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Synthetic studies on kinase inhibitors and cyclic peptides : strategies towards new antibiotics

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**Synthetic Studies on kinase Inhibitors and Cyclic Peptides:
Strategies Towards New Antibiotics**

Proefschrift

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de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof.mr. P.F. van der Heijden,
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Adriaan Willem Tuin

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List of Abbreviations

Å	Ångström	CaMK	Ca ²⁺ /Calmodulin dependent
AA	amino acid		kinase
AAA	azidoethoxy acetic acid	cAMP	cyclic adenosine
AAC	acyltransferase		monophosphate
Ac	acetyl	CAP	cationic antimicrobial peptide
AChE	acetylcholinesterase	Cdc	cell division cycle
ACN	acetonitrile	CDK	cyclin dependent kinase
AGC	PKA, PKG and PKC	cGMP	cyclic guadenosine
AIBN	azobisisobutyronitrile		monophosphate
Akt	V-akt murine thymoma viral oncogene homolog 1	CHAPS	3-[(3- cholamidopropyl) dimethylammonio]-1-
Ala (A)	alanine		propanesulfonate
AMP	adenosine monophosphate	CLK	Cdc2-like kinase
AMP-PNP	5'-adenylylimido-diphosphate	CK1	casein kinase 1
APH	aminoglycoside phosphotransferase	CMGC	CDK, MAPK GSK3 and CLK
aq.	aqueous	COSY	correlation spectroscopy
ARHGEF2	rho/rac guanine nucleotide exchange factor 2	C _q	quaternary carbon
Arg (R)	arginine	CTRL	control
arom	aromatic	Cys (C)	cysteine
AS160	Akt substrate of 160kD	D	dimensional
Asn (N)	asparagine	d	doublet
Asp (D)	aspartic acid	dd	doublet of doublets
ATP	adenosine triphosphate	Dab	2,4-diaminobutyric acid
ATCC	American Type Culture Collection	Dap	2,3-diaminopropanoic acid
BHA	benzylamine	DBU	1,8-diazabicyclo[5,4,0]undec- 7-ene
Bn	benzyl	DCM	dichloromethane
boc	<i>tert</i> -butyloxycarbonyl	DiBAL-H	diisobutylaluminium hydride
bs	broad singlet	DiPEA	<i>N,N</i> -diisopropyl- <i>N</i> -ethylamine
bu	butyl	DMF	<i>N,N</i> -dimethylformamide
BuChE	butyryl cholinesterase	DMSO	dimethylsulfoxide
		DNA	deoxyribonucleic acid
		DTT	1,4-Dithio-DL-threitol

EDTA	ethylenediaminetetraacetic acid	IRC	insulin responsive compartment
eq.	equivalents	<i>J</i>	coupling constant
ESI	electrospray interface	LCMS	liquid chromatography mass spectrometry
et	ethyl		
FAB	fast atom bombardment	Leu (L)	Leucine
FACS	fluorescence activated cell sorting	Lys (K)	lysine
FDA	Food and Drug Administration	M	molar
Fmoc	9 <i>H</i> -fluoren-9-ylmethoxycarbonyl	<i>m</i>	meta
Glu (E)	glutamic acid	m	multiplet
GLUT4	glucose transporter 4	MALDI-TOF	Matrix-assisted laser desorption/ionization time of flight
Gly (G)	glycine	MBHA	p-Methylbenzhydramine
GS	Gramicidin S	MIC	minimal inhibitory concentration
GSK	glucagon synthase kinase	MAPK	mitogen activated protein kinase
HCTU	2-(6-Chloro-1 <i>H</i> -benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate	me	methyl
hept	heptet	Met (M)	methionine
His (H)	histidine	MLCK	myosin light chain kinase
HM	hydrophobic motif	MLK	mixed lineage kinase
HMPB	4-(4-hydroxymethyl-3-methoxy-phenoxy)-butyric acid	MQ	Millipore water
HOBt	Hydroxybenzotriazole	Nle	norleucine
HPLC	high performance liquid chromatography	NMR	nuclear magnetic resonance
HTS	high throughput screening	NOESY	nuclear Overhauser enhancement spectroscopy
HWE	Horner-Wadsworth-Emmons	<i>o</i>	ortho
Hz	hertz	Orn (O)	ornithine
Ile (I)	isoleucine	<i>p</i>	para
IPA	isopropanol	PAF	Platelet-activating factor
<i>i</i> Pr.	isopropyl	PAK4	p21 protein (Cdc42/Rac)-activated kinase 4
		PAM2-Cl	2-pyridiniumalldoxim- <i>N</i> -methyl chloride

List of Abbreviations

PBS	phosphate buffered saline	r.t.	room temperature
PDK1	phosphoinositide dependent kinase 1	s	singlet
PE	petroleum ether	SAA	sugar amino acid
PH	Pleckstrin homology	SAR	structure activity relation
Phe (F)	phenylalanine	sat.	saturated
pip.	piperidine	SCV	<i>salmonella</i> containing vacuole
PKA	protein kinase A	SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel
PKB	protein kinase B		electrophoresis
PKC	protein kinase C	Ser (S)	serine
PKG	protein kinase G	siRNA	short interfering RNA
PNA	peptide nucleic acid	SopB	<i>Salmonella</i> outer protein B
ppm	parts per million	SPPS	solid phase peptide synthesis
PP2A	protein phosphatases II A	STE	homologues of yeast sterile kinase
Pr	propyl	Su	succinimide
Pro (P)	proline	t	triplet
PtdIns-3,4,5- P_3	phosphatidylinositol-3,4,5- triphosphate	TBAT	tetrabutylammonium triphenyldifluorosilicate
PTK	protein tyrosine kinase	<i>t</i> Bu	<i>tert</i> -butyl
PyBOP	benzotriazol-1-yl- oxytripyrrolidinophosphonium hexafluorophosphate	TEMPO	2,2,6,6-tetramethyl-1- piperidinyloxy, free radical
pyr.	pyridine	THF	tetrahydrofuran
q	quartet	Thr (T)	threonine
quant.	quantitative	TLC	thin layer chromatography
RAB-GAP	Rab GTPase activating protein	TOCSY	total correlation spectroscopy
RAC-PK	related to A and C protein kinase	TRIS	tris(hydroxymethyl) aminomethane
RC DC	reducing agent compatible and detergent compatible	Trp (W)	tryptophan
RGC	receptor guanylyl cyclase	Trt	trityl
RHOA	Ras homolog gene family, member A	Ts	<i>p</i> -toluenesulfonyl
RNA	ribonucleic acid	Tyr (Y)	tyrosine
RP	reversed phase	v	volume
		Val (V)	valine

Chapter 1 | General Introduction

Bacterial resistance against antibiotics

Shortly after the introduction of penicillin, bacterial strains that were insensitive towards treatment with this substance were discovered.¹ In spite of the continued development of novel antibiotics in the following decades, bacterial resistance has now become a global health threat.² To counteract bacterial resistance developing new antibiotics at established targets is not enough since the introduction of new antibiotics on the market is usually followed within months to years by bacterial resistance.^{2b} Therefore, the development of new classes of antibiotics should be accompanied by a search for new targets.

Aim and outline of this thesis

In **Chapter 2** of this thesis, a short introduction is given about the kinase superfamily and their key role in regulating a wide variety of physiological processes. Ill-regulated kinase activity has been implicated in many different tumors. Recently, over-stimulated activity of a host cell protein, an isoform of protein kinase B (PKB/Akt1), has been implicated in bacterial infection from pathogenic bacteria like *Salmonella*. Inhibitors for this class of enzymes have therefore not only attracted great interest in the field of oncology but can now also be

viewed as potential antibiotics. This chapter is concluded by an overview of currently developed inhibitors against PKB/Akt1. **Chapter 3** describes the development of chemical inhibitors against PKB/Akt1 based on the known pharmacophore H-89. First, the H-89 skeleton was evaluated for positions that could be modified to improve potency and selectivity. Next, the synthesis of a library of derivatives varying at two positions on the H-89 core structure resulted in the identification of a compound with desirable pharmacological properties in terms of selectivity between closely related kinases and *in vivo* potency for treating *Salmonella* infections. **Chapter 4** pursues the strategy described in chapter 3 by the construction of a 63-membered library in a parallel fashion using the Suzuki cross coupling reaction.

Besides the use of kinase inhibitors, cationic antimicrobial peptides (CAPs), like Gramicidin S (GS) can also be used to kill bacteria. This class of compounds disrupts the cell wall membrane of bacteria causing lysis of the cell. Although bacterial resistance to this class of compounds is low, their clinical applicability is hampered by their non-specific mode of action. Their hemotoxicity restricts this class of compounds to topical use. **Chapter 5** describes the perpetuation of previous finding regarding the structure-activity relationship of GS analogues. The synthesis and in depth structural and biological evaluation of 3 novel analogues is presented. **Chapter 6** deals with the synthesis of hybrid structures between GS and a related CAP, Loloatin C, and the analysis of their solution structures and biological activities.

Chapter 7 describes the development of a novel biotinylated methylfluorophosphonate and its use in a study directed towards the identification of novel targets of organophosphate based nerve agents. The same probe is also used in a preliminary study aimed at optimizing the isolation procedure of the human enzyme butyrylcholinesterase (HuBuChE) from plasma. This enzyme is currently being exploited as scavenger for organophosphate toxicants.

Chapter 8 discusses some future directions for the research described in this thesis.

References

¹ W.M.M. Kirby, *Science*, **1944**, 99, 219 – 220.

² For a review see: a) H. Yoneyama, R. Katsumata, *Biosci. Biotechnol. Biochem.*, **2006**, 70, 1060 – 1075. b) C. Walsh, *NATURE*, **2000**, 406, 775 – 781.

Chapter 2 | Kinase Inhibitors and Their Potential Role as Novel Antibiotics: An overview of the current state of the art

Introduction

With more than 500 members, the protein kinase family constitutes one of the largest families of proteins in man. Kinases are involved in a wide variety of cellular signaling pathways¹ and play key roles in regulating² most physiological processes including metabolism, cell growth, cell division, cell differentiation, cell motility, organelle trafficking, membrane transport, muscle contraction and immunity.³ Kinases alter the activity of their substrates by translocating the γ -phosphate group of ATP onto a suitable phosphate acceptor as depicted in Figure 1. This can be the hydroxyl group of a carbohydrate,⁴ a lipid⁵ and of a serine, threonine or tyrosine residue in proteins.³ Phosphorylation is not limited to oxygen based acceptors. Especially in prokaryotes, the nitrogen containing side chains in histidine, lysine and arginine can be phosphorylated through the action of kinases.⁶

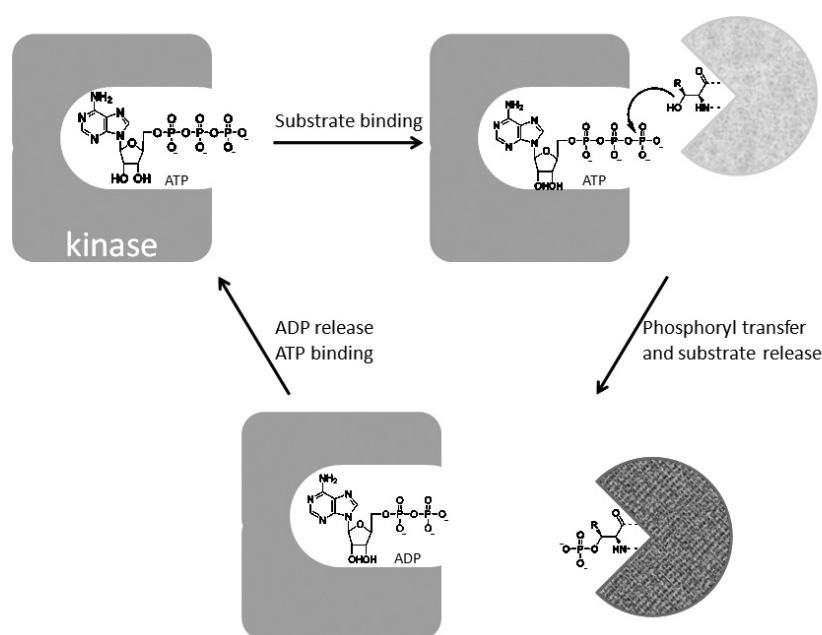


Figure 1; General mode of action of kinases

The kinase superfamily is normally divided into four major families (The AGC, CaMK, CMGC and PTK) complemented by four minor families (The PTK-like, RGC, CK1 and STE family), and this subdivision is primarily based on comparative genetic sequence analysis.⁷ Briefly, these eight subcategories have the following distinctive characteristics.

The AGC-family includes the cyclic AMP-dependent protein kinase (PKA) subfamily, the cyclic GMP-dependent protein kinase (PKG) subfamily and the diacylglycerol-activated/phospholipid-dependent protein kinase (PKC) subfamily. The preferred phosphorylation site for members of this family is a serine or threonine residue which is in close proximity to an N-terminal basic amino acid such as arginine or lysine. They regulate a vast amount of signaling processes including the response to insulin, protection against apoptosis and cytoskeletal organization.⁸

The Ca^{2+} /calmodulin dependent protein kinases⁹ (CaMK family) are also directed at Ser/Thr residues with nearby basic residues. Intracellular Ca^{2+} binds to calmodulin, a small protein that subsequently undergoes a conformational change, enabling binding to a number of other proteins including the members of this kinase family. CAM kinases regulate muscle contraction, ion channel permeability, gene transcription and protein synthesis.

The CMGC-family (containing the CDK, MAPK, GSK3, CLK subfamilies) preferentially phosphorylates Ser/Thr residues in a proline rich environment although the Cdc2-like kinase

family (CLK) also phosphorylates tyrosine residues. Family members regulate cell division (cyclin dependent kinases, CDKs),¹⁰ embryogenesis (mitogen activated protein kinases, MAPKs)¹¹ and tumor genesis (glycogen synthase kinase 3, GSK3).¹²

The protein tyrosine kinase family (PTKs) differs from the previously described families in their preferred substrate specificity.³ Members of this family possess a deeper and wider catalytic cleft preventing the smaller Ser/Thr residues to extend far enough into the cleft for efficient phosphorylation. PTKs play important roles in cell and tissue development.¹³

The PTK-like family is a diverse set of kinases including both Ser/Thr and Tyr kinases.¹ Amongst its members are the mixed lineage kinases (MLKs) that are Ser/Thr kinases involved in regulating inflammation, immunity and the cell cycle.¹⁴ Although they act as Ser/Thr kinases, three out of their eleven subdomains share sequence similarities with those found in tyrosine kinases.

The receptor guanylyl cyclases (RGCs) subfamily is a small group of enzymes involved in the production of cyclic GMP, an important second messenger molecule used by, for instance, protein kinase G.¹⁵ They contain a domain with significant homology to known kinase domains, however, it does not display kinase activity but rather acts as a structural regulator of the catalytic domain.¹⁶

Members of the casein kinase 1 family (CK1s) are Ser/Thr kinases that are co-factor-independent and constitutively active.¹⁷ Their activity is controlled by the tertiary structure of the substrate protein and restricted by the requirement of preceding phosphorylation of the substrate protein, by other kinases, of residues N-terminal to the target site of the CK1 kinase.

The STE kinase family (homologues of yeast sterile 7, sterile 11 and sterile 20 kinases) consists of kinases that preferentially phosphorylate serine or threonine residues with an arginine residue N-terminal to the phosphorylation site¹⁸ thereby regulating, amongst other things, MAPK activity.¹⁹

Many diseases, ranging from inflammatory disorders to various types of cancer, can be linked to ill-regulated kinase activity.²⁰ Overstimulation of a particular kinase may result in an increased or decreased activity of several, possibly hundreds²¹ of downstream effector proteins, some of which are kinases themselves. The prominent role of this class of enzymes in the development of numerous diseases should make them promising drug candidates.

Many AGC kinases are activated in response to the production of the lipid second messenger PtdIns-3,4,5- P_3 and they require translocation to the cell membrane. In addition, phosphorylation of their catalytic domain is imperative for full activation. Studies on PKA have revealed detailed insights in its regulatory mechanisms and the relation between the kinase structure and its biological function.²⁹ Ever since, PKA has served as a model protein for studying other AGC kinases, including Protein kinase B (PKB).

PKB (also known as Akt or Related to A and C-protein kinase, RAC-PK,) has three isoforms that share more than 90% sequence homology in their catalytic domain:³⁰ PKB α /Akt1, PKB β /Akt2 and PKB γ /Akt3. The physiological function of the specific isoforms has been studied using PKB/Akt knockout mice.³¹ PKB α /Akt1^{-/-} mice suffer from impaired placental development resulting in smaller body size and increased neonatal mortality, PKB β /Akt2^{-/-} mice develop severe diabetes in addition to a mild growth deficiency whereas PKB γ /Akt3^{-/-} mice develop a similar body size compared to wild type mice albeit with significant decreased brain size. Results with double mutant mice suggest extensive functional overlap between the isoforms *in vivo*. Studies using isoform selective and a dual Akt1/2 selective chemical inhibitor indicated that simultaneous inhibition of both Akt1 and Akt2 provided maximal apoptotic response and resulted in the sensitization of several tumor cells to treatment with a number of chemotherapeutic agents.³²

PKB/Akt consists of an N-terminal Pleckstrin Homology domain (PH) which binds to phosphatidylinositol-3,4,5-triphosphate (PtdIns-3,4,5- P_3) allowing localization of PKB/Akt to the membrane, a kinase domain (responsible for the display of activity) and a regulatory domain at the C-terminal containing the Hydrophobic Motif (HM).^{33c} Full activation of PKB/Akt requires phosphorylation on two residues, a threonine residing in the activation loop in the kinase domain and a serine residue in the HM domain.³³

The activation of PKB/Akt and the subsequent display of activity is the result of an intricate regulatory mechanism³⁴ that depends on many factors including cellular localization of PKB/Akt the activity of activating proteins (like PDK1) and inhibitory proteins (for instance Protein Phosphatase 2A, PP2A) and is schematically depicted in Figure 3.^{33c} Upon binding of the second messenger molecule, PtdIns-3,4,5- P_3 , to the PH domain of PKB/Akt, PKB/Akt is recruited to the membrane by lipid / PH domain interactions. Phosphorylation of the

activation loop Thr308 by PDK1 and of Ser473 (by an unidentified kinase, possibly PDK1) within the hydrophobic motif results in a 1000 fold increase in activity. Both phosphorylation processes can be reversed by phosphatases.³⁴

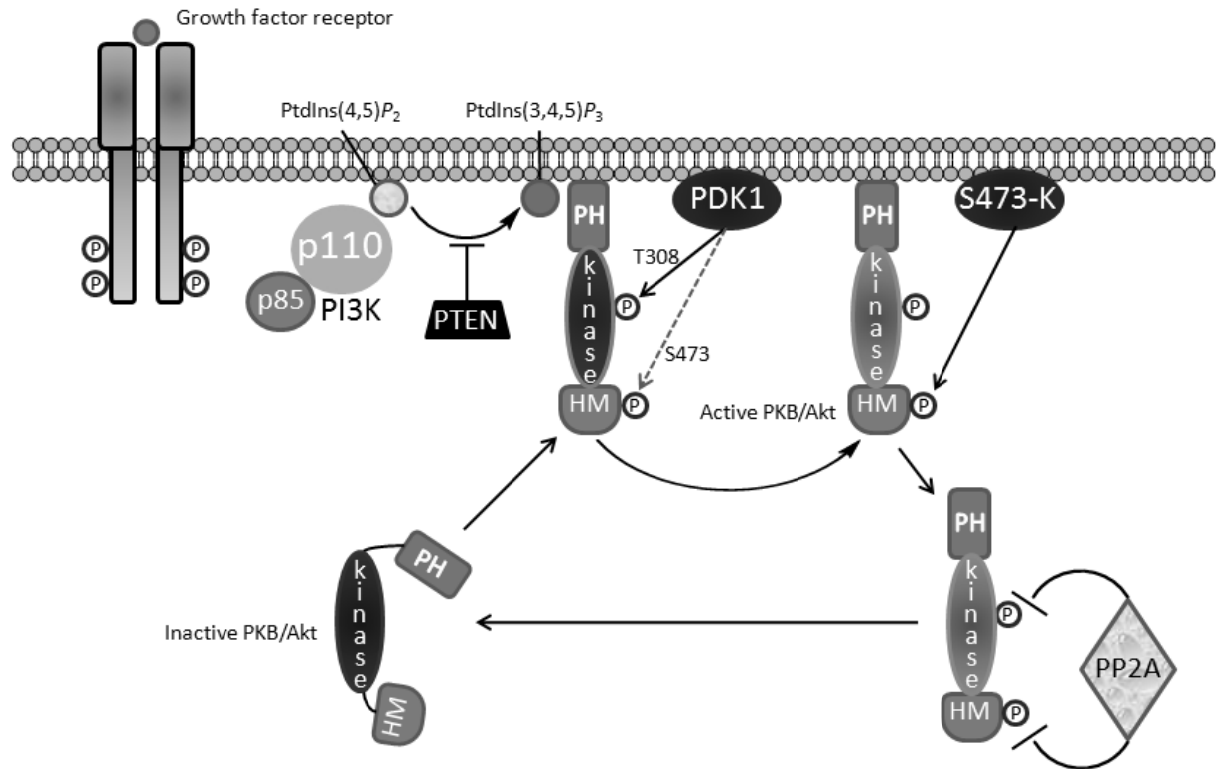


Figure 3; Regulatory mechanism of PKB/Akt

Crystal structure analysis of PKB/Akt in different states of activation³⁵ and in its activated form in the presence of a substrate protein³⁶ has revealed insights in the conformational changes resulting from both phosphorylations and the structural interactions of ATP and the substrate protein with PKB/Akt required for phosphorylation of the substrate.³⁷ The kinase domain of PKB/Akt consists of two lobes, namely the N-terminal lobe and the C terminal lobe (Figure 4).³⁸ The N-terminal lobe contains the PH domain and the phosphorylatable Ser473 (mutated as Asp473). The C-terminal lobe, containing the activation loop and the phosphorylatable Thr309, is connected to the N terminal lobe *via* the hinge region. Phosphorylation of Thr309 results in a large conformational change of the activation loop, thereby exposing the ATP (mimicked by AMP-PNP, an often used ATP analogue, (Figure 4) binding pocket and substrate peptide (GSK3 β) binding groove. Phosphorylation of Ser473 contributes to further stabilization of ATP and substrate binding.

