOH, 2.95 mg/mL, 0.2 mm cell).

CD [λ nm (Δε dm^2 mol^{-1})]: 268 (5.29), 225 (-3.09) (MeOH, 2.95 mg/mL, 0.2 mm cell).

Synthesis of $Co[N_4(AA)]Cl_2$:

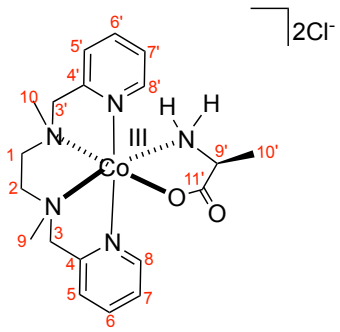
General Procedure:

A blue solution of $Co[N_4Cl_2]Cl$ (0.1 mmol, 1 equiv) in water (5 mL) turned to violet when warmed to 60 ^oC for 15 minutes. To that violet solution, amino acid (0.4 mmol, 4 equiv) was added and the solution was carefully adjusted to pH ~ 8 by adding 1 M $NaOH_{(aq)}$ The reaction was heated at 95 ^oC for ~ 2 h and then, after cooling, the resulting orange colored mixture was further diluted with water (4 mL). This diluted mixture was loaded on Sephadex CM-25 column and

eluted initially with distilled water followed by two column head of 0.1 M NaCl. Subsequently the desired complex $Co[N_4(AA)]Cl_2$, as a pink-orange color solution, was eluted with two column head of 0.3 M NaCl. The collected fractions were combined and dried *in vacuo* to give the desired complex with NaCl. The obtained residue was further washed with absolute ethanol and the filtrate was dried under reduced pressure to yield the title compound $Co[N_4(AA)]Cl_2$ as a pink-orange solid.

Λ,Δ-α-Co[{*N*, *N'*-Dimethyl-(*N, N'*-di(2-picolyl))ethane-1,2-diamine}{*S*-ala}]Cl_2

Λ,Δ-α- [Co(picenMe_2)(*S*-ala)]Cl_2 (185):



mixture of isomers (Λ / Δ : 1 / 0.2)

Following the general procedure as described above, the bischloro cobalt complex **171** (0.020 g, 0.05 mmol, 1 equiv) was complexed with *S*-Ala (0.018 g, 0.2 mmol, 4 equiv) to yield the title $Co[N_4(S\text{-ala})]Cl_2$ complex **185**, after Sephadex CM-25 column purification, as a mixture of isomers (Λ/Δ : 1/0.2) with Λ being the major isomer (0.01 g, 47%).

^{1}H NMR (600 MHz, CD_3OD): δ (Mixture of isomers) 9.46 (d, *J* = 6.0 Hz, 0.2x1H, H-8'), 9.45 (d, *J* = 6.0 Hz, 1H, H-8'), 8.46 (d, *J* = 6.0 Hz, 1H, H-8), 8.44

(d, *J* = 6.0 Hz, 0.2x1H, H-8), 8.34 – 8.29 (m, 1.2x2H, H-6', H-6), 7.97 – 7.84 (m, 1.2x4H, H-7', H-5', H-7, H-5), 4.83 (s, 1.2x2H, CH_2-3'), 4.54 (s, 1.2x2H, CH_2-3), 3.37 (q, *J* = 7.0 Hz, 1.2x1H, H-9'), 3.14 – 2.99 (m, 1.2x2H, H-1b, H-2b), 2.88 – 2.81 (m, 1.2x1H, H-1a), 2.79 (s, 1.2x3H, CH_3-10), 2.78 – 2.74 (m, 1.2x1H, H-2a), 2.50 (s, 1.2x3H, CH_3-9), 1.36 (d, *J* = 7.2 Hz, 0.2x3H, CH_3-10'), 1.30 (d, *J* = 7.2 Hz, 3H, CH_3-10').

^{13}C NMR (125 MHz, CD_3OD): δ (Mixture of isomers) 183.6, 183.4, 163.2, 163.1, 162.3, 162.0, 154.7, 154.6, 154.3, 154.0, 151.1, 151.0, 143.5, 143.4, 128.1, 128.0, 126.5, 126.4, 125.9, 125.7, 70.4, 70.3, 69.9, 69.2, 68.9, 68.7, 62.4, 62.3, 60.3, 60.2, 54.8, 53.6, 49.5, 49.6, 47.7, 47.6, 19.1, 19.0.

HRMS (ESI) *(m/z)*: Calc'd for $C_{19}H_{27}CoN_5O_2$ M*]$^+$: 416.1491, found 416.1482.

FTIR (thin film) cm^{-1}: 3387, 3030, 1665, 1616, 1480, 1451, 1383, 1351, 1263, 775.

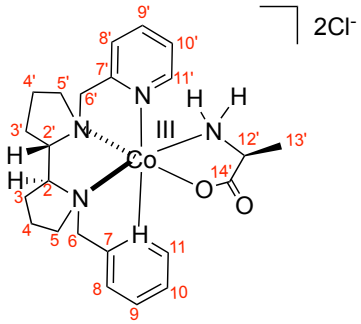
$[\alpha]_D$: 2 ($_C$ 0.041, H_2O).

UV-Vis [λ nm (ε x 10^{-3} dm^2 mol^{-1})]: 486 (0.53) (H_2O, 0.41 mg/mL, 1 cm cell).

CD [λ nm (Δε dm^2 mol^{-1})]: 501 (0.37), 358 (-0.09) (H_2O, 0.41 mg/mL, 1 cm cell).

Λ-α-Co[{(*S,S)*-(*N, N'*-Bis(2-picolyl))-2, 2'-bipyrrolidine}{(*S*-ala)]Cl_2

Λ-α-Co[(*S,S*-picbipyrro)(*S*-ala)]Cl_2 (186):



Following the general procedure described above, the bischloro cobalt complex **173** (0.020 g, 0.045 mmol, 1 equiv) was complexed with *S*-Ala (0.016 g, 0.18 mmol, 4 equiv) to yield the title Co[N_4(*S*-ala)]Cl_2 complex **186** (0.01 g, 42%).

^{1}H NMR (500 MHz, CD_3OD): δ 9.39 (d, *J* = 5.9 Hz, 1H, H-11'), 8.55 (d, *J* = 5.9 Hz, 1H, H-11), 8.30 (dt, *J* = 12.6, 7.7 Hz, 2H, H-9', H-9), 7.92 – 7.84 (m, 3H, H-10', H-8', H-10), 7.83 (d, *J* = 7.9 Hz, 1H, H-8), 4.75 (d, *J* = 16.2 Hz, 1H, H-6'b), 4.52 – 4.40 (m, 2H, H-6'a, H-6b), 4.28 (d, *J* = 16.5 Hz, 1H, H-6a), 4.03 (dd, *J* = 11.9, 6.7 Hz, 1H, H-5'b), 3.53 (dd, *J* = 11.8, 6.9 Hz, 1H, H-5b), 3.35 (q, *J* = 7.0 Hz, 1H, H-12'), 3.24 – 3.20 (m, 2H, H-2', H-2), 2.51 (td, *J* = 11.9, 7.2 Hz, 1H, H-5'a), 2.42 – 2.20 (m, 5H, CH_2-4', CH_2-4, H-5a), 2.08 – 1.98 (m, 2H, H-3b, H-3'b), 1.63 – 1.48 (m, 2H, H-3a, H-3'a), 1.30 (d, *J* = 7.1 Hz, 3H, CH_3-13').