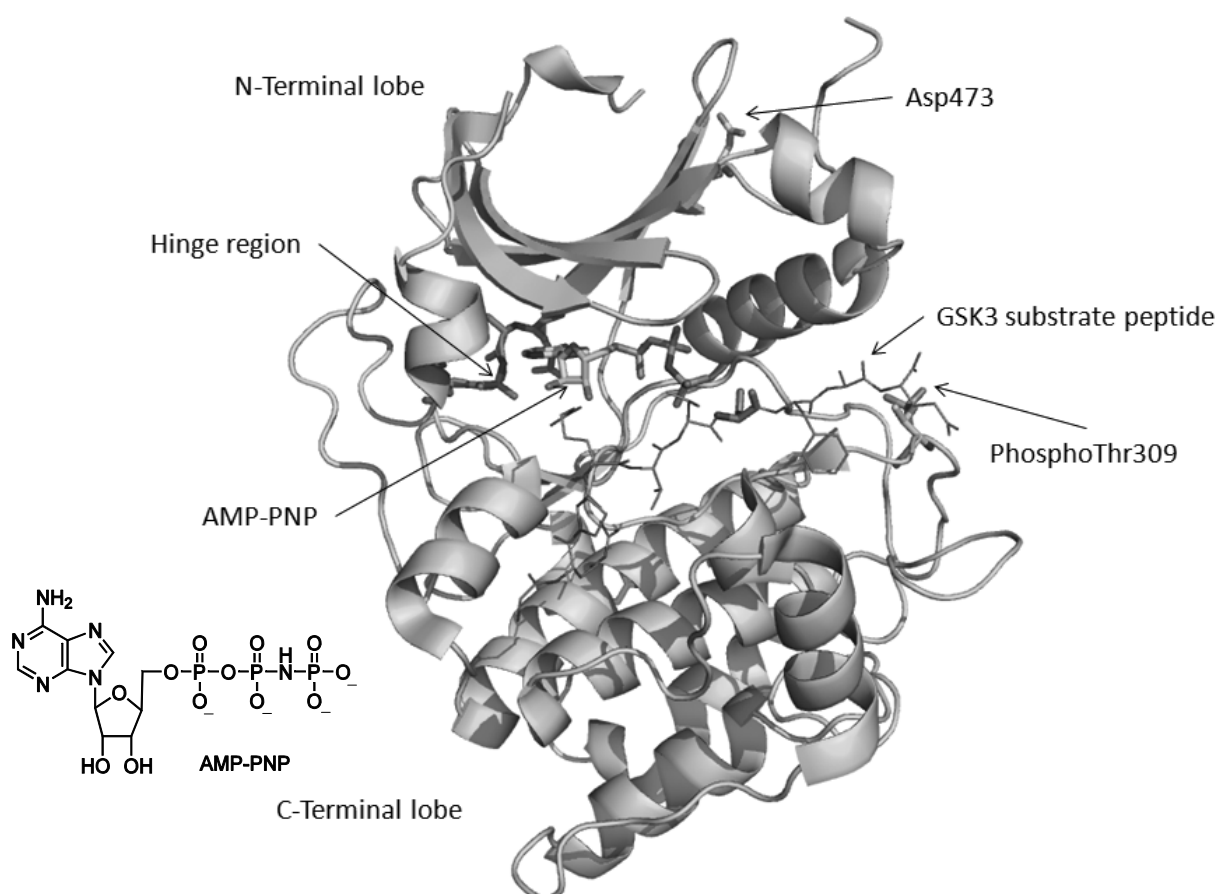


Figure 4; Crystal structure of activated PKB/Akt2 in the presence of AMP-PNP and substrate peptide GSK β ³⁸

In literature over a 100 proteins are suggested as PKB/Akt substrates, although less than 20 contain the minimally required sequence R-X-R-X-X-S/T.^{37d} Still, the involvement of PKB/Akt in a wide range of diseases has been well established.³⁹ For instance, GSK-3,⁴⁰ the first substrate protein of PKB/Akt that was identified,⁴¹ and that plays an important role in differentiation and cell fate determination, is deactivated upon phosphorylation by PKB/Akt1. Activated GSK-3 targets proteins that are implicated in Alzheimer's disease, neurological disorders and cancer. Another substrate of PKB/Akt is AS160 (Akt Substrate of 160kD), a RAB-GAP (RAB GTPase activating protein). Glucose uptake through the plasma membrane into muscle and fat cells is mediated by glucose transporter 4 (GLUT4).⁴² The majority of GLUT4 is stored in intracellular insulin responsive compartments (IRCs) with only 5% localized on the plasma membrane. The membrane of these IRCs contains several RAB proteins that are maintained in their inactive state by AS160. Upon insulin stimulation, these IRCs are translocated to the plasma membrane where AS160 is phosphorylated, and thereby inactivated, by PKB/Akt2, enabling the activated RAB proteins to mediate docking and fusion

of the IRCs with the plasma membrane where GLUT4 is released and glucose uptake enhanced.

Kinases also play a key role in the interaction between both viral⁴³ and bacterial⁴⁴ pathogens and host cells. Bacterial pathogens secrete proteins into the host cell that alter or mimic the activity of certain proteins, including kinases.⁴⁵ As a result, host cell signaling pathways that facilitate bacterial uptake into the host cell and / or prevent immune response mediated degradation are activated.⁴⁶ Inhibition of these kinases, that are essential for bacterial infection, is an appealing strategy to combat bacterial infection.⁴⁷ Hence, these kinases constitute a novel target for antibiotics and may be effective against multi-drug-resistant bacterial strains.

Kinases as targets for antibiotics

One example of a pathogen that actively induces uptake into the host cell, followed by avoiding the immune system is *Salmonella*. *Salmonellae* are Gram negative pathogenic bacteria⁴⁸ of which more than 2500 serotypes have been identified.⁴⁹ Some of these serotypes are pathogenic to humans only, for instance *S. Typhi* and *S. Paratyphi*, resulting in systemic infection and typhoid fever, whereas others, like *S. Typhimurium*, are pathogenic to animals as well, causing gastroenteritis.⁵⁰ Infection with *Salmonellae* usually results from oral ingestion of contaminated food or water. Bacteria that reach the smaller intestine interact with epithelial cells to avoid the immune system.⁵¹ Initially, the bacterium translocates virulence proteins *via* a Type III secretion system⁵² into the host cell. This results, amongst other things, in the modification of actin related signaling pathways resulting in endocytosis of the pathogen.⁵³ Once inside the cell, *Salmonella* resides in membrane bound compartments called a *Salmonella* Containing Vacuole (SCV) from where it injects a number of virulence proteins into the host cell cytosol. This results in the segregation of the SCV from the normal endocytic pathway⁵⁴ thereby avoiding lysosomal degradation *via* an until recently⁴⁵ not yet fully understood mechanism. *Salmonella* is known to inhibit the transport of these SCV's to the lysosome⁵⁵ and reactivating this dynein-mediated transport was shown to result in restored fusion of the SCV to the lysosome.⁵⁶ *Salmonella* activates the host cell kinase PKA⁵⁷ which might prevent dynein mediated transport of the SCV⁵⁸ which is crucial for pathogen replication.⁵⁶

The inhibition of host cell kinases to eliminate pathogens was studied using an interdisciplinary approach combining cell biology, bacteriology, biophysics, high throughput siRNA screening with automated microscopy and organic chemistry.⁴⁵ First the effects of a series of well-known kinase inhibitors on intracellular growth of *Salmonella* were tested. Only one kinase inhibitor (H-89) eliminated intracellular *Salmonella* without any effect on viability of the infected cell. H-89 (table 1, 1) is an often used Protein Kinase A (PKA) inhibitor, but is known to target other kinases as well. In fact, another PKA inhibitor (rp-cAMP) did not affect intracellular *Salmonella* growth suggesting that kinases other than PKA are involved in this process.

Subsequently H-89 and ten derivatives thereof were tested for inhibition of intracellular growth of *Salmonella* in human macrophages. The chemical profile or SAR indicated that only particular H-89 variants affected the kinase of interest, the genuine target. To further assess whether the putative kinase was only active on *Salmonella* or whether a more general cellular mechanism was targeted, the same set of H-89 variants was tested for inhibition of two unrelated intracellular bacteria; *M.smegmatis* and *M.tuberculosis*. Identical chemical profiles were found suggesting that particular kinase(s) were critical for intracellular survival of a set of bacterial pathogens and that a general cell biological mechanism was targeted by H-89 and some variants.

Genetic screens were subsequently employed to identify this kinase. The chemical inhibitors already revealed that inhibition of the target kinase allowed infection without notable effects on cell survival. This was critical information for the genetic screen because it showed that long lasting downregulation of the target by siRNA silencing techniques could be combined with infection. Chemistry was thus used to define the conditions of the genetic screen. Since the class of the tested compounds also pointed towards the class of enzymes (kinases) involved in controlling intracellular infection of bacterial pathogens, only the 'kinome' was silenced. Therefore 650 pooled siRNAs selectively silencing each kinase in the human genome were tested for effects on intracellular *Salmonella* growth. Cells were first transfected with the different siRNAs and infected with GFP-expressing *Salmonella* two days post-transfection (these two days are required for target proteins to 'disappear' and usually reflects two to four times their natural half-life). The effects on intracellular bacterial growth were monitored by automated microscopy, luminometry and FACS. This reverse genetic siRNA screen yielded ten kinases that reduced intracellular *Salmonella* growth. The ten

kinases were found to cluster in one network only, a network around the kinase PKB/Akt1. This suggests that the PKB/Akt1 pathway is critical for intracellular bacterial survival, at least for the bacterial species tested.

Then the actual target for H-89 was defined. Of the ten kinases identified in the genetic screen, five were selected (based on sequence homology to PKA) for *in vitro* SAR. The inhibition profile from *in vitro* kinase assays was subsequently cross-correlated with the biological effects of the compounds and only one kinase significantly cross-correlated: PKB/Akt. Due to the efforts of the pharmaceutical industry and academia to develop PKB/Akt inhibitors, the biological effects on control of bacterial infection could be confirmed with a competitive (table 1, **2**) and non ATP competitive PKB/Akt inhibitor (Table 1, **19**), strengthening the feasibility of this reciprocal chemical genetics approach. Thus, moving from chemistry to chemical profiling, then to genetic screenings and finally to biochemistry yielded a chemical lead, structural variants and a target critically involved in control of intracellular growth of a series of bacterial infections. One H-89 variant was further tested for biological activity in mice infected with *Salmonella typhimurium*. A delay in infection was observed without notable effects on tissue samples or mice, but the H-89 variants obviously have to be improved for further *in vivo* experiments.

The PKB/Akt1 controlled signaling pathways that are activated by *Salmonella* are depicted in Figure 5.⁴⁵ From within the phagosome, *Salmonella* injects effector proteins into the host cell cytosol. One of these proteins, SopB, activates PKB/Akt1. After binding of Cdc42 to the activated PAK4, ARHGEF2/GEF-H1 is phosphorylated. This prevents the phosphorylation of RHOA, instead, RAC1 is phosphorylated resulting in modified actin dynamics (lower pathway in Figure 5). A second pathway that is activated by *Salmonella* is depicted in the upper part of Figure 5. Here, SopB promoted activation of PKB/Akt1 results in the phosphorylation of AS160. This phosphorylation prevents binding of AS160 to the phagosomal membrane thereby arresting the transformation of the activated membrane-bound form of the GTP-ase RAB14 into an inactive cytosolic form. This in turn prevents the fusion of lysosomes with the phagosome and thereby degradation of *Salmonella*.

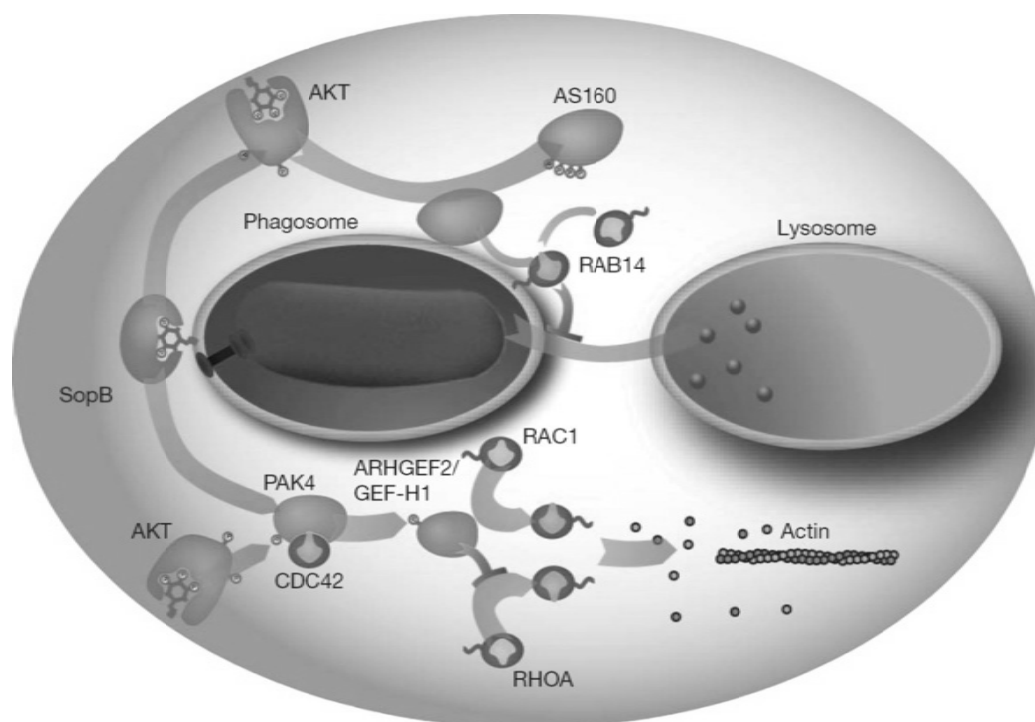


Figure 5; PKB/Akt pathway in salmonellosis. Following cellular uptake of *Salmonella* into the host cell, SopB is excreted into the cytosol resulting in the phosphorylation, and thereby activation, of PKB/Akt. This results in modified actin dynamics (lower pathway) and escape from lysosomal degradation (upper pathway).⁴⁵

The protein kinase PKB/Akt and the development of inhibitors

The pharmaceutical industry has great interest in developing small molecule inhibitors for PKB/Akt.⁵⁹ This interest is primarily based on the potential of PKB/Akt inhibitors as drugs for the treatment of cancer, but as discussed above, PKB/Akt inhibitors might also be applied in fighting infectious diseases.⁴⁵ The development of small molecule PKB/Akt inhibitors is mainly focused on ATP competitive inhibitors targeting the ATP-binding site. However, high sequence homology (>97%)³⁰ of the ATP binding pocket between Akt isoforms hampers the development of isoforms specific inhibitors. This is especially important as simultaneous inhibition of Akt1 and Akt2 is lethal.³¹ Therefore, other domains of PKB/Akt are being explored as potential targets for inhibitors as well; these include the Pleckstrin Homology domain, located at the N-terminus, and the substrate-binding site located at the C-terminus of PKB/Akt. Despite these challenges, several lead structures have been defined as PKB/Akt inhibitors. High throughput screening identified a number of lead structures, which are now further modified to create more potent and selective PKB/Akt inhibitors (Table 1).

One lead structure is H-89 (Table 1, **1**). Notably, H-89 is also a potent PKA inhibitor, since the ATP-binding site is very similar to PKB/Akt. H-89 has been modified in different regions of the core structure exemplified by **2**,⁶⁰ **3**⁶¹ and **4**.⁴⁵ To generate higher affinity inhibitors and inhibitors more selective for PKB/Akt over PKA various H-89 homologues have been tested. Modification of the isoquinoline moiety or the secondary amine abrogated PKB/Akt inhibition, whereas modifications in the bromophenyl part affected affinity but not selectivity over PKA.^{60, 61} The introduction of bulky substituents on the double bond (see also chapter 3) increased specificity for Akt1 over PKA. Further modifications are required to arrive at high affinity and more specific Akt1 inhibitors.

Screening large compound libraries has yielded several promising lead structures which are now further modified to create more potent and selective inhibitors. One such example is the discovery of bis-pyridinylethylene **5** as core structure.⁶² Further modifications of this structure yielded **6** which showed a 2.5 fold higher selectivity against PKA and an at least 15 fold preference for Akt1 over the other two isoforms.⁶² Compound **6** was further optimized by replacing the terminal pyridine with a methyl substituted indazole moiety resulting in **7**.⁶³ Although the activity improved significantly, the selectivity did not. Modifications of the central pyridine ring and the hinge binder in **5** did not improve⁶⁴ (**9**, GSK690693) or even reduced inhibition⁶⁵ (**8**). A related series of compounds with the central pyridine replaced by piperidine (e.g. compound **10**)⁶⁶ are active against Akt but not selective.

Structure-based design by scientists at the company Astex resulted in 6-phenylpurine **11** and phenylpyrazole **13** based Akt inhibitors. A series of compounds, represented by **12**⁶⁷ and **14**⁶⁸, was designed starting from either purine or pyrazole as hinge region binders, extended with a skeleton resembling the spatial features of H89.

The compounds discussed so far exemplify a wide range of Akt inhibitors with a similar SAR. They are characterized by a hinge binder, a crucial basic amine functionality, a connecting group and, in most cases, a substituted aryl moiety to improve activity. Several unrelated structures have been identified that bind to the ATP pocket of Akt, albeit with modest potency and selectivity and an unknown binding pattern. For instance, from a series of 1900 compounds tested, disubstituted triazine **15**⁶⁹ proved to be the most potent against Akt3 (IC_{50} = 1.0 μ M), however with similar potency against several other kinases. Pyrazolopyrimidine **16** was found to inhibit Akt1 with low micromolar activity.⁷⁰ Virtual screening of 50.000 compounds and experimentally testing the 200 best hits resulted in the

identification of **17** as Akt inhibitor.⁷¹ HTS identified pyrrolopyrimidine **18** in an *in vitro* kinase assay as promising lead structure, SAR optimization resulted in spiroindoline **19** as the most potent analogue.⁷¹

Table 1; ATP Competitive PKB/Akt Inhibitors

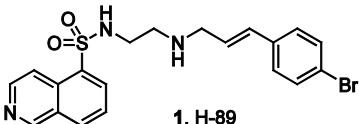
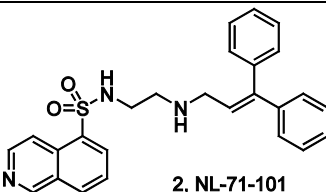
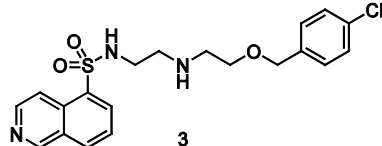
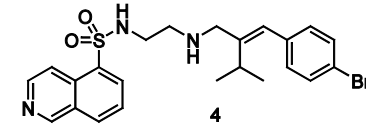
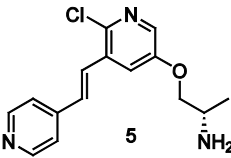
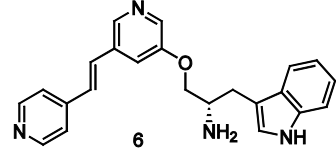
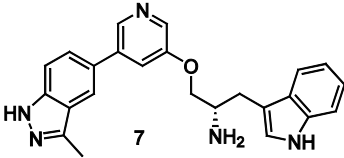
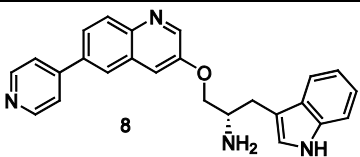
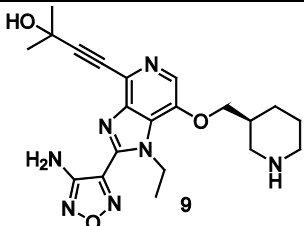
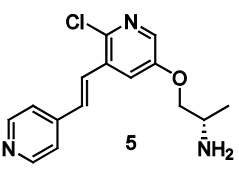
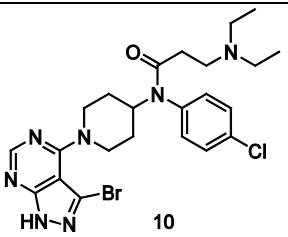
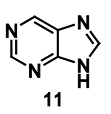
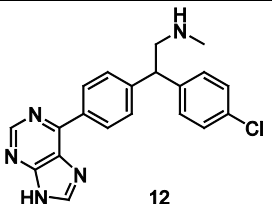
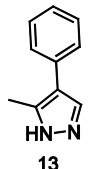
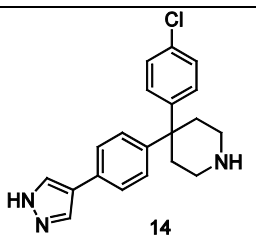
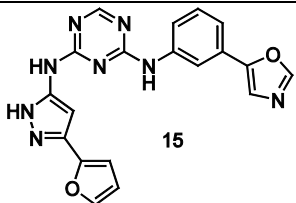
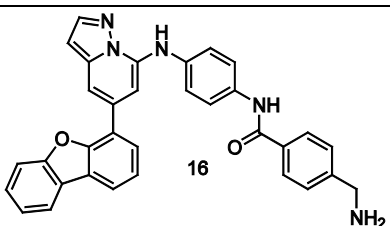
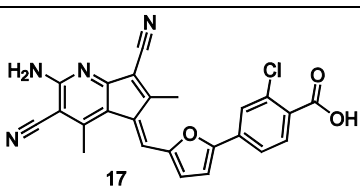
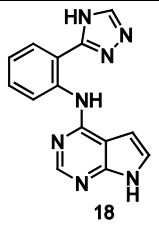
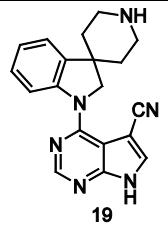
Lead structure	IC ₅₀ (nM)	Hit structure	IC ₅₀ (nM)
 1, H-89	PKA: 35 Akt: 2500	 2, NL-71-101	PKA: 9000 Akt: 3700
		 3	PKA: 170 Akt: 260
		 4	PKA: >10000 Akt: 1500
 5	Akt1: 5290	 6	PKA: 38 Akt1: 14
		 7	PKA: 6.3 Akt1: 0.16
		 8	PKA: N/A* Akt1: 300
		 9	PKA: N/A* Akt1: 2 Akt2: 8 Akt3: 9

Table 1; ATP Competitive PKB/Akt Inhibitors

Lead structure	IC ₅₀ (nM)	Hit structure	IC ₅₀ (nM)
 5	Akt1: 5290	 10	Akt: <50
 11	Akt2 >100000	 12	PKA: 15 ^a Akt2: 10
 13	Akt2: 80000	 14	PKA: N/A* Akt2: 18
		 15	Akt3: 1000
		 16	Akt1 < 1750
		 17	Akt1: 2600 K _i = 1100
 18	Akt1: 210	 19	Akt1: 2.4

^atested as racemate. * N/A: data is not available.

Although some very potent ATP competitive Akt inhibitors have been published, none of these display pharmaceutically desirable selectivity with respect to other members of the AGC kinase family, let alone between the three Akt isoforms. Given the high degree of homology of the ATP binding pocket between these kinases, this is not surprising. One strategy to improve selectivity is to target other, more distinctive, regions of the kinase, like the PH domain. Scientists at Merck have published several allosteric inhibitors, exemplified by **20**⁷² (Table 2), targeting the PH domain of Akt with good potency and excellent selectivity. Diphenylquinoxaline based inhibitor **21** was developed as dual akt1/akt2 inhibitor.⁷³ Several modifications have led to compounds with similar and improved physical properties.⁷⁴ Besides these allosteric inhibitors, compounds mimicking the natural ligands for the PH domain, PtdIns(3,4,5)P3 and PtdIns(4,5)P2, have been made. These compounds are composed of a polyhydroxylated carbocycle linked to a lipid tail *via* a phosphate or carbonate group exemplified by **22**.⁷⁵ Mimetics of the Akt substrate (**23**)⁷⁶ alone or conjugated to an ATP mimetic (**24**)⁷⁷ has yielded moderate improvements in terms of selectivity. Stepwise combinatorial evolution of a substrate mimetic ($K_i = 3.2$ mM) to a bisubstrate resulted in **25** ($K_i = 260$ nM).⁷⁸ High throughput screening for Akt1 inhibitors yielded lactoquinomycin **26** as potent inhibitor.⁷⁹ Unfortunately Akt2 was inhibited with similar potency (using 1 μ M **26** 92% of Akt1 and 99% of Akt2 was inhibited). Interestingly, this compound seems to covalently bind to a cysteine residue located on the activation loop.

Table 2; Non ATP Competitive PKB/Akt Inhibitors

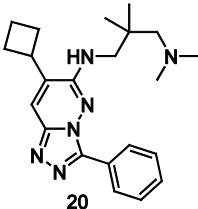
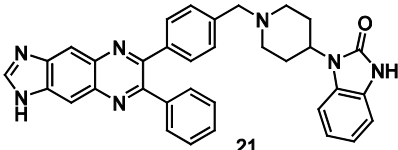
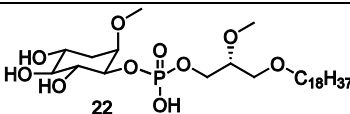
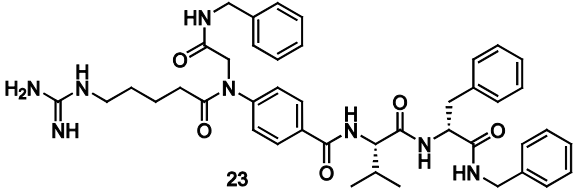
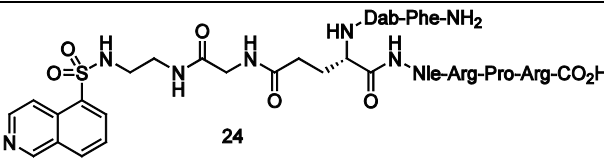
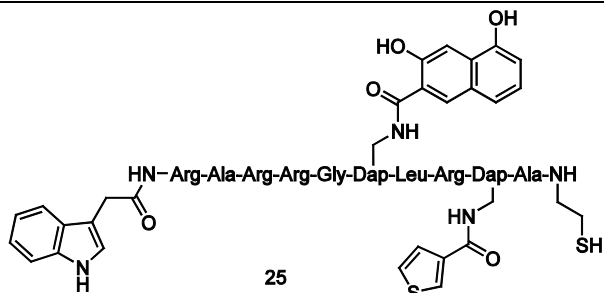
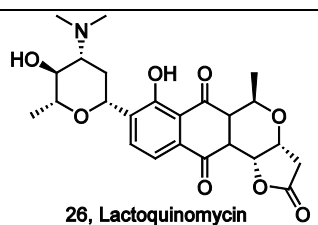
Structure	IC ₅₀ (nM)	Target
 20	Akt1: 4.600 Akt2: >250000 Akt3: >250000	PH domain
 21	Akt1: 58 Akt2: 210 Akt3: 2119	PH domain

Table 2; Non ATP Competitive PKB/Akt Inhibitors

Structure	IC ₅₀ (nM)	Target
 22	Akt: 4100	PH domain
 23	Akt1: 1400	Substrate binding grove
 24	PKA: 210 Akt1: 70	ATP pocket and substrate binding grove
 25	Akt1: K _i = 260nM	ATP pocket and substrate binding grove
 26, Lactoquinomycin	PKA: >200000 Akt1: 149	Irreversible binding with active site cysteine

Conclusion

This chapter highlights the prominent role played by members of the kinase family, focusing on PKB/Akt, in regulating almost every cellular process making them prime candidates for drug targets. The development of small molecule kinase inhibitors is met with various challenges regarding potency, selectivity and bioavailability which have to be overcome before these chemical entities become pharmaceutically relevant. This field of research has only recently started to yield marketable products. These pioneering achievements did initiate a revived interest in small molecule kinase inhibitors in both

industrial and academic surroundings. Major efforts are now being made to identify key kinases and their corresponding small molecule inhibitors.

A novel strategy that simultaneously tackles these goals, named reciprocal chemical genetics, is described and exemplified by the synchronal discovery of PKB/Akt1 as key regulator of bacterial pathogenesis and novel inhibitors thereof. High throughput gene silencing with siRNA libraries now allows identification of protein targets involved in defined biological processes. Chemical libraries can be screened for overlapping phenotypes. Combining these technologies then defines potential targets for chemical leads. If the lead is further modified for chemical profiling, then the profile can be used for *in vitro* enzyme assays to definitively identify the target of the compounds. This flowchart, combining chemistry and genetics, allows rapid identification of both lead and target. Currently the number of lead compounds and genes identified in chemical genetic and siRNA screens may be overwhelming. It is therefore often helpful to reduce the number of lead compounds and genes for follow up in subsequent assays. In the near future high content (multiple parameters) screening with compounds and siRNA for desired phenotype should be able to achieve this reduction. The multiple parameters (e.g. nuclear size, nuclear shape, vesicle distribution etc) obtained in a chemical genetic screen exemplified by Young *et al.*⁸⁰ can be cross correlated with the same parameters obtained in a genetic screen (e.g. siRNA or shRNA). Lead compounds and genes resulting in similar parameter values are then selected for further reciprocal chemical genetic screening.

In a proof-of-principle study, Akt1 was identified as key target in the inhibition of intracellular growth of pathogenic bacteria like *Salmonella* and *M.tuberculosis* by H-89 derivatives. Since PKB/Akt inhibitors are already in various phases of development as anti-cancer drugs, Akt1 inhibitors may successfully enter clinical practice. Such drugs may then not only be applied as anti-cancer drugs but also as antibiotics. Since PKB/Akt defines a new class of antibiotic targets (a host target rather than one of the pathogen) multidrug-resistant bacteria will still be susceptible to these compounds, as indeed shown for MDR-*M.tuberculosis*.⁴⁵ Given the growth of patients with multidrug-resistant bacteria, definition of new targets and corresponding inhibitors is urgent.

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Chapter 3 | Synthesis and Biological Evaluation of Novel Isoquinolinesulfonamide Based PKB/Akt1 Inhibitors

Introduction

A variety of bacterial strains, including *Salmonella typhimurium* and *Mycobacterium tuberculosis*, invade specific host cells and alter the activity of PKB/Akt1 to evade the host immune system and to promote intracellular survival. The discovery of this host / pathogen interaction was described in detail in chapter 2, and the overview includes a summary of PKB/Akt inhibitors that are described in the literature which, in addition to anticancer drugs, can now also be viewed as potential antibiotics. Briefly, infection with one of the above bacterial strains results in the uptake of these pathogens into the host cell where they reside in membrane enclosed vesicles. Effector molecules, expressed and excreted by the bacteria into the host cell cytosol, result in elevated PKB/Akt1 activity. This up-regulation of PKB/Akt1 blocks lysosomal interaction with these vesicles, thus escaping lysosomal degradation. Inhibition of PKB/Akt1 restores this interaction resulting in the destruction of the bacteria.

The development of PKB/Akt1 inhibitors is therefore of great interest, not only as potential antitumor agents (elevated PKB/Akt activity has been shown in several cancers including breast-, ovarian-, lung-, pancreatic-, prostate-, stomach- and melanocytic cancer)¹ but also as novel antibiotic, targeting host cell kinases rather than pathogen specific proteins.

The discovery of the involvement of PKB/Akt1 in bacterial infection was made with the aid of the literature compound H-89 (**1**)² and its derivative developed in the context of this theme (**2**, Figure 1). H-89 (**1**) was originally developed as a protein kinase A (PKA) inhibitor, which is closely related to PKB/Akt. However, it was established that the inhibition of PKB/Akt1, not PKA, gave rise to its antibiotic potency and eventually led to the identification of two positions (R¹ and R² in **2**, Figure 1) on the H89 scaffold amiable for functionalization towards developing more potent and selective inhibitors of PKB/Akt1.

In this chapter, novel functional variations of the isoquinolinesulfonamide scaffold are discussed. First, a literature survey on the development of this pharmacophore is presented. Next, the introduction of substituents on positions R¹ and R² (Figure 1) are discussed, ultimately leading to **3** as most potent and selective PKB/Akt1 inhibitor of this series.

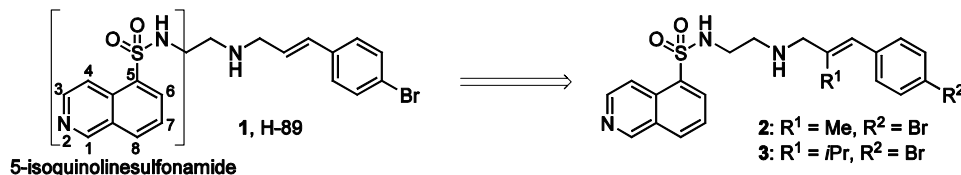


Figure 1; H-89 derived PKB/Akt1 inhibitors **2** and **3**

Isoquinolinesulfonamides as scaffold for small molecule kinase inhibitors

During the eighties and early nineties, the group of Hidaka³ has published several isoquinolinesulfonamide based small molecule inhibitors for protein kinases. The first 5-isoquinolinesulfonamides to be used as kinase inhibitors were comprised of an isoquinoline moiety attached *via* a sulfonamide to a short diamino spacer exemplified by H-7 (**4**), H-8 (**5**), and H-9 (**6**), Figure 2. These compounds were shown to have low micromolar K_i -values against a small panel of kinases (PKA, PKC, PKG, MLC kinase) which allowed these compounds to be used as ligands in affinity chromatography for the isolation of kinases from different biological samples. For this purpose, H-9 (**6**) can be directly attached to cyanogen bromide activated sepharose⁴ or equipped with a photoreactive group in combination with a fluorescent label (**7**) or a functionalization handle for solid phase attachment (**8**).⁵ A more

recent application of **4** - **6** is their ability to inhibit, albeit with moderate *in vitro* potency, two members of the aminoglycoside kinase family namely APH(3')-IIIA and AAC(6')-APH(2''), preventing the O-phosphorylation (and thereby the inactivation) of antibiotic aminoglycosides.⁶ Even though these compounds were not able to inverse antibiotic resistance *in vivo*, this study does present a noteworthy novel application of isoquinolinesulfonamides.

Interestingly, expanding the piperazine ring in H-7 (**4**) to a homopiperazine, (HMN-1179, **9**) resulted in a markedly different inhibition profile (Figure 2). A methyl scan covering all carbon atoms⁷ of the homopiperazine moiety (**9** - **13**) had only marginal effect on the inhibitory potency against PKA with IC_{50} values between 1.2 and 5.5 μ M.⁸ A similar trend was observed for calmodulin Kinase II (IC_{50} between 2.0 and 23 μ M). Interestingly, 7-methylated HMN-1180 (**13**) was found to selectively inhibit neuronal nitric oxide synthase (nNOS) with respect to endothelial- (eNOS) and inducible nitric oxide synthase (iNOS). Removing the methyl group altogether (HA1077, **14**) rendered it an antivasospasm drug with unidentified target.⁹

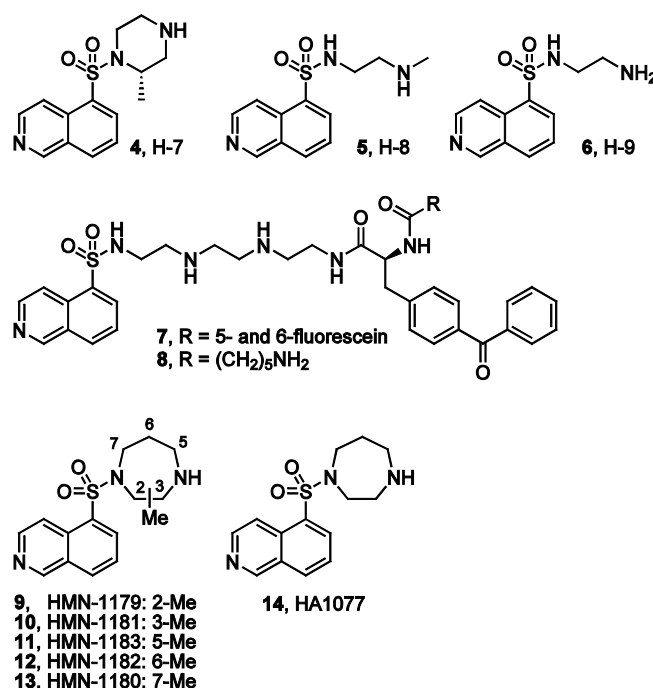


Figure 2; Isoquinolinesulfonamide based kinase inhibitors

In later years, more potent and selective inhibitors were developed (Figure 3). The tyrosinyl based bis-isoquinoline KN-62 (**15**) is a non-ATP-competitive inhibitor of Ca^{2+} /CaM kinase II ($K_i = 0.9 \mu$ M) that has no significant inhibitory potency against Myosin Light Chain

kinase (MLCK), PKA or PKC at concentrations up to 100 μM .¹⁰ The H-9 (**6**) derivative CKA-1306 (**16**) was found to inhibit PKA ($\text{IC}_{50} = 1.6 \mu\text{M}$) and Ca^{2+} /CaM kinase I ($\text{IC}_{50} = 2.5 \mu\text{M}$).¹¹

From a series of *N*-methylated isoquinolinesulfonamides **17** was identified as potent ($\text{MIC} = 2 \mu\text{g/mL}$) inhibitor of plasmodium falciparum MO15 related kinase (Pfmrk), a cyclin dependent kinase with important physiological properties in the life cycle of malaria.¹² An interesting attempt to optimize the potency of H-9 (**6**) towards PKC has been described by Sergheraert and Houdin.¹³ Sequence analysis of different PKC isoforms indicated the presence of a possible second ATP binding site in PKC α , PKC β and PKC γ . Although they were unable to bridge the distance between the two binding sites, these constructs could serve a role in the distinction between free cytosolic, inactive PKC, and membrane bound, active PKC, due to the increased local concentration of inhibitor.