^{1}H NMR (600 MHz, D_2O): δ 8.95 (d, *J* = 5.9 Hz, 1H, H-11'), 8.59 (d, *J* = 6.0 Hz, 1H, H-11), 8.30 (dtd, *J* = 17.2, 7.8, 1.3 Hz, 2H, H-9', H-9), 7.94 – 7.81 (m, 4H, H-10', H-8', H-10, H-8), 4.79 (d, *J* = 16.6 Hz, 1H, H-6'b), 4.46 – 4.38 (m, 2H, H-6'a, H-6b), 4.27 (d, *J* = 16.7 Hz, 1H, H-6a), 3.60 (p, *J* = 6.8, 6.4 Hz, 3H, H-5'b, H-5b, H-12'), 3.27 (dd, *J* = 4.9, 2.6 Hz, 2H, H-2', H-2), 2.61 (td, *J* = 12.0, 7.4 Hz,

1H, H-5'a), 2.43 – 2.25 (m, 5H, CH_2-4', CH_2-4, H-5a), 2.05 – 2.02 (m, 2H, H-3b, H-3'b), 1.64 – 1.50 (m, 2H, H-3a, H-3'a), 1.27 (d, J = 7.1 Hz, 3H, CH_3-13').

^{13}C NMR (125 MHz, CD_3OD): δ 183.8, 163.4, 162.4, 154.3, 150.5, 143.4, 143.2, 128.3, 126.7, 125.9, 76.2, 76.1, 74.3, 66.7, 66.1, 58.3, 57.8, 54.4, 24.2, 24.0, 23.9, 23.5, 19.0.

HRMS (ESI) *(m/z)*: Calc'd for $C_{23}H_{31}CoN_5O_2$ $[M^*]^+$: 468.1804, found 468.1803.

FTIR (thin film) cm^{-1}: 3378, 3023, 2930, 1669, 1450, 1380, 1270, 1036, 773, 559.

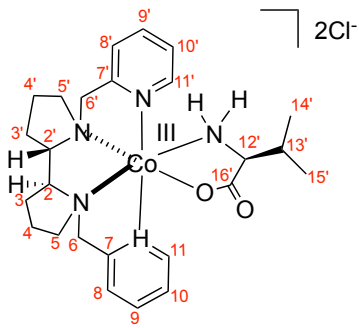
$[\alpha]_D$: 374 ($_C$ 0.046, H_2O).

UV-Vis [λ nm (ε x 10^{-3} dm^2 mol^{-1})]: 487 (1.54), 337 (3.42), 300 (17.60) (H_2O, 0.46 mg/mL, 1 cm cell).

CD [λ nm (Δε dm^2 mol^{-1})]: 498 (2.61), 358 (-0.21) (H_2O, 0.46 mg/mL, 1 cm cell).

Λ-α-Co[{(*S,S)*-(*N, N'*-Bis(2-picolyl))-2, 2'-bipyrrolidine}{(*S*-val)]Cl_2

Λ-α-Co[(*S,S*-picbipyrro)(*S*-val)]Cl_2 (190):



Following the general procedure described above, the bischloro cobalt complex **173** (0.020 g, 0.045 mmol, 1 equiv) was complexed with *S*-Val (0.021 g,

0.18 mmol, 4 equiv) to yield the title Co[N_4(*S*-val)]Cl_2 complex **190** (0.006 g, 27%).

^{1}H NMR (500 MHz, CD_3OD): δ 9.65 – 9.43 (m, 1H, H-11'), 8.69 – 8.53 (m, 1H, H-11), 8.31 (dtd, *J* = 20.7, 7.7, 1.3 Hz, 2H, H-9', H-9), 8.00 – 7.73 (m, 4H, H-10', H-8', H-10, H-8), 4.76 (d, *J* = 16.2 Hz, 1H, H-6'b), 4.59 – 4.46 (m, 2H, H-6'a, H-6b), 4.41 (d, *J* = 16.2 Hz, 1H, H-6a), 4.26 – 4.15 (m, 1H, H-12'), 3.58 – 3.49 (m, 1H, H-5'b), 3.28 (d, *J* = 3.1 Hz, 1H, H-5b), 3.24 – 3.20 (m, 2H, H-2', H-2), 2.55 – 2.46 (m, 1H, H-5'a), 2.43 – 2.22 (m, 5H, CH_2-4', CH_2-4, H-5a), 2.17 (ddt, *J* = 11.0, 7.0, 4.0 Hz, 1H, H-13'), 2.09 – 1.99 (m, 2H, H-3b, H-3'b), 1.64 – 1.48 (m, 2H, H-3a, H-3'a), 0.92 (d, *J* = 7.1 Hz, 3H, CH_3-14'), 0.68 (d, *J* = 7.1 Hz, 3H, CH_3-15').

^{13}C NMR (125 MHz, CD_3OD): δ 183.0, 163.3, 162.9, 154.2, 151.0, 143.5, 143.3, 128.2, 127.9, 126.8, 125.8, 76.3, 76.3, 74.3, 66.8, 66.3, 63.8, 58.3, 32.7, 24.1, 23.9, 23.9, 23.5, 18.2, 17.1.

HRMS (ESI) *(m/z)*: Calc'd for $C_{25}H_{35}CoN_5O_2[M^*]^+$: 496.2117, found 496.2107.

FTIR (thin film) cm^{-1}: 3381, 3029, 2961, 1668, 1615, 1448, 1379, 1258, 901, 779.

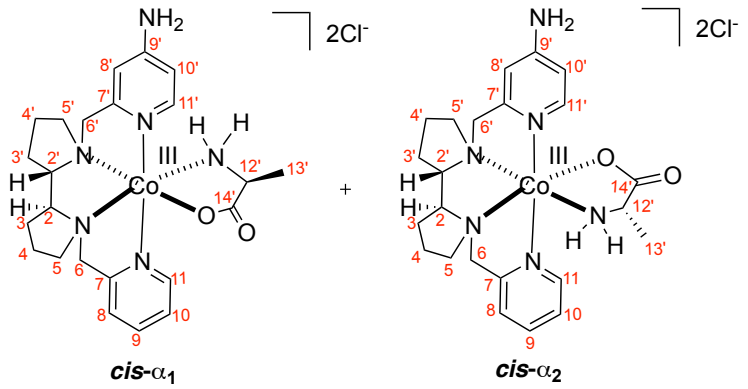
$[\alpha]_D$: 30 ($_C$ 0.05, H_2O).