Several groups have published conjugates of H-9 (**6**) and peptide sequences derived from or resembling a kinase substrate protein (**19-21**).¹⁴ Although the potency of these conjugates improved significantly with respect to H-9 (**6**), selectivity did not.

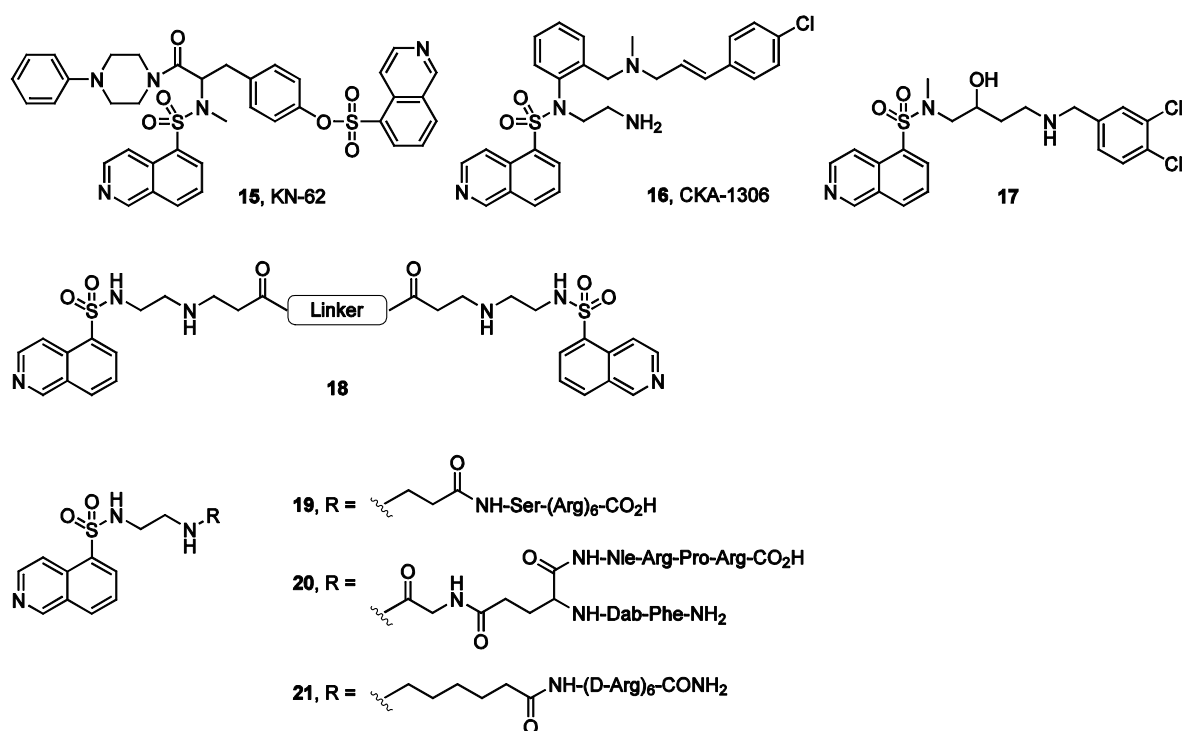


Figure 3; Advanced isoquinolinesulfonamide based kinase inhibitors

Analogues of 2-(Cinnamyl)-ethylamino-5-isoquinolinsulfonamides

In 1990, two novel isoquinolinesulfonamides (H-88 (**22**) and H-89 (**1**), Figure 4) appeared in the literature as part of a study aimed at the synthesis of selective inhibitors against PKA.² Whereas H-88 (**22**) was only marginally selective for PKA vs. PKG ($K_i = 0.38$ and $0.76 \mu\text{M}$ resp.), H-89 (**1**) was ten-fold more potent for PKA than for PKG ($K_i = 0.048$ and $0.48 \mu\text{M}$ resp.). Both inhibitors showed only double digit activities against a panel of 5 other related kinases (PKC, MLCK, CaMK II, Casein kinase I and II). Although highly controversial, H-89 (**1**)¹⁵ has long been considered a selective inhibitor for PKA, and is commercially available as reference compound for PKA inhibition,¹⁶ even though $10 \mu\text{M}$ H-89 (**1**) is able to inhibit at least eight different kinases by 80 - 100%, three of which with a similar or even greater potency than PKA.¹⁷ These results necessitate critical evaluation of earlier findings regarding the biological activity of H-89 (**1**) and evidence of PKA involvement should not solely rely on H-89-based experiments. Despite the drawbacks involved in H-89 (**1**) based assays, the chemical core structure of H-89 (**1**) has contributed to a great number of biochemical studies. The derivatization of H-89 (**1**) with a radiolabeled methylgroup on the sulfonamide nitrogen (**23**) allowed PET based experiments of PKA activity in the brain.¹⁸

The high sequence homology between PKA and PKB¹⁹ allowed the scaffold of H-89 (**1**) to be used as lead compound in the development of PKB inhibitors. Levitzki and co-workers²⁰ varied the H-89 (**1**) scaffold in the isoquinoline-, diamine- and styrene-region resulting in the identification of NL-71-101 (**24**) as kinase inhibitor with a 2.4 fold selectivity for PKB over PKA. In a similar study by McDonald and co-workers²¹ attempts were made to improve the pharmacological properties of this type of compounds by retaining the isoquinoline moiety and varying the linker region and the aryl group. This yielded **25** as most potent compound. Despite the loss of selectivity over PKA, the similar activity with respect to H-89 (**1**) indicated that the metabolically labile alkene moiety could be replaced by a more stable, and more hydrophilic, ether linkage.

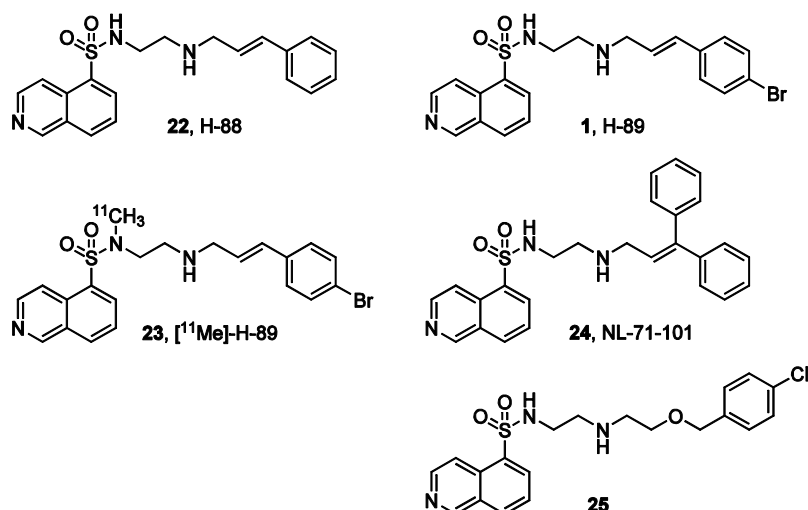


Figure 4; H-89 derivatives

Results and discussion

In the following section, the synthetic efforts of the transformation of H-89 into a potent and more selective inhibitor will be described. First, a small set of analogues is designed, aimed at identifying features of the H-89 (**1**) scaffold that can be modified to increase potency and selectivity. Next, the synthetic strategy to the lead compound resulting from this first library is adapted to allow larger quantities to be synthesized. Finally, a new strategy is described for the construction of a larger and more diverse library.

First small diversity set

A first set of H-89 (**1**) analogues was designed varying the degree of unsaturation, substitution of the linker and the presence or absence of the bromine on the styrene moiety (Figure 5).

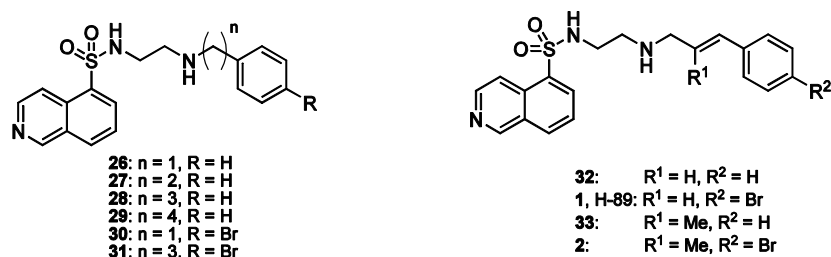
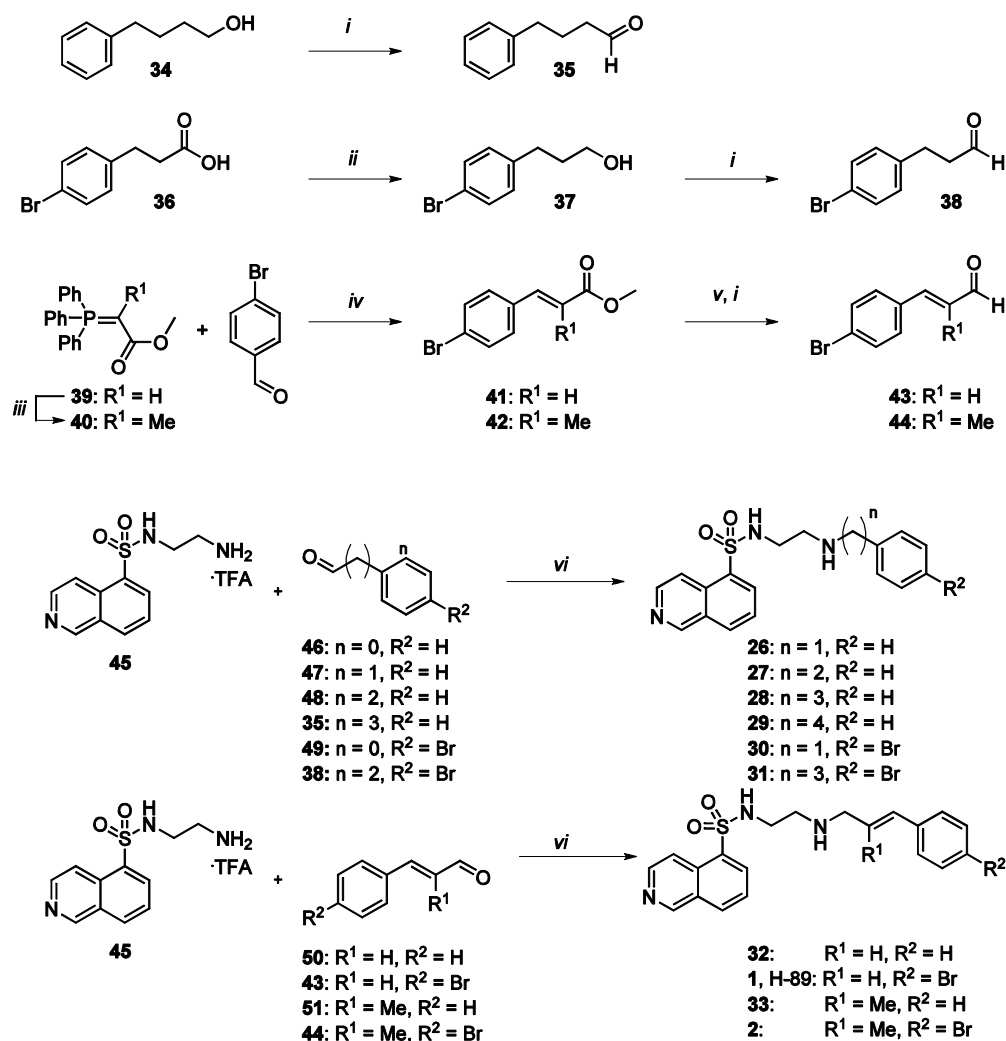


Figure 5; First library of isoquinolinesulfonamides

The synthesis of these isoquinolinesulfonamides is based on the reductive amination of amine **45**²² and the corresponding aldehydes (**35**, **38**, **43**, **44** and **46** - **51**, Scheme 1). Aldehydes (**46** - **51**) were commercially available, aldehyde **35** was prepared from 4-phenylbutanol by means of a Dess-Martin periodinane mediated oxidation and aldehyde **38** was synthesized from 3-[4-bromophenyl]-propionic acid *via* $\text{BH}_3 \cdot \text{Me}_2\text{S}$ mediated reduction (**37**) followed by Dess-Martin oxidation. Aldehydes **43** and **44** were obtained *via* the following sequence of reactions. Firstly, olefination of *p*-bromo-benzaldehyde with commercially available Wittig-reagent **39** or the methylated derivative **40**²³ in THF at 0°C using NaH as the base afforded methyl esters **41** and **42** in good yield. Performing this reaction in a different solvent (like DMF or DCM) or with another base (such as *n*-BuLi, KOtBu, DBU or NaH) suffered from an increase in side product formation and the requirement of longer reaction time according to TLC analysis. Selective reduction using DiBAL-H and Dess-Martin oxidation afforded the cinnamic aldehydes **43** and **44** which were purified by extraction only and were used as such in the following reactions. The crude aldehydes (**43** and **44**) were treated with amine **45** in MeOH under the agency of AcOH, Na_2SO_4 , followed by reduction with NaBH_4 to afford isoquinolinesulfonamides **1**, **2** and **26** – **33** in reasable to good yields after HPLC purification.

Biological results

This first set of isoquinolinesulfonamides (**1**, **2**, **26** – **33**) was tested against *Salmonella Thyphimurium* in primary human macrophages, (Figure 6). These results identified several features of the isoquinolinesulfonamides that determine potency against kinases. The optimal length of the linker connecting the phenyl group with the secondary amine proved to be three carbon atoms. The presence of a double bond in that linker improved potency, as does the bromine on the phenyl moiety. A small methyl substituent was well tolerated on the R¹ position (Scheme 1). This first SAR identified possible sites of the H-89 (**1**) scaffold suitable for modification that might increase potency and selectivity. At this stage it was decided to produce **2** on a sufficiently large scale to allow animal testing and to further diversify the library of H-89 (**1**) analogues by incorporating different alkyl substituents on the double bond and to replace the bromine with other halogens.



Scheme 1; Reagents and conditions: (i) 6 eq. Dess-Martin periodinane, DCM (ii) 5 eq. $BH_3 \cdot Me_2S$, THF, 0°C, 96% (iii) ref.23 (iv) 1.2 eq. MeLi (1.6M in Et_2O , THF, 0°C, 97% **41**, 99% **42** (v) 2.2 eq. DiBAL-H (1M in hexanes (vi) Na_2SO_4 , 0.2 eq. AcOH then 1.5 eq. $NaCNBH_3$ 43% **26**, 31% **27**, 27% **28**, 22% **29**, 10% **30**, 7% **31**, 41% **32**, 44% **1**, 44% **33**

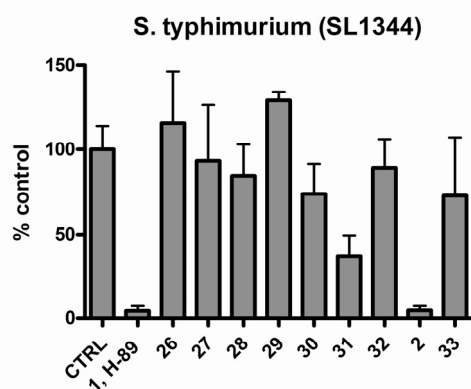
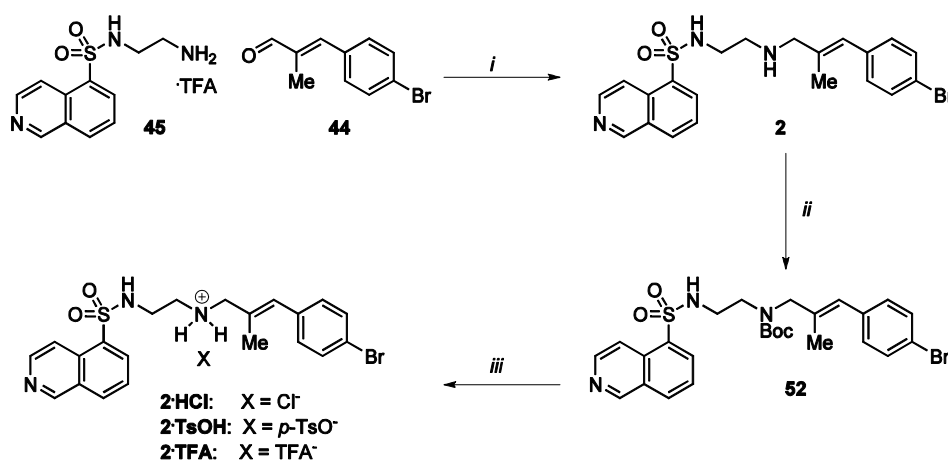


Figure 6; Potency against *salmonella*

Large scale synthesis of **2**

Both aldehyde **44** and amine **45** could be prepared on a multi gram scale without difficulties using the small scale procedures. The reductive amination proved to be more scale sensitive. Applying the small scale procedure on a 5 gram scale yielded less than 10% of the desired product. The use of titanium isopropoxide (acting as Lewis acid) and Na₂SO₄ instead of acetic acid/Na₂SO₄ increased the yield after silica column chromatography. However, the product could not be purified beyond a 90 - 95% purity as determined by ¹H-NMR. In order to remove the unidentified side product, compound **2** was *N*-protected using Boc-anhydride in THF to afford **52** in a 93% yield. Neither silica column chromatography nor HPLC resulted in complete removal of the side product as judged by ¹H-NMR analysis. Acidic treatment of **52** was expected to cause removal of the Boc protecting group and coinciding ammonium salt formation and, depending on the nature of the acid used, possibly enabling selective crystallization. Boc removal using an excess of a freshly prepared dry a methanolic HCl solution or a 2M stock solution of HCl in dioxane resulted in the formation of a crystalline product. Unfortunately, attempts to separate the byproducts by re-crystallization from different solvent systems failed. Boc removal using *p*-TsOH in dioxane, followed by recrystallization from dioxane/ether did result in the pure tosylate salt of **2** (X = tosylate) as determined by NMR. Salt exchange by alkaline extraction and re-acidification using TFA/DCM afforded pure **2** as the TFA salt (X = trifluoroacetate).²⁴ Further experiments revealed that the bacylation step could be omitted from the purification strategy and *p*-TsOH treatment followed by re-crystallization and anion exchange enabled the synthesis of **2** starting from 6.3 g **45** and 3.2 g **44** resulting in 1.6 g of the desired amine in 24% yield.

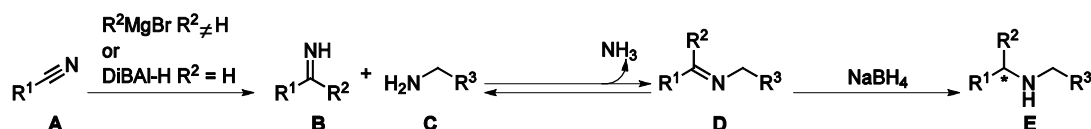


Scheme 2; Reagents and conditions: (i) 1.4 eq. Ti(O*i*Pr)₄, Na₂SO₄, MeOH, 3h. then 2.8 eq. NaCNBH₄ 16h. (ii) 1.5 eq. Boc₂O, THF, 93% (iii) 5 eq. HCl/MeOH, or 5 eq. HCl/dioxane or 5 eq. *p*-TsOH/dioxane

Transimination library

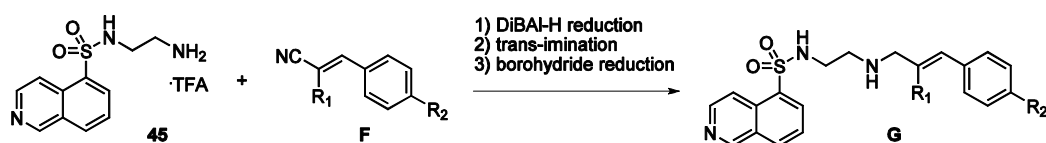
The promising *in vitro* results obtained with the first set of isoquinolinesulfonamides fomented the design of a synthesis scheme that would allow rapid diversification of the core isoquinolinesulfonamide scaffold focusing on bromine replacements and the double bond substitution. The new strategy should require starting materials which are readily available, and easily modified into building blocks that can be efficiently coupled with minimal purification steps yielding the desired library. The laborious preparation of α -alkylated cinnamic aldehydes (such as **43** and **44**, Scheme 1), together with the facile synthesis of amine **45**, directed our attention towards the development of an alternative strategy for the assembly of the secondary amine functionality as the final synthesis step.

An appealing alternative for the reductive amination was published by Van der Gen and Brussee²⁵ where they described the so-called one-pot Grignard-Transimination-Reduction sequence (Scheme 3). Grignard addition to a nitrile **A** yields primary imine **B** ($R^2 \neq H$), which upon addition of a primary amine **C**, undergoes a transimination reaction forming a secondary imine **D** under the liberation of ammonia. Sodium borohydride reduction of the secondary imine affords the α -alkylated amine **E**. Generating unalkylated imines **B** ($R^2 = H$)²⁶ could be accomplished *via* DiBAL-H reduction of nitriles **A** ultimately leading to secondary amines **D** after transimination and borohydride reduction in good yields.



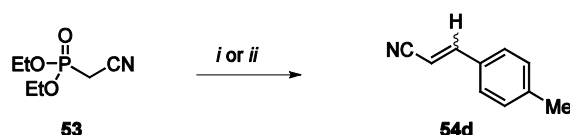
Scheme 3; General scheme for the trans imination protocol

Applying this reduction-transimination-reduction-sequence to generate a diverse library of isoquinolinesulfonamides of the general structure **G** would, besides amine **45**, require facile synthesis of nitriles **F**, bearing different substituents on positions R^1 and R^2 (Scheme 4).



Scheme 4; General synthesis of isoquinolinesulfonamides

First the synthesis of unsubstituted cinnamitriles **F** was investigated ($R^1 = H$, Scheme 5). It was envisaged that a Horner-Wadsworth-Emmons (HWE) reaction between *p*-substituted benzaldehydes and commercially available diethyl cyanomethylphosphonate **53** would result in α,β -unsaturated cinnamitriles **54a-i** ($R^1 = H$). This reaction proceeded in excellent yields with good selectivity ($E/Z > 10/1$). Attempts to increase the selectivity were met with moderate success and proved to be dependent on many variables including the nature of R^2 , reaction temperature and the speed of addition of the aldehyde. These findings prompted us to investigate reaction conditions that would result in a more equalized E/Z ratio resulting in an additional set of derivatives containing a *Z*-substituted double bond. Indeed, performing the reaction in the presence of additional sodium cations was found²⁷ to favor the formation of *Z*-substituted cinnamitriles resulting in E/Z ratio ranging from 4/1 to 1/1 according to ^1H -NMR.

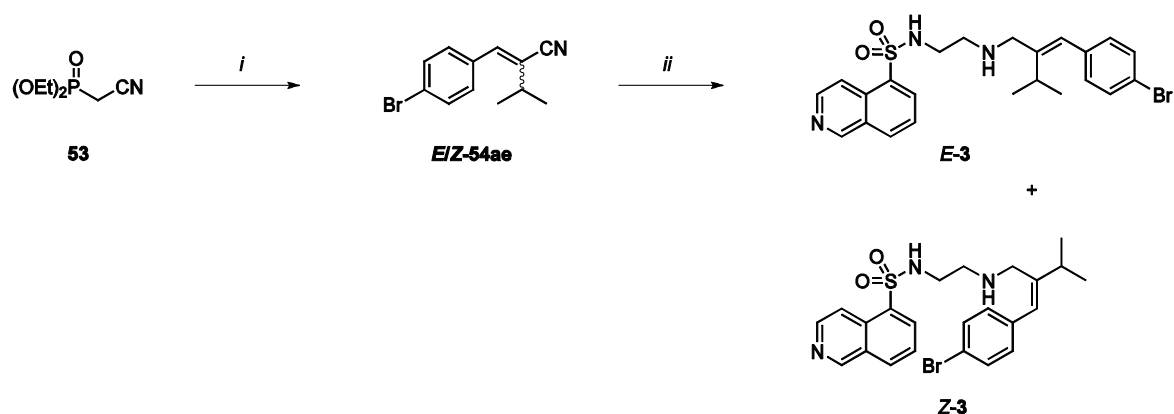


Scheme 5; Reagents and conditions: (i) 1.1 eq. NaH, 0.9 eq. *p*-tolylbenzaldehyde, DMF, $E/Z = 20/1$. (ii) 1.1 eq. NaH, 2.0 eq. NaI, 0.9 eq. *p*-tolylbenzaldehyde, DMF, $E/Z = 3:1$.

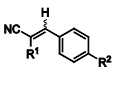
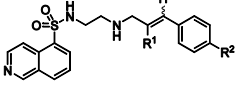
The synthesis of α -substituted cinnamitriles **54e-p** (Scheme 6) involved alkylation of phosphonate **53** using NaH and alkyl iodide followed by HWE reaction in a one-pot fashion. In these cases an increased prevalence of the *Z*-isomers was observed, probably arising from both steric hindrance and the presence of an additional equivalent of sodium cations.²⁷ Since separation of the isomers in the nitrile-stage was at best partially successful, and the nitriles were shown to isomerize during the trans-amination sequence, the nitriles were used in the following reactions as E/Z mixtures.

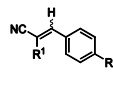
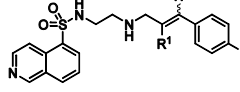
The thus obtained nitriles were subsequently used in the four-step-one-pot trans-amination procedure according to Brussee *et al.* (Scheme 6).²⁶ For example, diethyl cyanomethylphosphonate **54** is treated with NaH and alkylated using 2-iodopropane. The intermediate phosphonate is again deprotonated using NaH followed by the Horner-Wadsworth-Emmons reaction with 4-bromobenzaldehyde to afford **54ae** as 3/2 mixture of E/Z isomers in 73% yield. The nitrile is used as isomeric mixture and as such dissolved in dry ether at $-78^\circ C$ and treated with an excess DiBAL-H at $-78^\circ C$ before the excess reagent is quenched at $-100^\circ C$ with concomitant methanolysis of the iminium salt intermediate

towards the primary imine. Reaction with amine **45** at room temperature under the liberation of ammonia followed by overnight reduction of the resulting secondary imine with NaBH₄ furnished the final isoquinolinesulfonamides **55ae** as crude E/Z mixture in 80% yield after alkaline extraction. HPLC purification allowed the separation and isolation of both isomers. The remaining isoquinoline sulfonamides **55a-aj** could be synthesized in a similar fashion (Table 1). The separation of the E/Z isomers using HPLC was met with varying results. In most cases one or both of the isomers were obtained in a >95% purity. In some cases, however, neither of the two isomers could be obtained in >95% purity.



Scheme 6; Example of a HWE reaction and trans-imation sequence: *Reagents and conditions*: (i) a: NaH (1.2 eq.), 2-iodopropane (1.5 eq.), DMF, 1h., 0°C; b: NaH (1.2 eq.) 4-bromobenzaldehyde, 0°C tot r.t.; 16h. (ii) a: DiBAL-H (2 eq.), Et₂O, -78°C to 0°C, 30 min. b: MeOH, -100°C c: **45** (1.5 eq.), r.t., 3h. d: NaBH₄ (2 eq.) -18°C to r.t., 16h. Yields and E/Z ratios of the complete library are given in Table 1 and in the experimental section.

				
	R ¹	R ²	53 → 54 % (E:Z)	54 → 55 % (E), % (Z)
a	H	H	91 (4:1)	31.6 (E)
b	H	F	84 (5:2)	34.0 (E)
c	H	Cl	88 (2:1)	18.2 (E)
d	H	Br	87 (5:2)	19.4 (E)
e	H	Me	quant (5:2)	38.3 (E)
f	H	CF ₃	92 (5:2)	14.0 (E)
g	H	OMe	quant (3:1)	45.8 (E)
h	H	OPh	90 (3:1)	23.2 (E)
i	H	NO ₂	92 (2:1)	27.7 (E)
j	Me	H	60 (1:1)	9.1 (E), 2.5 (Z)
k	Me	F	45 (1:1)	11.5 (E), 4.2 (Z)
l	Me	Cl	38 (3:2)	10.5 (E)
m	Me	Br	63 (1:1)	12.4 (E), 2.0 (E/Z = 5/3)
n	Me	Me	35 (1:0)	10.5 (E), 13.5 (Z)
o	Me	CF ₃	58 (1:1)	8.5 (E), 7.0 (Z)
p	Me	OMe	20 (1:1)	21.9 (E), 5.7 (Z)
q	Me	OPh	45 (1:1)	10.3 (E)
r	Me	NO ₂	56 (1:1)	7.4 (E/Z = 6/1), 7.1 (Z)

				
	R ¹	R ²	53 → 54 % (E:Z)	54 → 55 % (E), % (Z)
s	Et	H	49 (1:1)	14.1 (E), 4.6 (E/Z = 1/5)
t	Et	F	55 (4:3)	8.5 (E), 5.9 (E/Z = 1/4)
u	Et	Cl	39 (1:1)	2.4% (E)
v	Et	Br	64 (1:1)	6.5 (E/Z = 5/1), 2.6 (Z)
w	Et	Me	16 (1:1)	19.0 (E/Z = 1/2), 10.7 (Z)
x	Et	CF ₃	60 (3:2)	0.8 (E), 7.4 (Z)
y	Et	OMe	51 (1:1)	5.6 (E/Z = 5/1)
z	Et	OPh	51 (1:1)	14.2 (E), 4.8 (Z)
aa	Et	NO ₂	55 (1:1)	10.1% (E/Z = 3/2)
ab	iPr	H	67 (3:2)	3.1 (E), 3.3 (Z)
ac	iPr	F	67 (3:2)	4.6 (E), 4.2 (E/Z = 3/2)
ad	iPr	Cl	34 (3:2)	13.4 (E), 7.5 (Z)
ae	iPr	Br	73 (3:2)	8.3 (E), 16.6 (Z)
af	iPr	Me	67 (1:1)	1.6 (E), 7.8 (Z)
ag	iPr	CF ₃	62 (3:2)	15.2 (E), 13.3 (Z)
ah	iPr	OMe	84 (1:1)	6.6 (E/Z = 1/4)
ai	iPr	OPh	34 (1:1)	9.6 (E/Z = 1/1)
aj	iPr	NO ₂	23 (5:1)	20.6 (E), 10.7 (Z)

Biological evaluation

In a preliminary *in vitro* biological evaluation, the inhibitory effect of the isoquinolinesulfonamides **E-55a-d, j-m, s-v, ab-ae** (R¹ = H, Me, Et or *i*Pr; R² = H, F, Cl or Br) against PKA and PKB/Akt1 are depicted in Figure 7A and B. Activity against *salmonella* in primary human macrophages is depicted in Figure 7C.²⁸ In the case of PKA, with a proton or a methyl group on the double bond, larger halogens provided better inhibition. With an ethyl or isopropyl, however, the bromine is no longer tolerated on that position. Inhibitory potency against PKB increases with halogen size and is largely unaffected by the size of R¹. The potency of **E-55a-d, j-m** (R¹ = H or Me) is strongly dependent on the nature of R². Compounds **E-55s-v, ab-ae** (R¹ = Et or *i*Pr) are generally more active and less dependent on the nature of R².

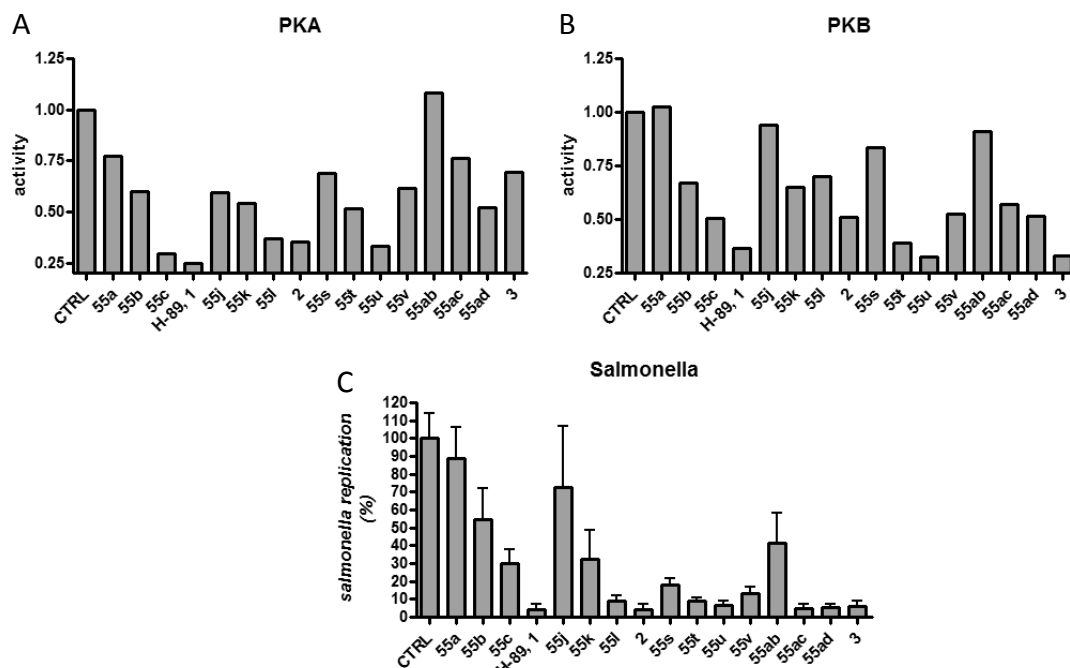


Figure 7; a: PKA activity as determined in an *in vitro* kinase reaction in the absence or presence of 10 μ M compound. Results are normalized to the activity detected in the absence of any compound (CTRL, containing DMSO only). b: Similar for PKB. c: Effect of 10 μ M compound in intracellular growth of *Salmonella* in human primary macrophages compared to DMSO.

Conclusion

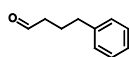
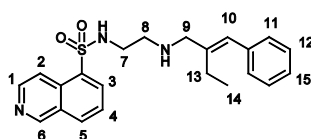
Initial SAR has identified a novel substitution site on the skeleton of H-89 that allows for the construction of more selective inhibitors for PKB. Substitution of the double bond with aliphatic sidechains with increasing size reduces potency against PKA rendering it more selective towards PKB. These results indicate that the ATP binding pocket of PKB, although very similar to PKA in amino acid composition, does contain a cavity situated around the double bond that can be occupied by a bulky apolar group. Replacing the bromine does not result in increased selectivity, however, activity is changed. Smaller halogens decrease activity probably due to reduced van der Waals interaction. Up-scaling the synthesis of the lead compound **2** proved to be less than trivial and could only be optimized to a maximum yield of 24% after silica column purification and re-crystallization, whereas the small scale procedure yielded 44% after HPLC purification. Diversification of the isoquinolinesulfonamide scaffold was achieved *via* the transimination protocol allowing the synthesis of 64 novel H-89 derivatives varying in the double bond configuration, the double bond substitution and the aromatic substituent. HPLC purification and separation of the two geometrical isomers was successful in most cases. Preliminary *in vitro* biological tests show a

decrease activity against PKA with bulky groups for R^1 while activity against PKB is unaffected. Activity against both enzymes increases with increasing size of R^2 . In the *in vivo* experiments, the effect of R^2 seems to correlate with the size of R^1 . For $R^1 = H$ or Me, activity increases with the size of R^2 . For $R^1 = Et$ or *i*Pr, the influence of R^2 on the inhibitory potency is largely abolished.

Experimental Section:

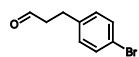
General: PE with a boiling range of 40 - 60°C was used. THF and Et₂O were distilled over LiAlH₄ prior to use. DCM was distilled over CaH₂ prior to use. All other solvents used under anhydrous conditions were stored over molecular sieves (4Å) except for methanol which was stored over 3Å molecular sieves. Solvents used for work-up and column chromatography were of technical grade and distilled before use. Unless stated otherwise, solvents were removed by rotary evaporation under reduced pressure at 40°C. Reactions were monitored by TLC-analysis using Merck 25 DC plastikfolien 60 F₂₅₄ with detection by spraying with 20% H₂SO₄ in EtOH, (NH₄)₆Mo₇O₂₄·4H₂O (25 g/L) and (NH₄)₄Ce(SO₄)₄·2H₂O (10 g/L) in 10% sulfuric acid or by spraying with a solution of ninhydrin (3 g/L) in EtOH / AcOH (20/1 v/v), followed by charring at approx. 150°C. Column chromatography was performed on Fluka silicagel (0.04 – 0.063 mm). For LC/MS analysis, an JASCO HPLC-system (detection simultaneously at 214 and 254 nm) equipped with an analytical C₁₈ column (4.6 mmD × 250 mmL, 5μ particle size) in combination with buffers A: H₂O, B: MeCN and C: 0.5% aq. TFA and coupled to a mass instrument with a custom-made Electrospray Interface (ESI) was used. For reversed-phase HPLC purification of the final compounds, an automated HPLC system supplied with a semi-preparative C₁₈ column (10.0 mmD × 250 mmL, 5μ particle size) was used. The applied buffers were A: H₂O, B: MeCN and C: 1.0% aq. TFA. High resolution mass spectra were recorded by direct injection (2 μL of a 2 μM solution in water/acetonitrile; 50/50; v/v and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 °C) with resolution $R = 60000$ at m/z 400 (mass range $m/z = 150-2000$) and dioctylphthalate ($m/z = 391.28428$) as a "lock mass".²⁹ The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). ¹H- en ¹³C-NMR spectra were measured on a Joel JNM-FX-200 (200/50 Mhz), a Brüker AV-400 (400/100 MHz), a Brüker AV-500 (500/125 MHz) or a Brüker DMX-600 (600/125 MHz). Chemical shifts are given in ppm (δ) relative to TMS (0 ppm) or MeOD (3.30 ppm) and coupling constants are given in Hz.