UV-Vis [λ nm (ε x 10^{-3} dm^2 mol^{-1})]: 485 (0.62) (H_2O, 0.5 mg/mL, 1 cm cell).

CD [λ nm (Δε dm^2 mol^{-1})]: 492 (0.77), 359 (-0.09) (H_2O, 0.5 mg/mL, 1 cm cell).

Λ-α$_1$-α$_2$-Co[{(*S*,*S*)-(*N*-(2-Picolyl)-*N'*-(4-amino-2-methyl)pyridine)-2, 2'-bipyrrolidine}{*S*-ala}]Cl$_2$

Λ-α$_1$-α$_2$-Co[(*S*,*S*-picbipyrro-NH$_2$) (*S*-ala)]Cl$_2$ (191):



(2:1 mixture of diastereomers)

Following the general procedure described above, the bischloro cobalt complex **174** (0.010 g, 0.021 mmol, 1 equiv) was complexed with *S*-ala (0.008 g, 0.084 mmol, 4 equiv) to yield the title Co[N$_4$(*S*-ala)]Cl$_2$ complex **191** (0.003 g, 29%), after Sephadex CM-25 column purification, as a mixture of α$_1$ and α$_2$ isomers with a major/minor isomer in ratio of 2:1. X-ray crystallographic studies are required to deduce the configuration of the major isomer (either α$_1$ or α$_2$).

^{1}H NMR (500 MHz, CD$_3$OD): δ (Mixture of isomers) 9.38 (d, *J* = 6.0 Hz, 1.5x1H, H-11), 8.99 (s, 1.5x1H, H-11), 8.49 (d, *J* = 5.7 Hz, 1.5x1H, H-8), 8.30 – 8.25 (m, 1.5x1H, H-9), 7.90 (s, 1.5x1H, H-8'), 7.87 – 7.80 (m, 1.5x2H, H-10, H-10'), 4.70 (d, *J* = 16.2 Hz, 1.5x1H, H-6'b), 4.47 – 4.40 (m, 1.5x2H, H-6'a, H-6b), 4.17 (d, *J* = 16.2 Hz, 1.5x1H, H-6a), 4.07 (dd, *J* = 11.9, 6.8 Hz, 0.5x1H, H-5'b), 3.98 (dd, *J* = 11.9, 6.8 Hz, 1H, H-5'b), 3.57 – 3.55 (m, 0.5x1H, H-5b), 3.54 – 3.51 (m, 1H, H-5b), 3.38 – 3.28 (m, 1.5x1H, H-12'), 3.21 – 3.18 (m, 1.5x2H, H-2', H-

2), 2.51 (td, *J* = 11.8, 7.2 Hz, 1.5x1H, H-5'a), 2.44 – 2.19 (m, 1.5x5H, CH_2-4', CH_2-4, H-5a), 2.15 – 2.09 (m, 0.5x2H, H-3b, H-3'b), 2.05 – 1.99 (m, 2H, H-3b, H-3'b), 1.64 – 1.58 (m, 0.5x2H, H-3a, H-3'a), 1.57 – 1.51 (m, 2H, H-3a, H-3'a), 1.33 (d, *J* = 7.2 Hz, 0.5x3H, CH_3-13'), 1.30 (d, *J* = 7.2 Hz, 3H, CH_3-13').

^{13}C NMR (125 MHz, CD_3OD): δ (Mixture of isomers) 184.1, 183.9, 163.4, 162.4, 156.8, 155.7, 154.3, 153.0, 152.6, 150.5, 148.4, 144.5, 143.4, 143.2, 128.2, 128.1, 126.6, 125.8, 118.8, 118.7, 116.3, 115.0, 76.5, 76.1, 74.6, 74.1, 66.7, 66.1, 65.2, 65.0, 58.2, 58.1, 57.8, 57.6, 54.5, 54.4, 24.1, 24.0, 23.9, 23.8, 23.7, 23.6, 23.5, 23.4, 19.0, 18.9.

HRMS (ESI) *(m/z)*: Calc'd for $C_{23}H_{32}CoN_6O_2$ $[M^*]^+$: 483.1913, found 483.1901.

FTIR (thin film) cm^{-1}: 3324, 3078, 2985, 1665, 1628, 1496, 1453, 1383, 1351, 1270, 1123, 898, 717, 677.

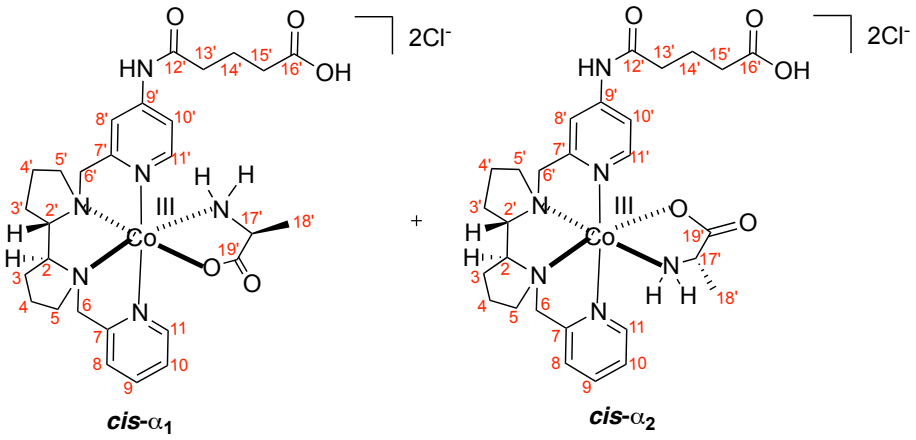
$[\alpha]_D$: 67 (*C* 0.047, H_2O).

UV-Vis [λ nm (ε x 10^{-3} dm^2 mol^{-1})]: 488 (0.96) (H_2O, 0.47 mg/mL, 1 cm cell).

CD [λ nm (Δε dm^2 mol^{-1})]: 489 (0.87), 368 (-0.24) (H_2O, 0.47 mg/mL, 1 cm cell).

Λ-α$_1$-α$_2$-Co[{(*S*,*S*)-(*N*-(2-Picolyl)- *N*'-(2-picolyl-4-amidoglutaric acid))-2, 2'-bipyrrolidine}{*S*-ala}]Cl$_2$

Λ-α$_1$-α$_2$-Co[(*S*,*S*-picbipyrro-4-amidoglutaric acid)(*S*-ala)]Cl$_2$ (192):



(1:0.7 mixture of diastereomers)

Following the general procedure described above, the bischloro cobalt complex **161** (0.010 g, 0.017 mmol, 1 equiv) was complexed with *S*-Ala (0.006 g, 0.068 mmol, 4 equiv) to yield the title Co[N$_4$(*S*-ala)]Cl$_2$ complex **192** (0.004 g, 39%), after Sephadex CM-25 column purification, as a mixture of α$_1$ and α$_2$ isomers with a major/minor isomer in ratio of 1:0.7. X-ray crystallographic studies are required to deduce the configuration of the major isomer (either α$_1$ or α$_2$).