The Isoquinoline sulfonic acid-(2-amino-ethyl) amides are numbered as follows:

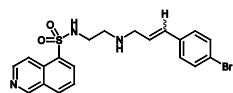


4-Phenyl-butyraldehyde **35**: Dess-Martin periodinane (2.4 g, 6 mmol, 6 equiv.) was added to a solution of 4-phenyl-1-butanol (150 mg, 1 mmol) in CH₂Cl₂ (30 mL). After stirring the reaction

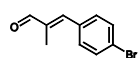
mixture at room temperature for 1 h a solution of 1M Na₂S₂O₃ (30 mL) was added and stirred vigorously for 5 min. The aqueous layer was extracted with CH₂Cl₂ (3 × 10 mL). The combined organic layers were dried (MgSO₄) and concentrated. The aldehyde was used without further purification.



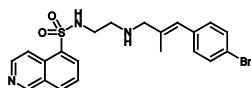
3-(4-Bromophenyl)propanal **38**: 3-(4-Bromophenyl)propionic acid (2.0 g, 8.7 mmol) was treated for 16 h with Me₂S in THF at 0°C to rt after which TLC analysis (20% EtOAc/PE) indicated complete conversion of the starting material into a higher running spot. The reaction mixture was cooled to 0°C and MeOH (10 mL) was slowly added (gas evolution) and stirring was continued for 1 h. Evaporation of all volatiles yielded the intermediate 3-(4-bromophenyl)propanol **37** (1.8 g, 8.4 mmol, 96%). All physical data was in agreement with published data³⁰. 3-(4-bromophenyl)propanol was oxidized to 3-(4-bromophenyl)propanal **38** using the same procedure as described for **35** and was used as crude aldehyde in the next reaction.



H-89 **1**: Amine **45** (276 mg, 1.1 mmol, 1.1 eq.) was co-evaporated with dry toluene/MeOH 1/1 to remove traces of water and dissolved in dry methanol and Na₂SO₄. Crude aldehyde **43** and acetic acid (11 µl, 0.2 mmol, 0.2 eq.) were added and stirring was continued until TLC analysis indicated complete disappearance of the aldehyde after which NaCNBH₃ (100 mg, 1.6 mmol, 1.6 eq.) was added. The reaction mixture was allowed to stir overnight before it was concentrated, redissolved in DCM (10 ml) and washed with brine. Filtration over a path of silica and reversed phase HPLC purification afforded the title compound (202 mg, 0.44mmol, 44%) as colorless oil. ¹H NMR (400 MHz, MeOD): δ 9.34 (s, 1H, H6), 8.63 – 8.60 (d, 1H, H1, *J* = 6.2), 8.56 – 8.53 (d, 1H, H2, *J* = 6.2), 8.49 – 8.45 (dd, 1H, H3, *J* = 1.1, 7.3), 8.38 – 8.34 (d, 1H, H5, *J* = 8.4), 7.83 – 7.76 (t, 1H, H4, *J* = 6.9, 8.1), 7.49 – 7.44 (d, 2H, CH_{phenyl}, *J* = 8.8), 7.12 – 7.08 (d, 2H, CH_{phenyl}, *J* = 8.4), 6.19 (s, 1H, =CHPh), 3.10 (s, 2H, NCH₂), 3.07 – 3.00 (t, 2H, H8, *J* = 6.2), 2.58 – 2.51 (t, 2H, H7, *J* = 6.2), 1.72 (d, 3H, CH₃, *J* = 1.1); ¹³C-NMR (100 MHz, MeOD) : δ 154.1, 144.8, 137.6, 137.0, 136.0, 134.7, 134.5, 132.3, 132.1 – 131.5, 130.2, 127.5, 126.8, 121.0, 118.9, 57.7, 48.5, 42.8, 16.7. MS: *m/z* = 460.1, 461.2 1 : 1 (M+H)⁺



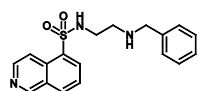
(E)-3-(4-bromophenyl)-2-methylacrylaldehyde **44**: The aldehyde was prepared *via* the intermediate (E)-3-(4-bromo-phenyl)-2-methyl-prop-2-en-1-ol which was prepared according to literature procedures³¹ on a 5 mmol scale from *p*-bromobenzaldehyde *via* a Wittig reaction with [(methoxycarbonyl)methyl]triphenylphosphonium iodide²³ followed by a DiBAL-H reduction affording the intermediate alcohol as off white solid (3.4 mmol, 67% over two steps). All physical data was in agreement with published data³¹. Oxidation using Dess-Martin periodane as described for **36** afforded crude aldehyde **52** which was used without further purification.

Large scale synthesis of Isoquinoline-5-sulfonic acid 2-((E)-3-(4-bromo-phenyl)-2-methyl-allylamino)-ethyl)-amide **2.**

Dess-Martin Periodinane (12.7 g, 30 mmol, 1.5 eq.) was dissolved in DCM (50 mL) under an argon atmosphere before a solution of (E)-3-(4-bromo-phenyl)-2-methyl-prop-2-en-1-ol (4.5 g, 20 mmol) in DCM (50 mL) was added. Stirring was continued until TLC analysis indicated complete disappearance of the starting material. The reaction was quenched by addition of Na₂S₂O₃ (25 mL, 2M) and sat. aq. NaHCO₃ (25 mL) and stirring was continued for 30 minutes after which the organic phase was separated, washed with brine, dried (MgSO₄) and concentrated. The residue was applied to silica column chromatography using diethylether / hexanes to afford aldehyde **44** as white solid (3.19 g, 14.3 mmol, 91%). ¹H-NMR (200 MHz, MeOD): δ 9.46 (s, 1H, CHO), 7.43 (d, 2H, 2x H_{arom}, J = 8.8), 7.4 (d, 2H, 2x H_{arom}, J = 8.8), 7.1 (s, 1H, CH=C), 1.93 (s, 3H, CH₃). ¹³C-NMR (50 MHz, CDCl₃): δ 194.7 (CH=O), 147.7 (C=CH), 138.3 (MeC=CH), 133.6 (C_qarom), 131.5 (CHarom), 131.1 (CHarom), 123.5 (C_qarom), 10.5 (CH₃). MS: m/z = 224.8, 226.8 1: 1 (M+H)⁺.

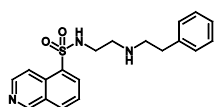
Amine **45** (6.3 g, 25 mmol, 1.8 eq.) was co-evaporated with dry toluene / dry methanol 1/1 to remove traces of water and dissolved in dry methanol (250 mL). Na₂SO₄ was added as drying agent. Aldehyde **44** (3.19 g, 14.3 mmol, 1 eq.) and Ti(OⁱPr)₄ (5.8 mL, 20 mmol, 1.4 eq.) were added and stirring was continued until TLC indicated complete disappearance of the aldehyde. The reaction mixture was cooled to 0°C and NaCNBH₃ (2.5 g, 40 mmol, 2.8 eq.) was added. The reaction mixture was allowed to stir overnight at r.t. before it was diluted with water (500 mL) and extracted with DCM (3x, 250 mL). The combined organic fractions were washed with sat. aq. NaHCO₃ (200 mL) and brine 200 mL), dried (MgSO₄), filtrated and concentrated. The residue was purified using column chromatography (MeOH / DCM) to afford the title compound with a minor impurity (~5% according to NMR). The compound was further purified as follows. The residue was dissolved in dioxane and *p*-toluenesulfonic acid monohydrate was added. The resulting precipitate was collected by filtration and washed with ether. ¹H-NMR (200 MHz, MeOD): 9.91 (s, 1H, H₆), 9.13 (d, 1H, H₁, J = 7.0), 8.83 (dd, 1H, H₃, J = 1.1, 7.3), 8.78 (m, 2H, H₂ + H₅), 8.13 (dd, 1H, H₄, J = 8.4, 7.3), 7.66 (m, 4H, 4x H_{arom}), 7.51 (d, 2H, 2x H_{arom}), 7.21 (m, 6H, 6x H_{arom}), 6.63 (s, 1H, CH=C), 3.79 (s, 2H, N-CH₂-), 3.28 – 3.24 (m, 4H, H₇ + H₈), 2.35 (s, 6H, 2x CH₃ pTsOH), 1.95 (d, 3H, =C-CH₃, J = 1.5 Hz). MS: m/z = 460.1, 461.2 1: 1 (M+H)⁺.

Anion exchange was performed as follows. The solid was suspended in DCM (100 mL) and neutralized with sat. aq. NaHCO₃ (50 mL). The organic layer was washed with brine, dried (MgSO₄), filtered and concentrated to afford the title compound as colorless oil (1.6 g, 3.5 mmol, 24%). Analytical data were identical to those obtained by the small scale procedure.

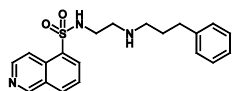


N-(2-(benzylamino)ethyl)isoquinoline-5-sulfonamide **26**: Isoquinolinesulfonamide **26** was prepared according to the procedure described for **1** yielding the title compound (30 mg, 0.09 mmol, 43 %) as light yellow oil. ¹H-NMR (200 MHz, MeOD): δ 9.34 (s, 1H, H₆), 8.60 – 8.57 (d, 1H, H₁, J = 6.2), 8.53 – 8.50 (d, 1H, H₂, J = 6.2), 8.45 – 8.42 (d, 1H, H₃, J = 7.3), 8.36 – 8.32 (d, 1H, H₅, J = 8.4), 7.81 – 7.73 (t, 1H, H₄, J = 7.3, 8.4), 7.27 – 7.10 (m, 5H, H_{arom}), 3.54 (s, 2H, N-CH₂-Ph), 3.03 – 2.97 (t, 2H, H₇, J = 6.2), 2.57 – 2.50 (t, 2H, H₈, J = 6.2). ¹³C-NMR (50 MHz, MeOD): δ 154.0 (C₆), 144.6 (C₁), 139.8 (C_qPh), 136.1 (C₉), 134.6 (C₃),

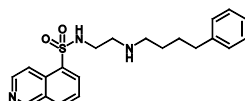
134.4 (C5), 132.3 (C10), 130.3 (C11), 129.2, 129.0 (CH_{phenyl}), 127.9 (C4), 127.4 (CH_{phenyl}), 118.8 (C2), 53.6 (C8), 49.6 (CH₂Ph), 42.7 (C7); MS: m/z = 342.0 (M+H)⁺



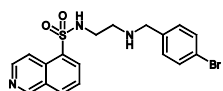
N-(2-(phenethylamino)ethyl)isoquinoline-5-sulfonamide **27**: Isoquinolinesulfonamide **27** was synthesized according to the procedure described for **1** yielding the title compound (22 mg, 0.06 mmol, 31 %) as colorless oil. ¹H-NMR (200 MHz, MeOD) : δ 9.37 (s, 1H, H6), 8.62 – 8.59 (d, 1H, H1, J = 6.2), 8.54 – 8.50 (d, 1H, H2, J = 6.2), 8.47 – 8.43 (d, 1H, H3, J = 7.3), 8.40 – 8.35 (d, 1H, H5, J = 8.4), 7.84 – 7.76 (dd, 1H, H4, J = 8.0, J = 7.3), 7.29 – 7.08 (m, 5H, CH_{arom}), 2.99 – 2.93 (t, 2H, H7, J = 6.2), 2.62 – 2.55 (m, 6H, 2x NH-CH₂ + CH₂-Ph). ¹³C-NMR (50 MHz, MeOD) : δ 154.1 (C6), 144.6 (C1), 140.4 (Cq_{phenyl}), 136.0 (C9), 134.6 (C3), 134.5 (C5), 132.3 (C10), 130.4 (C11), 129.3, (2x CH_{phenyl}), 127.4 (C4), 127.0 (CH_{phenyl}), 118.9 (C2), 51.2 (C8), 49.0 (NCH₂-CH₂Ph) 42.7 (C7), 36.4 (NCH₂-CH₂Ph); MS: m/z = 356.1 (M+H)⁺.



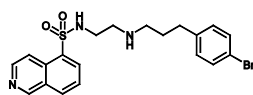
N-(2-(3-phenylpropylamino)ethyl)isoquinoline-5-sulfonamide **28**: Product **28** was synthesized according to the procedure described for **1** yielding the title compound (20 mg, 0.05 mmol, 27 %) as a colorless oil. ¹H-NMR (200 MHz, MeOD) : δ 9.39 – 9.38 (d, 1H, H6, J = 1.1), 8.65 – 8.62 (d, 1H, H1, J = 6.2), 8.56 – 8.52 (dt, 1H, H2, J = 1.1, 6.2), 8.49 – 8.44 (dd, 1H, H3, J = 1.09, 7.3), 8.41 – 8.37 (d, 1H, H5, J = 8.4), 7.86 – 7.78 (dd, 1H, H4, J = 7.3, 8.4), 7.27 – 7.09 (m, CH_{phenyl}), 3.03 – 2.97 (t, 2H, H7, J = 6.6), 2.68 – 2.48 (m, 6H, 2x, NH-CH₂ + CH₂-Ph, 1.76 – 1.61 (m, 2H, CH₂-CH₂-Ph). ¹³C-NMR (50 MHz, MeOD) : δ 154.1 (C6), 144.6 (C1), 142.7 (Cq_{phenyl}), 136.1 (C9), 134.6 (C3), 134.5 (C5), 132.4 (C10), 130.4 (C11), 129.1 (2x CH_{phenyl}), 127.5 (C4), 126.6 (CH_{phenyl}), 50.0 (C8), 49.2 (NH-CH₂), 42.6 (C7), 34.1 (CH₂-Ph), 31.8 (CH₂-CH₂-Ph); MS: m/z = 370.1 (M+H)⁺



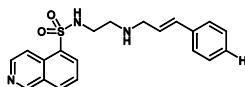
N-(2-(4-phenylbutylamino)ethyl)isoquinoline-5-sulfonamide **29**: This product was synthesized analogously to the procedure described for **1** using 4-phenylbutyraldehyde **35** (30 mg, 0.2 mmol, 0.8 equiv.) to give **29** (17 mg, 0.04 mmol, 22%) as a colorless oil. ¹H NMR (200 MHz, MeOD) : δ 9.38 (s, 1H, H6), 8.64 – 8.61 (d, 1H, H2, J = 6.2), 8.55 – 8.52 (d, 1H, H2, J = 6.2), 8.48 – 8.44 (d, 1H, H3, J = 7.3), 8.40 – 8.36 (d, 1H, H5, J = 8.0), 7.85 – 7.77 (dd, 1H, H4, J = 7.3, 8.4), 7.28 – 7.09 (m, 5H, CH_{phenyl}), 3.06 – 2.99 (t, 2H, H8, J = 6.6), 2.70 – 2.53 (m, 4H, CH₂-Ph + NH-CH₂), 2.49 – 2.42 (t, 2H, H7, J = 6.9), 1.61 – 1.19 (m, 4H, NH-CH₂-CH₂ + CH₂-CH₂-Ph); MS: m/z = 384.2 (M+H)⁺.



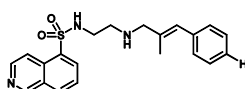
N-(2-(4-bromobenzylamino)ethyl)isoquinoline-5-sulfonamide **30**: This compound is prepared according to the procedure described for **1** using *p*-bromobenzaldehyde. Yield: 5.3 mg, 9.85 μmol 10%. ¹H NMR (200 MHz, MeOD): δ 9.65 (bs, 1H, H6), 8.78 – 8.77 (d, 1H, H2, J = 5.6), 8.70 (bs, 1H, H1), 8.61 – 8.60 (d, 1H, H5, J = 7.3), 8.56 – 8.55 (d, 1H, H3, J = 8.2), 7.96 – 7.94 (t, 1H, H4, J = 7.8, 7.9), 7.59 – 7.58 (d, 2H, H10, J = 8.3), 7.40 – 7.38 (d, 2H, H11, J = 8.3), 3.2 (s, 2H, H9), 3.18 – 3.15 (m, 4H, H7+H8); ¹³C NMR (50 MHz, MeOD): δ 152.4, 140.5, 137.0, 136.2, 136.0, 134.0, 133.4, 133.0, 131.5, 129.2, 124.9, 121.1, 51.4, 48.0, 39.9; MS: m/z = 420.5, 422.2 1:1 (M+H)⁺; HRMS: calcd for [C₁₈H₁₈BrN₃O₂S + H]⁺ = 420.03759, found 420.03757



N-(2-(3-(4-bromophenyl)propylamino)ethyl)isoquinoline-5-sulfonamide **31:** This compound is prepared according to the general procedure using 3-(4-bromophenyl)-propanal. Yield: 4.2 mg, 7.45 μ mol 7.5%. $^1\text{H-NMR}$ (MeOD): δ 9.47 (bs, 1H, H6), 8.66 (bs, 1H, H1), 8.57 – 8.56 (d, 1H, H2, $J_1 = 5.46$ Hz), 8.48 – 8.44 (m, 2H, H3/5), 7.87 – 7.84 (t, 1H, H4, $J_1 = 7.80$ Hz, $J_2 = 7.86$ Hz), 7.45 – 7.43 (d, 2H, H12, $J_1 = 8.22$ Hz), 7.16 – 7.15 (d, 2H, H13, $J_1 = 8.22$ Hz), 3.11 – 3.07 (m, 4H, H7/8), 3.03 – 3.00 (t, 2H, H9, $J_1 = 8.16$ Hz, $J_2 = 7.92$ Hz), 2.69 – 2.66 (t, 2H, H11, $J_1 = J_2 = 7.26$ Hz), 2.00 – 1.95 (m, 2H, H10); $^{13}\text{C-NMR}$ (MeOD): δ 154.11, 144.33, 140.80, 135.55, 135.38, 135.33, 132.77, 131.41, 128.01, 121.16, 48.29, 39.97, 32.86, 28.58; MS: $m/z = 448.40, 450.27$ 1:1 ($\text{M}+\text{H}$) $^+$; HRMS: calcd for $[\text{C}_{20}\text{H}_{22}\text{BrN}_3\text{O}_2\text{S} + \text{H}]^+ = 448.06889$, found 448.06890

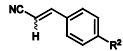


Isoquinoline-5-sulfonic acid (2-((E)-3-phenyl-allylamino)-ethyl)-amide **32:** Compound **32** was synthesized according to the general procedure using cinnamic aldehyde (191 μ l, 1.36 mmol) yielding, after HPLC purification, **32** (233 mg, 0.56 mmol, 41%) as yellowish oil. $^1\text{H NMR}$ (CDCl_3): δ 9.76 (bs, 1H, H6), 8.97 – 8.93 (d, 1H, H1, $J = 6.57$ Hz), 8.72 – 8.59 (m, 3H, H2, H3, H5), 8.05 – 7.97 (t, 1H, H4, $J = 8.04$ Hz), 7.34 – 7.26 (m, 5H, H_{arom}), 6.85 – 6.77 (d, 1H, PhCH= , $J = 16.08$ Hz), 6.29 – 6.21 (m, 1H, $\text{CH}_2\text{CH=}$), 3.85 – 3.62 (d, 2H, NCH_2 , $J = 7.30$ Hz), 3.21 (bs, 4H, H7, H8). $^{13}\text{C-NMR}$ (CDCl_3): δ 151.59 (C6), 139.88 (C1), 137.03 (C3), 136.57 (C9), 136.57 (C5), 135.58 (C10), 129.57 – 127.69 ($\text{CH}_2\text{CH=}$, $\text{CH}_{\text{phenyl}}$), 121.35 (CH, C2), 118.83 (CH= , C14), 50.30 (CH_2 , C12), 47.30 (CH_2 , C11), 39.92 (CH_2 , C10). MS: $m/z = 368.1$ ($\text{M}+\text{H}$) $^+$

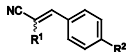


Isoquinoline-5-sulfonic acid (2-((E)-2-methyl-3-phenyl-allylamino)-ethyl)-amide **33:** Compound **33** was synthesized according to the general procedure using α -methyl cinnamic aldehyde (1.36 mmol) yielding, after HPLC purification, **33** (0.6 mmol, 44%) as yellowish oil. $^1\text{H NMR}$ (CDCl_3): δ 9.60 (s, 1H, H6), 8.74 (m, 2H, H1, H2), 8.64 – 8.46 (m, 2H, H3, H5), 7.99 – 7.91 (dd, 1H, H4, $J_1 = 7.31$ Hz, $J_2 = 8.04$ Hz), 7.42 – 7.17 (m, 5H, H_{phenyl}), 6.71 (s, 1H, CMe=CHPh), 3.80 (s, 2H, NCH_2), 3.21 (s, 4H, H7, H8), 1.98 (s, 3H, CH_3). $^{13}\text{C-NMR}$ (CDCl_3): δ 152.9, 144.4, 137.2, 135.5, 134.2, 133.3, 133.0, 130.9, 128.7, 128.5 – 129.9, 125.8, 117.2, 57.0, 47.1, 42.1, 16.1. MS: $m/z = 382.2$ ($\text{M}+\text{H}$) $^+$

General procedure for the synthesis of cinnamonitriles.

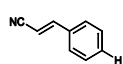
a) para-substituted cinnamonitriles with the general structure  were prepared as follows:

To an ice-cold solution of NaH (516 mg, 12.9 mmol, 60% mineral oil) and NaI (1.9 g, 12.9 mmol) in DMF (40 mL) diethyl cyanomethylphosphonate (2.27 g, 12.8 mmol) was slowly added and allowed to stir for 15 minutes before addition of the aldehyde (13.5 mmol). The reaction was allowed to stir until completion (TLC 10% EtOAc/PE) and quenched by addition of freshly prepared sat. aq. Na_2HSO_3 (50 mL). The mixture was diluted with H_2O (150 mL) and Et_2O (50 mL), the layers were separated, the aqueous phase washed with Et_2O (3 x 50 mL) and the combined organic phase was washed with sat. aq. bicarb. (1 x 25 mL) and brine (1 x 25 mL), dried over Na_2SO_4 , filtered and concentrated. The residue was further purified by silica column chromatography (0 - 3% Et_2O /PE) to afford the cinnamonitriles.

b) para-substituted, α -alkylated cinnamonnitriles with the general structure  were prepared similarly to the cinnamonnitriles described above with the following adaptations:

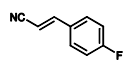
- No NaI was used

- diethyl cyanomethylphosphonate was first deprotonated at 0°C using NaH (516mg, 12.9 mmol, 60% mineral oil) and subsequently alkylated with alkyl iodide (13 mmol) for 1h at r.t. after which the reaction mixture was cooled to 0°C and treated as described above to afford the α -alkylated cinnamonnitriles as E/Z mixtures which were used as E/Z mixtures in the following reactions.



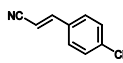
(E/Z)-Cinnamonnitrile **54a** was obtained *via* method A in 91% yield as white solid (E/Z = 4/1).

^1H NMR (400 MHz, CDCl_3) δ 7.87 – 7.81 (m, 2H), 7.48 – 7.42 (m, 7H), 7.41 (s, 1H), 7.37 (s, 1H), 7.15 (d, J = 12.1, 1H), 5.89 (d, J = 16.7, 1H), 5.47 (d, J = 12.1, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ 150.3, 148.5, 133.4, 133.2, 131.09, 130.7, 128.9, 128.8, 128.7, 127.2, 118.0, 117.2, 96.1, 94.8. MS: m/z = 130.0 ($\text{M}+\text{H}$) $^+$.



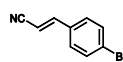
(E/Z)-3-(4-fluorophenyl)acrylonitrile **54b** was obtained *via* method A in 84 % yield as white solid

with an E/Z ratio of 5/2. ^1H NMR (400 MHz, CDCl_3) δ : 7.74 – 7.65 (m, 2H), 7.39 – 7.31 (m, 3H), 7.23 (d, J = 16.7, 1H), 7.03 – 6.94 (m, 4H), 5.75 (d, J = 16.7, 1H), 5.37 (d, J = 12.1, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ 164.84, 164.4, 162.3, 161.9, 148.4, 146.6, 130.6, 130.5, 129.4, 129.4, 129.3, 129.0, 128.9, 117.6, 116.8, 115.6, 115.4, 115.2, 95.5, 95.5, 94.1, 77.2, 53.2. MS: m/z = 148.3 ($\text{M}+\text{H}$) $^+$.



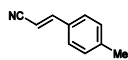
(E/Z)-3-(4-chlorophenyl)acrylonitrile **54c** was obtained *via* method A in 88 % yield as white solid

with an E/Z ratio of 2/1. ^1H NMR (400 MHz, CDCl_3) δ 7.76 (d, J = 8.5, 2H), 7.45 – 7.33 (m, 7H), 7.12 (d, J = 10.0, 1H), 5.88 (d, J = 16.7, 1H), 5.50 (d, J = 12.1, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ 149.0, 147.1, 137.1, 131.9, 130.1, 129.3, 129.0, 128.4, 117.7, 96.9, 95.6. MS: m/z = 163.9 ($\text{M}+\text{H}$) $^+$.



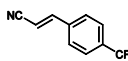
(E/Z)-3-(4-bromophenyl)acrylonitrile **54d** was obtained *via* method A in 87 % yield as a white solid

with an E/Z ratio of 5/2. ^1H NMR (400 MHz, CDCl_3) δ 7.68 (d, J = 8.6, 2H), 7.59 – 7.51 (m, 3H), 7.38 – 7.30 (m, 4H), 7.08 (d, J = 12.1, 1H), 5.90 (d, J = 16.6, 1H), 5.51 (d, J = 12.1, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ 149.0=1, 147.2, 132.2, 132.0, 130.2, 128.6, 125.5, 125.2, 117.7, 97.0, 95.7. MS: m/z = 207.9 : 209.9 1:1 ($\text{M}+\text{H}$) $^+$.



(E)-3-p-tolylacrylonitrile **54e** was obtained *via* method A in 99 % yield as white solid with an E/Z

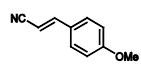
ratio of 5/2. ^1H NMR (400 MHz, CDCl_3) δ 7.65 (d, J = 8.1, 2H), 7.25 – 7.09 (m, 7H), 6.99 (d, J = 12.1, 1H), 5.70 (d, J = 16.6, 1H), 5.30 (d, J = 12.1, 1H), 2.34 (s, 3H), 2.30 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 149.3, 147.0, 141.0, 140.7, 130.4, 130.2, 129.1, 129.0, 128.9, 128.3, 126.7, 117.8, 117.0, 94.3, 93.0, 20.7. MS: m/z = 144.0 ($\text{M}+\text{H}$) $^+$.



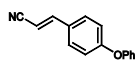
(E/Z)-3-(4-(trifluoromethyl)phenyl)acrylonitrile **54f** was obtained *via* method A in 92 % yield as

yellowish solid with an E/Z ratio of 5/2. ^1H NMR (400 MHz, CDCl_3) δ 7.92 (d, J = 8.2, 2H), 7.75 – 7.66 (m, 3H), 7.59 (d, J = 8.2, 2H), 7.45 (d, J = 16.7, 2H), 7.21 (d, J = 12.1, 1H), 6.02 (d, J = 16.7, 1H), 5.63 (d, J =

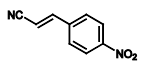
12.1, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ 148.7, 146.9, 136.7, 132.4, 129.1, 127.5, 126.0, 126.0, 125.8, 125.8, 124.9, 122.2, 117.3, 116.6, 99.2, 97.9. MS: m/z = 197.8 ($\text{M}+\text{H}$) $^+$.



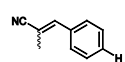
(E/Z)-3-(4-methoxyphenyl)acrylonitrile **54g**. ^1H NMR (400 MHz, CDCl_3) δ 7.36 (d, J = 8.8, 2H), 7.27 (d, J = 16.6, 1H), 6.90 (d, J = 8.8, 2H), 5.68 (d, J = 16.6, 1H), 3.82 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 161.7, 149.6, 128.8, 126.0, 118.4, 114.2, 93.0, 55.1. MS: m/z = 160.2 ($\text{M}+\text{H}$) $^+$



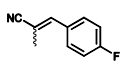
(E/Z)-3-(4-phenoxyphenyl)acrylonitrile **54h** was obtained *via* method A in 90 % yield as white solid with an E/Z ratio of 3/1. ^1H NMR (400 MHz, CDCl_3) δ 7.83 (d, J = 8.7, 2H), 7.47 – 7.34 (m, 7H), 7.22 (t, J = 7.4, 1H), 7.06 (m, 9H), 5.79 (d, J = 16.6, 1H), 5.38 (d, J = 12.1, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ 160.23, 155.64, 149.53, 147.62, 130.83, 129.92, 129.02, 128.12, 124.33, 124.29, 119.80, 119.76, 118.23, 117.98, 94.58, 93.15. MS: m/z = 222.0 ($\text{M}+\text{H}$) $^+$.



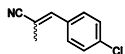
(E/Z)-3-(4-(trifluoromethyl)phenyl)acrylonitrile **54i** was obtained *via* method A in 92 % yield as yellow solid with an E/Z ratio of 2/1. ^1H NMR (400 MHz, CDCl_3) δ 8.29 (m, 3H), 7.97 (d, J = 8.7, 2H), 7.66 (m, 3H), 7.49 (d, J = 16.7, 1H), 7.27 (d, J = 12.2, 1H), 6.09 (d, J = 16.7, 1H), 5.73 (d, J = 12.1, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ 147.71, 146.01, 139.16, 129.68, 129.57, 128.10, 124.27, 124.03, 123.81, 116.96, 100.93, 99.56, 44.16, 30.22.



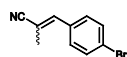
(E/Z)-2-methyl-3-phenylacrylonitrile **54j** was obtained *via* method B in 60 % yield as off white solid with an E/Z ratio of 1/1. ^1H NMR (400 MHz, CDCl_3) δ 7.74 – 7.68 (m, 2H), 7.46 – 7.33 (m, 6H), 7.31 (d, J = 7.0, 2H), 7.16 (s, 1H), 6.92 (s, 1H), 2.12 (s, 3H), 2.10 (d, J = 1.3, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 149.96, 148.20, 143.85, 143.55, 133.65, 133.46, 130.72, 130.47, 129.35, 128.93, 128.86, 128.64, 128.56, 128.46, 128.32, 128.25, 127.98, 126.97, 120.81, 118.78, 109.20, 105.63, 95.95, 94.64, 21.63, 16.32.



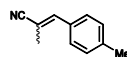
(E/Z)-3-(4-fluorophenyl)-2-methylacrylonitrile **54k** was obtained *via* method B in 45 % yield as colorless oil with an E/Z ratio of 1/1. ^1H NMR (400 MHz, CDCl_3) δ 7.72 – 7.48 (m, 2H), 7.33 – 7.18 (m, 2H), 7.13 – 6.92 (m, 5H), 6.87 – 6.77 (m, 1H), 2.11 – 2.00 (m, 6H). ^{13}C NMR (100 MHz, CDCl_3) δ 163.93, 163.62, 161.44, 161.13, 142.48, 142.11, 130.99, 130.91, 129.99, 129.91, 129.83, 129.72, 120.62, 118.58, 115.36, 115.15, 108.95, 105.38, 21.29, 16.07.



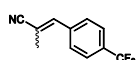
(E/Z)-3-(4-chlorophenyl)-2-methylacrylonitrile **54l** was obtained *via* method B in 38 % yield as yellowish solid with an E/Z ratio of 3/2. ^1H NMR (400 MHz, CDCl_3) δ 7.56 (d, J = 8.5, 2H), 7.34 – 7.24 (m, 4H), 7.19 (d, J = 8.5, 2H), 7.05 (s, 1H), 6.81 (s, 1H), 2.07 (s, 3H), 2.04 (d, J = 1.0, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 142.37, 142.01, 134.97, 134.67, 132.03, 131.86, 130.19, 129.19, 128.42, 120.45, 118.40, 109.80, 106.36, 21.54, 16.29. MS: m/z = 177.9 ($\text{M}+\text{H}$) $^+$.



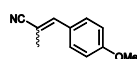
(E/Z)-3-(4-bromophenyl)-2-methylacrylonitrile **54m** was obtained *via* method B in 63 % yield as white solid with an E/Z ratio of 1/1. ^1H NMR (400 MHz, CDCl_3) δ 7.57 – 7.45 (m, 6H), 7.17 (d, J = 8.4, 2H), 7.09 (s, 1H), 6.85 (s, 1H), 2.12 (s, 3H), 2.09 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 142.67, 142.30, 132.59, 132.39, 131.60, 131.57, 130.50, 129.54, 123.59, 123.25, 120.60, 118.56, 110.10, 106.68, 21.82, 16.53.



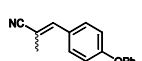
(E/Z)-2-methyl-3-(4-methylphenyl)acrylonitrile **54n** was obtained *via* method B in 35 % yield as colorless oil with an E/Z ratio of 1/1. ^1H NMR (400 MHz, CDCl_3) δ 7.63 (d, J = 8.1, 1H), 7.24 – 7.16 (m, 7H), 7.12 (s, 1H), 6.87 (s, 1H), 2.38 (s, 3H), 2.37 (s, 3H), 2.11 (s, 6H). ^{13}C NMR (100 MHz, CDCl_3) δ 143.83, 143.53, 139.60, 139.13, 130.95, 130.76, 129.01, 128.96, 128.65, 127.98, 121.06, 118.99, 108.03, 104.26, 21.57, 20.91, 16.36. MS: m/z = 157.9 ($\text{M}+\text{H}$) $^+$.



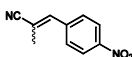
(E/Z)-2-methyl-3-(4-(trifluoromethyl)phenyl)acrylonitrile **54o** was obtained *via* method B in 58 % yield as yellow oil with an E/Z ratio of 1/1. Yield 58%, colorless oil. ^1H NMR (400 MHz, CDCl_3) δ 7.77 (d, J = 8.2, 2H), 7.65 (d, J = 8.1, 2H), 7.61 (d, J = 8.1, 2H), 7.42 (d, J = 8.1, 2H), 7.20 (s, 1H), 6.97 (s, 1H), 2.16 (s, 3H), 2.11 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 148.67, 146.93, 136.67, 132.35, 129.08, 127.52, 126.00, 125.96, 125.79, 125.75, 124.89, 122.19, 117.32, 116.55, 99.16, 97.90. MS: m/z = 212.1 ($\text{M}+\text{H}$) $^+$.



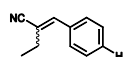
(E/Z)-3-(4-methoxyphenyl)-2-methylacrylonitrile **54p** was obtained *via* method B in 20 % yield as yellow oil with an E/Z ratio of 1/0. ^1H NMR (400 MHz, CDCl_3) δ 7.63 (d, J = 8.8, 2H), 6.86 (d, J = 8.8, 2H), 6.78 (s, 1H), 3.74 (s, 3H), 2.04 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 160.28, 143.07, 129.61, 126.14, 119.26, 113.63, 102.35, 54.76, 21.39. MS: m/z = 174.1 ($\text{M}+\text{H}$) $^+$.



(E/Z)-2-methyl-3-(4-phenoxyphenyl)acrylonitrile **54q** was obtained *via* method B in 45 % yield as colorless oil with an E/Z ratio of 1/1. ^1H NMR (400 MHz, CDCl_3) δ 7.72 (d, J = 8.7, 2H), 7.43 – 7.33 (m, 4H), 7.30 (d, J = 8.7, 2H), 7.18 (dd, J = 7.0, 13.5, 2H), 7.13 (s, 1H), 7.10 – 6.97 (m, 8H), 6.87 (s, 1H), 2.12 (s, 3H), 2.11 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 158.34, 158.02, 155.58, 142.99, 142.67, 130.81, 129.73, 129.54, 129.50, 128.37, 128.20, 123.77, 123.67, 121.01, 119.23, 119.20, 118.95, 117.67, 117.65, 107.67, 103.94, 21.48, 16.33. MS: m/z = 236.1 ($\text{M}+\text{H}$) $^+$.

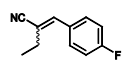


(E/Z)-2-methyl-3-(4-nitrophenyl)acrylonitrile **54r** was obtained *via* method B in 56 % yield as yellow oil with an E/Z ratio of 1/1. ^1H NMR (400 MHz, CDCl_3) δ 8.30 – 8.16 (m, 4H), 7.84 (d, J = 8.6, 2H), 7.51 (d, J = 8.7, 2H), 7.26 (s, 1H), 7.05 (s, 1H), 2.22 (s, 3H), 2.16 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 147.71, 147.48, 141.61, 141.20, 139.94, 139.62, 129.88, 128.98, 123.72, 123.63, 120.01, 118.06, 113.42, 110.79, 22.09, 16.74. MS: m/z = 188.9 ($\text{M}+\text{H}$) $^+$.

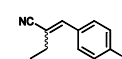


(E/Z)-2-benzylidenebutanenitrile **54s** was obtained *via* method B in 49 % yield as colorless oil with an E/Z ratio of 1/1. ^1H NMR (400 MHz, CDCl_3) δ 7.77 – 7.70 (m, 2H), 7.45 – 7.33 (m, 6H), 7.28 (d, J = 7.1, 2H), 7.15 (s, 1H), 6.93 (s, 1H), 2.47 (dd, J = 7.5, 15.0, 2H), 2.44 (dd, J = 7.5, 15.0, 2H), 1.28 – 1.21 (m, 6H).

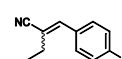
^{13}C NMR (100 MHz, CDCl_3) δ 142.95, 141.98, 133.59, 133.45, 129.32, 128.76, 128.65, 128.28, 128.24, 128.07, 119.64, 118.22, 116.71, 112.42, 29.09, 22.48, 12.50, 12.30. MS: m/z = 158.0 ($\text{M}+\text{H}$) $^+$.



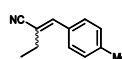
(E/Z)-2-(4-fluorobenzylidene)butanenitrile **54t** was obtained *via* method B in 55 % yield as colorless oil with an E/Z ratio of 4/3. ^1H NMR (400 MHz, CDCl_3) δ 7.73 – 7.66 (m, 2H), 7.31 – 7.23 (m, 2H), 7.11 – 7.01 (m, 4H), 6.88 (s, 1H), 2.49 – 2.35 (m, 4H), 1.27 – 1.19 (m, 6H). ^{13}C NMR (100 MHz, CDCl_3) δ 164.16, 163.83, 161.67, 161.34, 141.87, 140.79, 130.86, 130.77, 130.27, 130.18, 129.88, 129.85, 119.65, 118.25, 116.76, 115.59, 115.55, 115.37, 115.34, 112.38, 29.12, 22.54, 12.55, 12.32. MS: m/z = 176.1 ($\text{M}+\text{H}$) $^+$.