^{1}H NMR (500 MHz, D$_2$O): δ (Mixture of isomers) 8.95 (d, *J* = 6.0 Hz, 1.7x1H), 8.58 (d, *J* = 6.0 Hz, 1H), 8.34 – 8.26 (m, 1.7x2H), 7.90 – 7.79 (m, 1.7x3H), 4.70 (d, *J* = 15.4 Hz, 1.7x1H), 4.47 – 4.29 (m, 1.7x2H), 4.21 (d, *J* = 17.7 Hz, 1.7x1H), 3.62 – 3.54 (m, 1.7x3H), 3.26 – 3.20 (m, 1.7x2H), 2.65 – 2.57 (m, 1.7x2H), 2.54 –

2.52 (m, 1.7x2H), 2.38 – 2.25 (m, 1.7x6H), 2.08 – 1.98 (m, 1.7x4H), 1.62 – 1.50 (m, 1.7x2H), 1.29 (d, J = 7.2 Hz, 0.7x3H), 1.28 (d, J = 7.2 Hz, 3H).

^{13}C NMR (125 MHz, CD_3OD): δ (Mixture of isomers) 182.7, 182.5, 173.7, 173.4, 162.1, 161.9, 160.9, 159.8, 158.7, 158.5, 158.2, 152.7, 152.6, 152.4, 150.9, 150.2, 149.2, 146.7, 141.9, 141.8, 141.6, 126.7, 126.5, 124.9, 124.8, 124.3, 116.0, 115.9, 113.5, 112.5, 111.7, 108.7, 107.5, 74.7, 74.3, 72.7, 72.5, 65.2, 65.1, 64.6, 64.5, 63.9, 56.7, 56.6, 56.3, 56.2, 53.2, 53.0, 52.9, 50.7, 35.5, 32.3, 22.1, 19.8, 17.7, 17.6.

HR/LC-MS (ESI) *(m/z)*: System B

t_{major} = 3.2 min [***m/z*** Calc'd for $C_{28}H_{38}CoN_6O_5$ $[M^*]^+$: 597.2236, found 597.2243; Calc'd for $C_{28}H_{39}CoN_6O_5$ $[M+H]^+$: 598.2308, found 598.2302.

t_{minor} = 3.9 min [***m/z*** Calc'd for $C_{28}H_{38}CoN_6O_5$ $[M^*]^+$: 597.2236, found 597.2229; Calc'd for $C_{28}H_{39}CoN_6O_5$ $[M+H]^+$: 598.2308, found 598.2303.

FTIR (thin film) cm^{-1}: 3388, 2980, 1722, 1653, 1522, 1452, 1280, 1036, 902, 857, 774.

$[\alpha]_D$: 138 ($_C$ 0.056, H_2O).

UV-Vis [λ nm (ε x 10^{-3} dm^2 mol^{-1})]: 480 (1.50) (H_2O, 0.56 mg/mL, 1 cm cell).

CD [λ nm (Δε dm^2 mol^{-1})]: 495 (1.05), 421 (-0.44), 369 (-0.38) (H_2O, 0.56 mg/mL, 1 cm cell).

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Appendix: X-ray Crystal Structure of 11β-Aminoprogesterone (76)

STRUCTURE REPORT

XCL Code: JCV1003 **Date:** 21 May 2010

Compound: 11β-Aminoprogesterone
Formula: $C_{21}H_{31}NO_2$

Supervisor: J. C. Vederas **Crystallographer:** R. McDonald

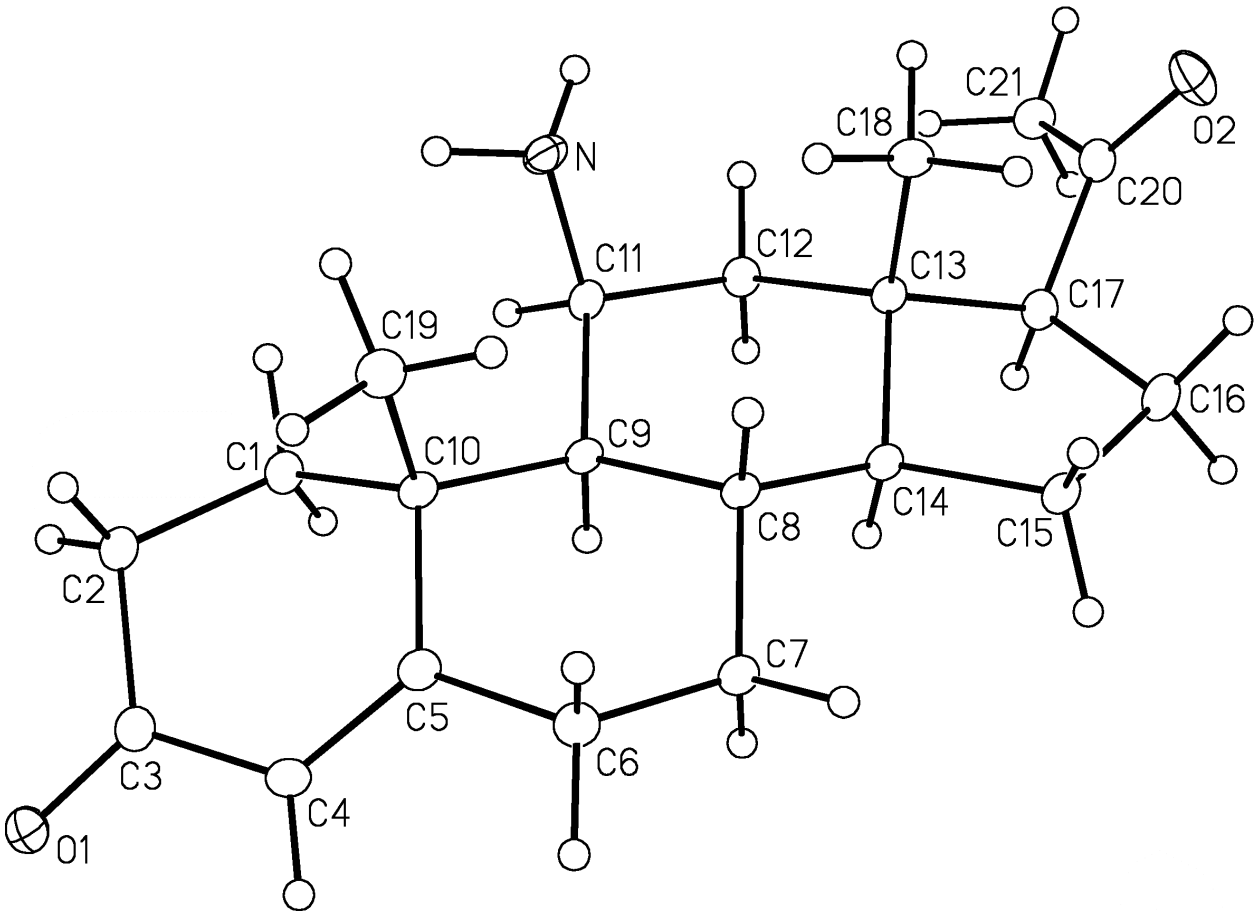
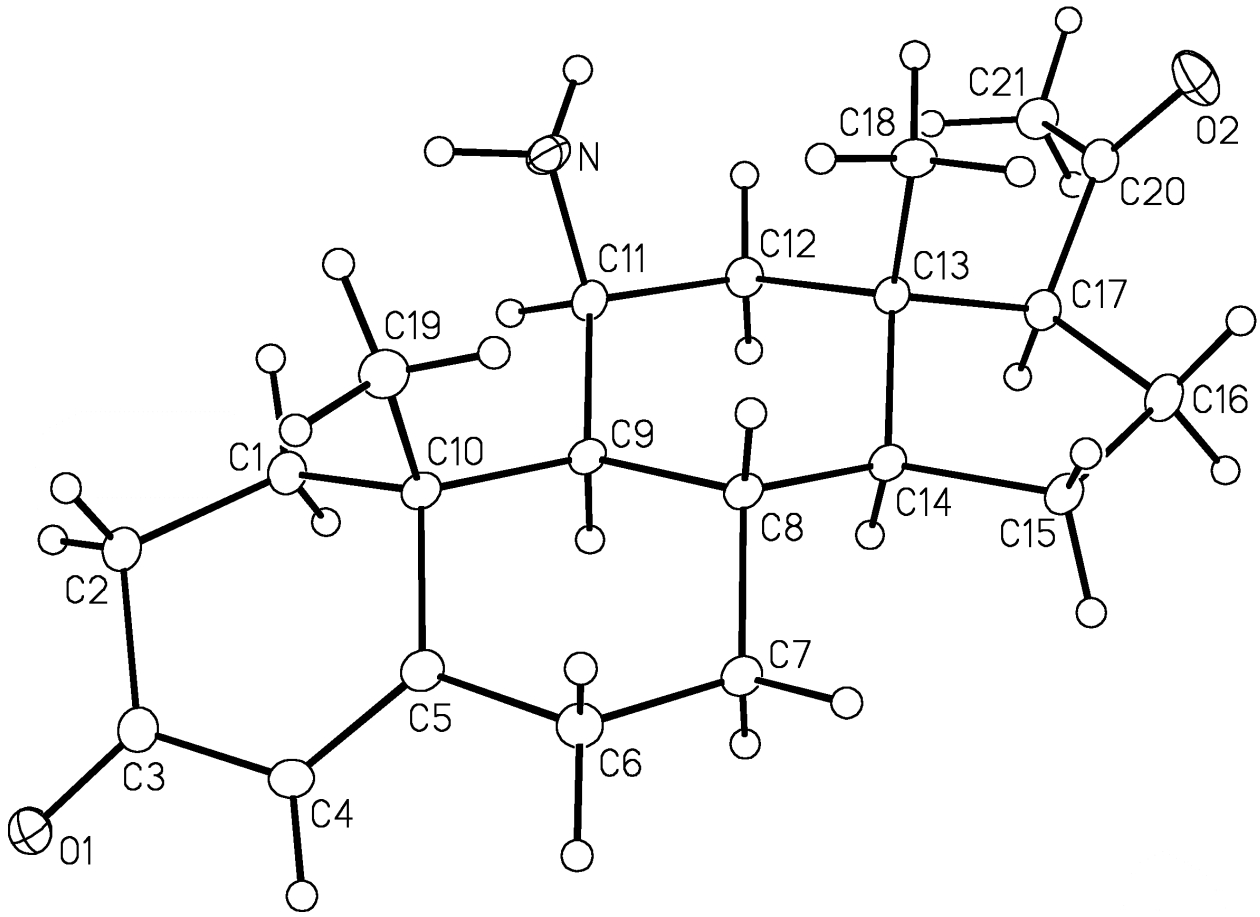


Figure Legends

Figure 1. Perspective view of the 11β-aminoprogesterone molecule showing the atom labelling scheme. Non-hydrogen atoms are represented by Gaussian ellipsoids at the 20% probability level. Hydrogen atoms are shown with arbitrarily small thermal parameters.

Figure 2. Illustration of hydrogen-bonded interactions between adjacent molecules of 11β-aminoprogesterone within the crystal lattice. Primed atoms are related to unprimed ones via the crystallographic rotational-translational symmetry operation ($1–x$, $^{-}1/2+y$, $^{1}/2–z$). Double-primed atoms are related to unprimed ones eq \o(z,¯)). Starred (*) atoms are related to unprimed ones via the rotational-translational symmetry operation ($1–x$, $^{1}/2+y$, $^{1}/2–z$). Atoms marked with an octothorpe (#) are related to unprimed ones via the rotational-translational eq \o(z,¯)).


C21
O2
C18
N
C20
C11
C12
C13
C17
C19
C16
C1
C10
C9
C14
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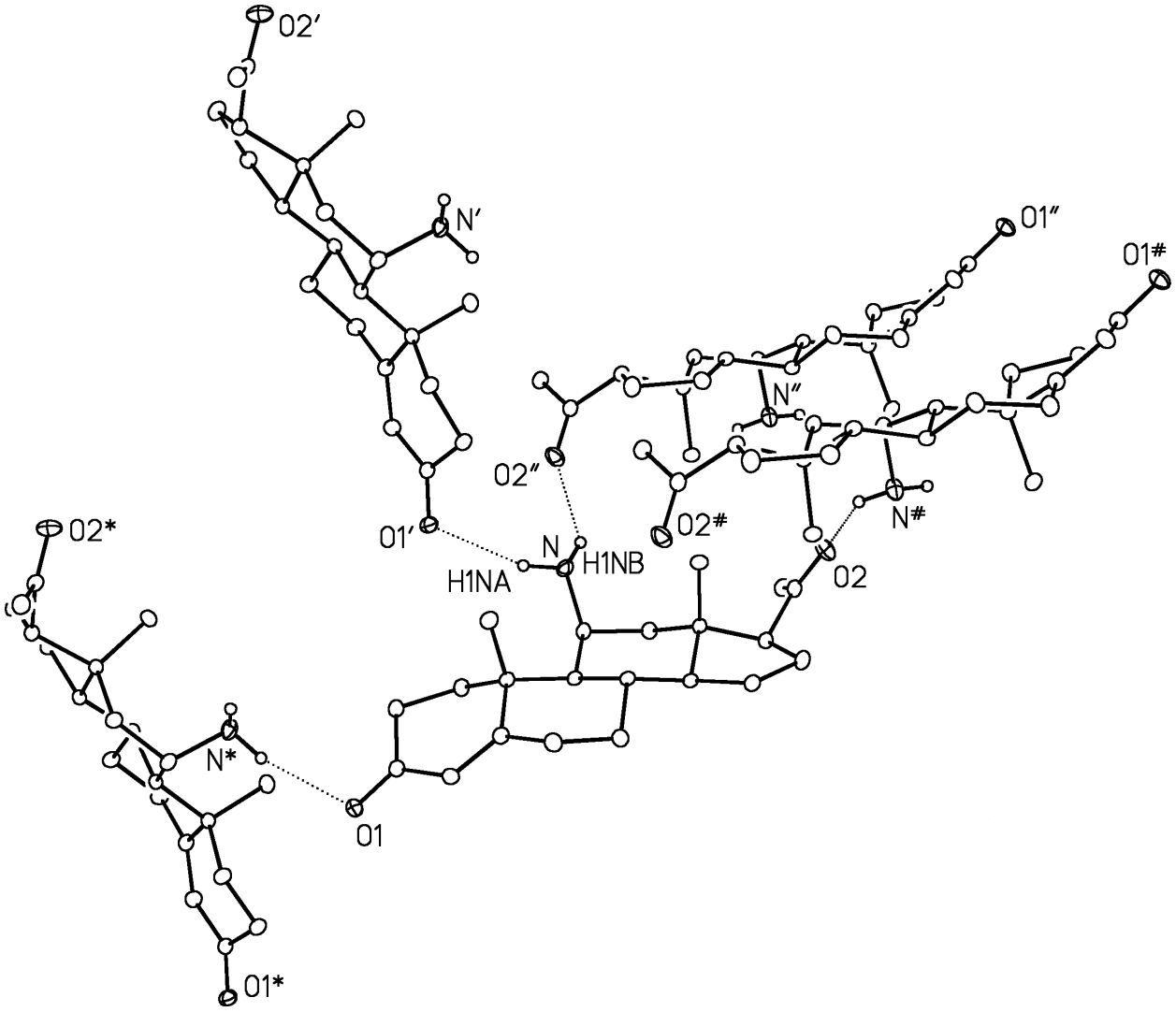
C15
C2
C7
C5
C6
C3
C4
O1


O2′
N′
O1″
O1#
N″
O2″
O2#
N#
O1′
N
H1NA
H1NB
O2
O2*
N*
O1
O1*

List of Tables

Table 1. Crystallographic Experimental Details

A. Crystal Data	
formula	$C_{21}H_{31}NO_2$
formula weight	329.47
crystal dimensions (mm)	0.88 × 0.23 × 0.21
crystal system	orthorhombic
space group	$P2_12_12_1$ (No. 19)
unit cell parameters[a]	
a (Å)	7.4436 (3)
b (Å)	11.5809 (4)
c (Å)	20.5000 (7)
V (Å^3)	1767.17 (11)
Z	4
ρ_{calcd} (g cm^{-3})	1.238
μ (mm^{-1})	0.078
B. Data Collection and Refinement Conditions	
diffractometer	Bruker D8/APEX II CCD[b]
radiation (λ [Å])	graphite-monochromated Mo Kα (0.71073)
temperature (°C)	–100
scan type	ω scans (0.3°) (20 s exposures)
data collection 2θ limit (deg)	55.06
total data collected	15583 ($-9 \le h \le 9$, $-15 \le k \le 15$, $-26 \le l \le 26$)
independent reflections	2340 (R_{int} = 0.0198)
number of observed reflections (*NO*)	2210 [$F_o^2 \ge 2\sigma(F_o^2)$]
structure solution method	direct methods (*SHELXD*[c])
refinement method	full-matrix least-squares on F^2 (*SHELXL–97*[d])
absorption correction method	Gaussian integration (face-indexed)
range of transmission factors	0.9839–0.9347
data/restraints/parameters	2340 / 0 / 221
Flack absolute structure parameter[e]	1.6(15)
goodness-of-fit (*S*)[f] [all data]	1.067
final *R* indices[g]	
R_1 [$F_o^2 \ge 2\sigma(F_o^2)$]	0.0330
wR_2 [all data]	0.0930
largest difference peak and hole	0.293 and –0.143 e Å^{-3}

[a]Obtained from least-squares refinement of 9965 reflections with $5.30° < 2\theta < 55.02°$.

[b]Programs for diffractometer operation, data collection, data reduction and absorption correction were those supplied by Bruker.

(continued)

Table 1. Crystallographic Experimental Details (continued)

[c]Schneider, T. R.; Sheldrick, G. M. *Acta Crystallogr.* **2002**, *D58*, 1772-1779.

[d]Sheldrick, G. M. *Acta Crystallogr.* **2008**, *A64*, 112–122.

[e]Flack, H. D. *Acta Crystallogr.* **1983**, *A39*, 876–881; Flack, H. D.; Bernardinelli, G. *Acta Crystallogr.* **1999**, *A55*, 908–915; Flack, H. D.; Bernardinelli, G. *J. Appl. Cryst.* **2000**, *33*, 1143–1148. The Flack parameter will refine to a value near zero if the structure is in the correct configuration and will refine to a value near one for the inverted configuration. The low anomalous scattering power of the atoms in this structure (none heavier than oxygen) implies that the data cannot be used for absolute structure assignment, thus the Flack parameter is provided for informational purposes only. The absoulute stereochemistry has been assigned based on the known stereochemistry of the progesterone-derived precursor compounds.

[f]$S = [\Sigma w(F_o^2 - F_c^2)^2/(n - p)]^{1/2}$ (n = number of data; p = number of parameters varied; $w = [\sigma^2(F_o^2) + (0.0609P)^2 + 0.2143P]^{-1}$ where $P = [\text{Max}(F_o^2, 0) + 2F_c^2]/3$).

[g]$R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$; $wR_2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^4)]^{1/2}$.

Table 2. Atomic Coordinates and Equivalent Isotropic Displacement Parameters

Atom	*x*	*y*	*z*	U_{eq}, Å^2
O1	0.2891(2)	0.47718(10)	0.25310(6)	0.0406(3)*
O2	0.3316(2)	-0.27713(12)	-0.12857(7)	0.0464(4)*
N	0.4959(2)	-0.05509(14)	0.11388(7)	0.0372(3)*
C1	0.4547(2)	0.22060(15)	0.17402(8)	0.0290(3)*
C2	0.4368(2)	0.29665(15)	0.23461(8)	0.0319(4)*
C3	0.2842(2)	0.38139(14)	0.22791(8)	0.0300(3)*
C4	0.1275(2)	0.34079(15)	0.19160(8)	0.0323(4)*
C5	0.1208(2)	0.23908(15)	0.15948(8)	0.0275(3)*
C6	-0.0521(2)	0.19721(15)	0.12957(9)	0.0308(4)*
C7	-0.0253(2)	0.15290(14)	0.05995(8)	0.0284(3)*
C8	0.12394(19)	0.06229(13)	0.05529(7)	0.0221(3)*
C9	0.29932(19)	0.11407(13)	0.08285(7)	0.0214(3)*
C10	0.2804(2)	0.15737(13)	0.15505(7)	0.0236(3)*
C11	0.4720(2)	0.04286(14)	0.06918(8)	0.0261(3)*
C12	0.48010(19)	-0.00077(14)	-0.00207(7)	0.0256(3)*
C13	0.3069(2)	-0.05846(13)	-0.02545(7)	0.0219(3)*
C14	0.1539(2)	0.02895(13)	-0.01587(7)	0.0229(3)*
C15	-0.0036(2)	-0.02176(14)	-0.05473(8)	0.0283(3)*
C16	0.0869(2)	-0.08535(16)	-0.11263(8)	0.0343(4)*
C17	0.2925(2)	-0.07814(14)	-0.10081(7)	0.0266(3)*
C18	0.2705(2)	-0.17267(13)	0.01008(8)	0.0280(3)*
C19	0.2361(3)	0.06037(15)	0.20518(8)	0.0333(4)*
C20	0.3976(2)	-0.18178(15)	-0.12545(8)	0.0296(3)*
C21	0.5897(2)	-0.16202(16)	-0.14564(9)	0.0335(4)*

Anisotropically-refined atoms are marked with an asterisk (*). The form of the anisotropic displacement parameter is: $\exp[-2\pi^2(h^2a^{*2}U_{11} + k^2b^{*2}U_{22} + l^2c^{*2}U_{33} + 2klb^*c^*U_{23} + 2hla^*c^*U_{13} + 2hka^*b^*U_{12})]$.

Table 3. Selected Interatomic Distances (Å)

Atom1	Atom2	Distance	Atom1	Atom2	Distance
O1	C3	1.224(2)	C9	C10	1.569(2)
O2	C20	1.210(2)	C9	C11	1.552(2)
N	C11	1.469(2)	C10	C19	1.558(2)
C1	C2	1.528(2)	C11	C12	1.547(2)
C1	C10	1.540(2)	C12	C13	1.529(2)
C2	C3	1.507(2)	C13	C14	1.536(2)
C3	C4	1.461(2)	C13	C17	1.565(2)
C4	C5	1.350(2)	C13	C18	1.534(2)
C5	C6	1.506(2)	C14	C15	1.534(2)
C5	C10	1.522(2)	C15	C16	1.551(2)
C6	C7	1.530(2)	C16	C17	1.552(2)
C7	C8	1.531(2)	C17	C20	1.519(2)
C8	C9	1.5437(19)	C20	C21	1.506(2)
C8	C14	1.525(2)			

Table 4. Selected Interatomic Angles (deg)

Atom1	Atom2	Atom3	Angle	Atom1	Atom2	Atom3	Angle
C2	C1	C10	113.93(13)	C9	C10	C19	114.26(12)
C1	C2	C3	111.53(13)	N	C11	C9	113.44(13)
O1	C3	C2	121.96(16)	N	C11	C12	109.39(13)
O1	C3	C4	122.01(17)	C9	C11	C12	112.12(12)
C2	C3	C4	116.00(14)	C11	C12	C13	113.93(12)
C3	C4	C5	123.98(16)	C12	C13	C14	107.29(12)
C4	C5	C6	120.76(15)	C12	C13	C17	115.51(12)
C4	C5	C10	122.84(15)	C12	C13	C18	112.15(12)
C6	C5	C10	116.28(13)	C14	C13	C17	99.86(12)
C5	C6	C7	112.11(13)	C14	C13	C18	112.11(12)
C6	C7	C8	112.50(13)	C17	C13	C18	109.33(12)
C7	C8	C9	108.95(12)	C8	C14	C13	113.40(12)
C7	C8	C14	109.82(12)	C8	C14	C15	118.81(13)
C9	C8	C14	108.96(12)	C13	C14	C15	104.36(12)
C8	C9	C10	113.17(12)	C14	C15	C16	104.31(12)
C8	C9	C11	115.32(12)	C15	C16	C17	106.46(13)
C10	C9	C11	114.48(12)	C13	C17	C16	103.27(13)
C1	C10	C5	110.31(12)	C13	C17	C20	114.08(13)
C1	C10	C9	108.33(12)	C16	C17	C20	114.43(14)
C1	C10	C19	110.78(13)	O2	C20	C17	121.96(16)
C5	C10	C9	108.97(12)	O2	C20	C21	120.62(16)
C5	C10	C19	104.11(13)	C17	C20	C21	117.41(15)

Table 5. Hydrogen-Bonded Interactions

D–H···A	D–H (Å)	H···A (Å)	D···A (Å)	∠D–H···A (deg)	Note
N–H1NA···O1[a]	0.91	2.33	3.1841(19)	156.4	[a]At 1–*x*, −1/2+*y*, 1/2–*z*.
N–H1NB···O2[b]	0.91	2.41	3.180(2)	142.2	eq \o(*z*,¯).

Table 6. Torsional Angles (deg)

Atom1	Atom2	Atom3	Atom4	Angle
C10	C1	C2	C3	-54.21(19)
C2	C1	C10	C5	44.07(18)
C2	C1	C10	C9	163.27(13)
C2	C1	C10	C19	-70.66(17)
C1	C2	C3	O1	-147.83(17)
C1	C2	C3	C4	34.3(2)
O1	C3	C4	C5	175.48(17)
C2	C3	C4	C5	-6.7(3)
C3	C4	C5	C6	173.04(16)
C3	C4	C5	C10	-2.9(3)
C4	C5	C6	C7	133.72(17)
C10	C5	C6	C7	-50.1(2)
C4	C5	C10	C1	-15.9(2)
C4	C5	C10	C9	-134.72(16)
C4	C5	C10	C19	102.97(18)
C6	C5	C10	C1	168.00(14)
C6	C5	C10	C9	49.19(18)
C6	C5	C10	C19	-73.12(17)
C5	C6	C7	C8	52.74(18)
C6	C7	C8	C9	-56.23(17)
C6	C7	C8	C14	-175.49(13)
C7	C8	C9	C10	57.23(16)
C7	C8	C9	C11	-168.26(12)
C14	C8	C9	C10	177.03(11)
C14	C8	C9	C11	-48.46(16)
C7	C8	C14	C13	177.77(12)
C7	C8	C14	C15	-59.11(17)
C9	C8	C14	C13	58.51(16)
C9	C8	C14	C15	-178.37(12)
C8	C9	C10	C1	-172.91(12)
C8	C9	C10	C5	-52.87(16)
C8	C9	C10	C19	63.09(16)
C11	C9	C10	C1	52.19(16)
C11	C9	C10	C5	172.23(12)
C11	C9	C10	C19	-71.82(17)
C8	C9	C11	N	-80.33(16)
C8	C9	C11	C12	44.20(17)
C10	C9	C11	N	53.58(17)
C10	C9	C11	C12	178.12(12)
N	C11	C12	C13	78.33(16)
C9	C11	C12	C13	-48.42(17)
C11	C12	C13	C14	56.20(16)
C11	C12	C13	C17	166.52(13)
C11	C12	C13	C18	-67.32(16)
C12	C13	C14	C8	-62.45(16)
C12	C13	C14	C15	166.79(11)
C17	C13	C14	C8	176.75(12)
C17	C13	C14	C15	46.00(14)
C18	C13	C14	C8	61.09(16)
C18	C13	C14	C15	-69.66(15)
C12	C13	C17	C16	-156.43(14)
C12	C13	C17	C20	78.76(17)
C14	C13	C17	C16	-41.78(15)
C14	C13	C17	C20	-166.58(13)
C18	C13	C17	C16	75.98(16)
C18	C13	C17	C20	-48.83(17)
C8	C14	C15	C16	-159.73(13)
C13	C14	C15	C16	-32.24(16)
C14	C15	C16	C17	5.24(18)
C15	C16	C17	C13	22.90(18)
C15	C16	C17	C20	147.48(14)
C13	C17	C20	O2	89.8(2)
C13	C17	C20	C21	-89.73(17)
C16	C17	C20	O2	-28.9(2)
C16	C17	C20	C21	151.64(15)

Table 7. Anisotropic Displacement Parameters (U_{ij}, Å^2)

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O1	0.0512(8)	0.0309(6)	0.0398(7)	-0.0062(5)	-0.0129(6)	0.0032(6)
O2	0.0407(7)	0.0370(7)	0.0615(9)	-0.0158(6)	0.0077(7)	-0.0103(6)
N	0.0394(8)	0.0419(8)	0.0303(7)	-0.0025(6)	-0.0088(6)	0.0185(7)
C1	0.0207(7)	0.0344(8)	0.0319(8)	-0.0042(7)	-0.0040(6)	-0.0005(7)
C2	0.0298(8)	0.0344(8)	0.0316(8)	-0.0038(7)	-0.0081(7)	0.0010(7)
C3	0.0346(8)	0.0299(7)	0.0255(7)	0.0009(6)	-0.0022(7)	-0.0012(7)
C4	0.0304(8)	0.0298(7)	0.0368(8)	-0.0028(6)	-0.0057(7)	0.0076(7)
C5	0.0208(7)	0.0324(8)	0.0292(7)	-0.0007(6)	-0.0004(6)	0.0027(6)
C6	0.0191(7)	0.0357(8)	0.0376(8)	-0.0052(7)	0.0002(7)	0.0024(6)
C7	0.0177(7)	0.0323(7)	0.0353(8)	-0.0050(6)	-0.0043(6)	0.0029(6)
C8	0.0170(6)	0.0232(6)	0.0260(7)	0.0030(6)	-0.0017(5)	-0.0003(6)
C9	0.0162(6)	0.0241(6)	0.0240(6)	0.0029(5)	-0.0009(5)	-0.0004(6)
C10	0.0191(7)	0.0259(7)	0.0257(7)	0.0018(6)	-0.0017(6)	0.0012(6)
C11	0.0166(6)	0.0341(8)	0.0276(7)	-0.0038(6)	-0.0029(6)	0.0016(6)
C12	0.0167(6)	0.0317(7)	0.0286(7)	-0.0016(6)	0.0023(6)	-0.0019(6)
C13	0.0180(6)	0.0245(7)	0.0233(6)	0.0015(5)	-0.0006(5)	-0.0017(6)
C14	0.0197(7)	0.0238(7)	0.0252(7)	0.0033(5)	-0.0025(6)	-0.0013(6)
C15	0.0214(7)	0.0314(7)	0.0321(8)	-0.0008(6)	-0.0063(6)	0.0009(6)
C16	0.0275(8)	0.0453(9)	0.0303(8)	-0.0045(7)	-0.0070(7)	0.0013(8)
C17	0.0258(7)	0.0299(7)	0.0242(7)	0.0009(6)	-0.0007(6)	-0.0025(7)
C18	0.0252(7)	0.0259(7)	0.0328(8)	0.0055(6)	0.0016(6)	0.0001(6)
C19	0.0363(9)	0.0360(8)	0.0276(7)	0.0046(7)	0.0048(7)	0.0007(8)
C20	0.0303(8)	0.0343(8)	0.0243(7)	-0.0025(6)	-0.0008(6)	-0.0019(7)
C21	0.0297(8)	0.0389(9)	0.0318(8)	0.0010(7)	0.0025(7)	0.0016(8)

The form of the anisotropic displacement parameter is:
$\exp[-2\pi^2(h^2a^{*2}U_{11} + k^2b^{*2}U_{22} + l^2c^{*2}U_{33} + 2klb^*c^*U_{23} + 2hla^*c^*U_{13} + 2hka^*b^*U_{12})]$

Table 8. Derived Atomic Coordinates and Displacement Parameters for Hydrogen Atoms

Atom	*x*	*y*	*z*	U_{eq}, Å^2
H1NA	0.5332	-0.0287	0.1534	0.045
H1NB	0.5796	-0.1042	0.0973	0.045
H1A	0.5500	0.1626	0.1819	0.035
H1B	0.4934	0.2693	0.1369	0.035
H2A	0.4158	0.2472	0.2733	0.038
H2B	0.5503	0.3394	0.2416	0.038
H4	0.0242	0.3889	0.1905	0.039
H6A	-0.1402	0.2613	0.1291	0.037
H6B	-0.1022	0.1344	0.1568	0.037
H7A	0.0047	0.2188	0.0312	0.034
H7B	-0.1392	0.1187	0.0442	0.034
H8	0.0897	-0.0077	0.0810	0.026
H9	0.3168	0.1865	0.0571	0.026
H11	0.5766	0.0958	0.0757	0.031
H12A	0.5072	0.0654	-0.0310	0.031
H12B	0.5801	-0.0567	-0.0061	0.031
H14	0.1910	0.1010	-0.0391	0.027
H15A	-0.0843	0.0401	-0.0706	0.034
H15B	-0.0739	-0.0762	-0.0276	0.034
H16A	0.0549	-0.0478	-0.1544	0.041
H16B	0.0474	-0.1669	-0.1142	0.041
H17	0.3390	-0.0076	-0.1233	0.032
H18A	0.1600	-0.2074	-0.0070	0.034
H18B	0.2568	-0.1579	0.0569	0.034
H18C	0.3714	-0.2256	0.0030	0.034
H19A	0.3471	0.0208	0.2179	0.040
H19B	0.1533	0.0047	0.1853	0.040
H19C	0.1799	0.0945	0.2439	0.040
H21A	0.6481	-0.2365	-0.1536	0.040
H21B	0.6535	-0.1210	-0.1108	0.040
H21C	0.5926	-0.1156	-0.1856	0.040