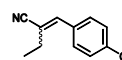
(E/Z)-2-(4-chlorobenzylidene)butanenitrile **54u** was obtained *via* method B in 39 % yield as colorless oil with an E/Z ratio of 1/1. ^1H NMR (400 MHz, CDCl_3) δ 7.37 – 7.30 (m, 7H), 7.20 (d, J = 8.5, 2H), 7.09 (s, 1H), 2.48 – 2.37 (m, 4H), 1.26 – 1.19 (m, 6H). ^{13}C NMR (100 MHz, CDCl_3) δ 141.68, 140.62, 136.68, 134.75, 132.11, 131.68, 130.06, 129.87, 129.41, 128.94, 128.75, 128.55, 128.25, 119.48, 117.49, 117.45, 29.13, 22.59, 12.49, 12.32. MS: m/z = 191.1 ($\text{M}+\text{H}$) $^+$.



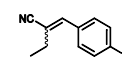
(E/Z)-2-(4-bromobenzylidene)butanenitrile **54v** was obtained *via* method B in 64 % yield as colorless oil with an E/Z ratio of 1/1. ^1H NMR (400 MHz, CDCl_3) δ 7.59 – 7.55 (m, 2H), 7.54 – 7.47 (m, 4H), 7.19 – 7.13 (m, 2H), 7.10 (s, 1H), 6.87 (s, 1H), 2.49 – 2.39 (m, 4H), 1.27 – 1.22 (m, 6H). ^{13}C NMR (100 MHz, CDCl_3) δ 141.98, 140.90, 132.67, 132.49, 132.20, 131.74, 131.72, 130.75, 130.40, 129.78, 123.74, 123.30, 119.66, 118.22, 117.73, 113.57, 29.40, 22.80, 12.69, 12.54. MS: m/z = 235.9 : 238.1 1:1 ($\text{M}+\text{H}$) $^+$.



(E/Z)-2-(4-methylbenzylidene)butanenitrile **54w** was obtained *via* method B in 16 % yield as yellow oil with an E/Z ratio of 1/1. ^1H NMR (400 MHz, CDCl_3) δ 7.66 (d, J = 8.1, 2H), 7.24 – 7.19 (m, 4H), 7.13 (s, 1H), 6.91 (s, 1H), 2.51 (q, J = 7.5, 3H), 2.47 – 2.41 (m, 3H), 2.39 (s, 3H), 2.38 (s, 3H), 1.30 – 1.23 (m, 6H). ^{13}C NMR (100 MHz, CDCl_3) δ 143.10, 142.09, 139.71, 139.15, 130.98, 130.83, 129.09, 129.07, 128.84, 128.19, 120.01, 118.56, 115.79, 111.25, 29.20, 22.62, 21.02, 20.96, 12.68, 12.41. MS: m/z = 172.0 ($\text{M}+\text{H}$) $^+$.

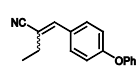


(E/Z)-2-(4-(trifluoromethyl)benzylidene)butanenitrile **54x** was obtained *via* method B in 60 % yield as colorless oil with an E/Z ratio of 3/2. ^1H NMR (400 MHz, CDCl_3) δ 7.81 (d, J = 8.2, 2H), 7.68 – 7.61 (m, 4H), 7.40 (d, J = 8.2, 2H), 7.20 (s, 1H), 6.99 (s, 1H), 2.52 – 2.42 (m, 4H), 1.30 – 1.23 (m, 6H). ^{13}C NMR (100 MHz, CDCl_3) δ 141.50, 140.44, 137.31, 137.10, 131.13, 130.81, 130.48, 129.09, 128.55, 127.70, 125.43, 125.40, 125.36, 124.99, 122.29, 119.47, 119.24, 117.85, 115.83, 29.35, 22.81, 12.42, 12.36. MS: m/z = 226.2 ($\text{M}+\text{H}$) $^+$.

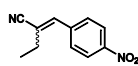


(E/Z)-2-(4-methoxybenzylidene)butanenitrile **54y** was obtained *via* method B in 51 % yield as colorless oil with an E/Z ratio of 1/1. ^1H NMR (400 MHz, CDCl_3) δ 7.69 (d, J = 8.8, 2H), 7.25 (d, J = 8.8, 2H), 7.07 (s, 1H), 6.93 – 6.88 (m, 4H), 6.84 (s, 1H), 3.81 (s, 3H), 3.80 (s, 3H), 2.48 (q, J = 7.5, 2H), 2.39 (q, J = 7.5, 2H), 1.28 – 1.19 (m, 6H). ^{13}C NMR (100 MHz, CDCl_3) δ 160.47, 160.07, 142.78, 141.74, 130.66, 129.90,

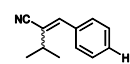
126.42, 126.30, 120.35, 118.90, 114.24, 113.86, 113.82, 109.52, 55.00, 54.98, 29.38, 29.15, 22.60, 12.79, 12.41.
MS: $m/z = 187.9$ (M+H)⁺.



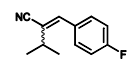
(E/Z)-2-(4-phenoxybenzylidene)butanenitrile **54z** was obtained *via* method B in 51 % yield as yellow oil with an E/Z ratio of 1/1. ¹H NMR (400 MHz, CDCl₃) δ 7.82 (d, $J = 6.9$, 2H), 7.72 (d, $J = 8.8$, 1H), 7.44 – 6.95 (m, 16H), 6.89 (s, 1H), 2.49 (q, $J = 7.5$, 2H), 2.40 (q, $J = 7.5$, 2H), 1.29 – 1.21 (m, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 190.27, 158.48, 158.14, 155.72, 154.75, 142.35, 141.31, 131.55, 130.93, 130.66, 129.94, 129.79, 129.61, 129.57, 128.38, 128.28, 124.55, 123.83, 123.74, 119.98, 119.94, 119.32, 119.28, 118.52, 117.81, 117.19, 116.01, 115.40, 110.89, 29.09, 22.55, 12.66, 12.36. MS: $m/z = 250.1$ (M+H)⁺.



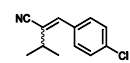
(E/Z)-2-(4-nitrobenzylidene)butanenitrile **54aa** was obtained *via* method B in 55 % yield as yellow oil with an E/Z ratio of 1/1. ¹H NMR (400 MHz, CDCl₃) δ 8.20 – 8.12 (m, 4H), 7.81 (d, $J = 8.8$, 2H), 7.45 (d, $J = 8.7$, 2H), 7.20 (s, 1H), 7.03 (s, 1H), 2.51 – 2.41 (m, 4H), 1.26 – 1.18 (m, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 147.47, 147.26, 140.60, 139.85, 139.59, 139.51, 129.58, 129.51, 128.93, 123.47, 123.44, 120.41, 118.80, 117.43, 117.26, 29.27, 22.79, 12.25. MS: $m/z = 203.0$ (M+H)⁺.



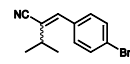
(E/Z)-2-benzylidene-3-methylbutanenitrile **54ab** was obtained *via* method B in 67 % yield as colorless oil with an E/Z ratio of 3/2. ¹H NMR (400 MHz, CDCl₃) δ 7.78 – 7.72 (m, 2H), 7.47 – 7.33 (m, 6H), 7.29 (d, $J = 7.0$, 2H), 7.11 (s, 1H), 6.97 (s, 1H), 3.19 – 3.08 (m, 1H), 2.74 – 2.62 (m, 1H), 1.27 (d, $J = 6.8$, 6H), 1.23 (d, $J = 6.8$, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 141.74, 140.65, 133.74, 133.55, 129.41, 128.75, 128.63, 128.51, 128.38, 128.29, 122.80, 118.37, 117.96, 117.48, 34.72, 27.31, 21.16, 21.07. MS: $m/z = 172.2$ (M+H)⁺.



(E/Z)-2-(4-fluorobenzylidene)-3-methylbutanenitrile **54ac** was obtained *via* method B in 67 % yield as colorless oil with an E/Z ratio of 3/2. ¹H NMR (400 MHz, CDCl₃) δ 7.74 – 7.67 (m, 2H), 7.29 – 7.23 (m, 2H), 7.11 – 7.01 (m, 5H), 6.91 (s, 1H), 3.06 (hept, $J = 6.7$, 1H), 2.65 (hept, $J = 6.8$, 1H), 1.23 (d, $J = 6.8$, 6H), 1.20 (d, $J = 6.7$, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 140.33, 139.20, 135.13, 134.69, 132.15, 132.04, 129.86, 129.56, 128.61, 128.56, 123.46, 118.72, 118.07, 117.22, 34.72, 27.39, 21.10, 21.00. MS: $m/z = 189.9$ (M+H)⁺.

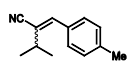


(E/Z)-2-(4-chlorobenzylidene)-3-methylbutanenitrile **54ad** was obtained *via* method B in 34 % yield as colorless oil with an E/Z ratio of 3/2. ¹H NMR (400 MHz, CDCl₃) δ 7.63 (d, $J = 8.6$, 2H), 7.37 – 7.28 (m, 4H), 7.19 (d, $J = 8.5$, 2H), 7.02 (s, 1H), 6.89 (s, 1H), 3.04 (hept, $J = 6.7$, 1H), 2.65 (hept, $J = 6.8$, 1H), 1.23 (d, $J = 6.9$, 6H), 1.19 (d, $J = 6.8$, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 140.33, 139.20, 135.13, 134.69, 132.15, 132.04, 129.86, 129.56, 128.61, 128.56, 123.46, 118.72, 118.07, 117.22, 34.72, 27.39, 21.10, 21.00. MS: $m/z = 206.1$ (M+H)⁺.

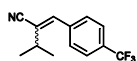


(E/Z)-2-(4-bromobenzylidene)-3-methylbutanenitrile **54ae** was obtained *via* method B in 73 % yield as yellowish oil with an E/Z ratio of 3/2. ¹H NMR (400 MHz, CDCl₃) δ 7.56 (d, $J = 8.6$, 2H), 7.52 – 7.45 (m, 4H), 7.12 (d, $J = 8.4$, 2H), 7.01 (s, 1H), 6.88 (s, 1H), 3.03 (hept, $J = 6.7$, 1H), 2.65 (hept, $J = 6.7$,

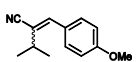
1H), 1.23 (d, $J = 6.8$, 6H), 1.19 (d, $J = 6.8$, 6H). ^{13}C NMR (100 MHz, CDCl_3) δ 140.49, 139.36, 132.60, 132.46, 131.63, 131.59, 130.10, 129.79, 123.57, 123.04, 118.89, 118.13, 117.26, 34.78, 27.45, 21.14, 21.06. MS: $m/z = 249.8 : 252.0$ 1:1 ($\text{M}+\text{H}$) $^+$.



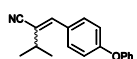
(E/Z)-3-methyl-2-(4-methylbenzylidene)butanenitrile **54af** was obtained *via* method B in 67 % yield as yellow oil with an E/Z ratio of 1/1. ^1H NMR (400 MHz, CDCl_3) δ 7.67 (d, $J = 8.2$, 2H), 7.25 – 7.17 (m, 6H), 7.07 (s, 1H), 6.93 (s, 1H), 3.16 (hept, $J = 6.7$, 1H), 2.67 (hept, $J = 6.8$, 1H), 2.39 (s, 3H), 2.38 (s, 3H), 1.27 (d, $J = 6.8$, 6H), 1.24 (d, $J = 6.8$, 6H). ^{13}C NMR (100 MHz, CDCl_3) δ 141.72, 140.58, 139.61, 138.92, 130.92, 130.80, 129.04, 128.57, 128.26, 121.80, 118.53, 117.64, 116.62, 34.65, 27.24, 21.16, 21.02. MS: $m/z = 185.9$ ($\text{M}+\text{H}$) $^+$.



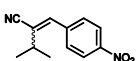
(E/Z)-3-methyl-2-(4-(trifluoromethyl)benzylidene)butanenitrile **54ag** was obtained *via* method B in 62 % yield as yellow oil with an E/Z ratio of 3/2. ^1H NMR (400 MHz, CDCl_3) δ 7.82 (d, $J = 8.3$, 2H), 7.69 – 7.61 (m, 4H), 7.40 (d, $J = 8.2$, 2H), 7.14 (s, 1H), 7.01 (s, 1H), 3.05 (hept, $J = 6.7$, 1H), 2.72 (hept, $J = 6.8$, 1H), 1.28 (d, $J = 6.8$, 6H), 1.22 (d, $J = 6.8$, 6H). ^{13}C NMR (100 MHz, CDCl_3) δ 140.13, 139.04, 137.42, 137.17, 131.14, 130.81, 130.44, 128.89, 128.66, 127.72, 125.46, 125.42, 125.39, 125.01, 122.31, 121.24, 119.60, 117.88, 117.06, 34.96, 27.72, 21.06, 21.03. MS: $m/z = 239.9$ ($\text{M}+\text{H}$) $^+$.



(E/Z)-2-(4-methoxybenzylidene)-3-methylbutanenitrile **54ah** was obtained *via* method B in 84 % yield as yellow oil with an E/Z ratio of 1/1. ^1H NMR (400 MHz, CDCl_3) δ 7.71 (d, $J = 8.8$, 2H), 7.25 (d, $J = 8.8$, 2H), 7.03 (s, 1H), 6.95 – 6.87 (m, 5H), 3.82 (s, 3H), 3.82 (s, 3H), 3.15 (hept, $J = 6.7$, 1H), 2.64 (hept, $J = 6.8$, 1H), 1.27 – 1.21 (m, 12H). ^{13}C NMR (100 MHz, CDCl_3) δ 160.51, 160.05, 141.55, 140.34, 130.47, 130.07, 126.41, 126.35, 120.54, 118.99, 118.11, 115.06, 113.95, 113.87, 55.06, 55.04, 34.73, 27.29, 21.37, 21.18. MS: $m/z = 202.0$ ($\text{M}+\text{H}$) $^+$.



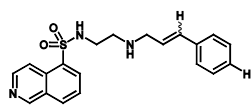
(E/Z)-3-methyl-2-(4-phenoxybenzylidene)butanenitrile **54ai** was obtained *via* method B in 34 % yield as yellow oil with an E/Z ratio of 1/1. ^1H NMR (400 MHz, CDCl_3) δ 7.75 (d, $J = 5.8$, 2H), 7.42 – 7.35 (m, 4H), 7.29 (d, $J = 8.6$, 2H), 7.22 – 7.15 (m, 2H), 7.11 – 6.99 (m, 9H), 6.94 (s, 1H), 3.17 (hept, $J = 6.7$, 1H), 2.69 (hept, $J = 6.8$, 1H), 1.30 – 1.24 (m, 12H). ^{13}C NMR (100 MHz, CDCl_3) δ 158.52, 158.14, 155.82, 155.76, 141.10, 139.92, 130.47, 130.11, 129.67, 129.63, 128.40, 128.36, 123.88, 123.78, 121.61, 119.38, 119.32, 118.62, 117.91, 117.76, 116.43, 34.71, 27.32, 21.28, 21.13. MS: $m/z = 263.9$ ($\text{M}+\text{H}$) $^+$.



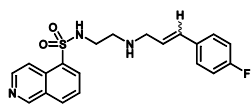
(E/Z)-3-methyl-2-(4-nitrobenzylidene)butanenitrile **54aj** was obtained *via* method B in 23 % yield as colorless oil with an E/Z ratio of 5/1. ^1H NMR (400 MHz, CDCl_3) δ 8.18 (d, $J = 8.8$, 2H), 8.15 (d, $J = 8.8$, 2H), 7.82 (d, $J = 8.8$, 2H), 7.43 (d, $J = 8.6$, 2H), 7.13 (s, 1H), 7.04 (s, 1H), 3.00 (hept, $J = 6.7$, 1H), 2.71 (hept, $J = 6.7$, 1H), 1.23 (d, $J = 6.9$, 6H), 1.18 (d, $J = 6.7$, 6H). ^{13}C NMR (100 MHz, CDCl_3) δ 147.46, 147.26, 140.00, 139.95, 139.67, 139.17, 138.08, 130.19, 129.47, 129.38, 129.03, 123.44, 122.98, 122.60, 117.44, 116.63, 34.86, 27.70, 23.85, 21.38, 20.89. MS: $m/z = 216.9$ ($\text{M}+\text{H}$) $^+$.

General procedure for the transimination

A solution of nitrile (1 mmol) in anhydrous Et₂O (5 mL) is cooled to -78°C before dropwise addition of DiBAL-H (2 mL, 2 mmol, 1M soln. in hexanes) and the reaction mixture was allowed to warm to 0°C in 30 min. under stirring. The reaction mixture was cooled to -100°C followed by rapid addition of MeOH (5 mL). After 5 min., a soln. of amine **45** (2 mmol) in MeOH (5 mL) was slowly added and the reaction mixture was allowed to stir at r.t. for 3 h. Then, the reaction mixture was cooled to -18°C and NaBH₄ (2mmol) was added and the reaction mixture was allowed to stir for 16 h. at r.t.. The reaction mixture was diluted with 0.5M aq. NaOH (10 mL), the layers were separated and the aqueous phase was washed with CHCl₃ (3 x 10 mL). The combined organic phase was washed with H₂O (3 x 10 mL) and brine (5 mL), dried, filtered and concentrated. The residue was purified by HPLC. The LCMS spectrum often showed the presence of a minor sideproduct with a mass of four daltons higher than the expected mass of the desired product. This probably arises from reduction of the isoquinoline ring towards the 1,2,3,4-tetrahydroisoquinoline analogue under the agency of residual NaBH₄ and TFA from the HPLC eluents. This product could, in most cases, be separated from the desired product except for **55l**, **55q** and **55u**. The Z isomer usually has a shorter retention time then the E isomer. This allowed, in most cases, the isolation of the individual isomers. However, in some cases, neither of the isomers could be obtained in >95% purity and were used as mixtures. The double bond configuration could be established based on the coupling constants of the double bond protons (**55a** – **55i**) or by NOESY analysis (**55j** – **55aj**).

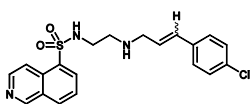


(E)-N-(2-(cinnamylamino)ethyl)isoquinoline-5-sulfonamide **55a**. Yield: 152.2 mg, 316 μmol, 31.6 %. ¹H NMR (400 MHz, MeOH) δ 9.59 (bs, 1H), 8.79 (d, *J* = 6.3, 1H), 8.68 (bs, 1H), 8.61 (d, *J* = 7.4, 1H), 8.49 (d, *J* = 8.2, 1H), 7.96 – 7.88 (m, 1H), 7.41 (d, *J* = 6.8, 2H), 7.35 – 7.25 (m, 3H), 6.81 (d, *J* = 15.8, 1H), 6.31 – 6.22 (m, 1H), 3.84 (d, *J* = 7.2, 2H), 3.24 (d, *J* = 4.4, 2H), 3.21 (d, *J* = 4.3, 2H). ¹³C NMR (100 MHz, MeOH) δ 152.05, 140.17, 139.98, 137.00, 136.69, 136.05, 135.85, 133.84, 129.76, 129.70, 129.14, 127.81, 121.10, 118.98, 50.44, 49.85, 47.44, 40.07. HRMS: calcd for [C₂₀H₂₁N₃O₂S + H]⁺ = 368.14272, found 368.14286.



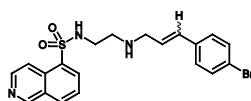
(E)-N-(2-(3-(4-fluorophenyl)allylamino)ethyl)isoquinoline-5-sulfonamide **55b**. Yield: 228.7 mg, 458 μmol, 34%.

¹H-NMR (MeOD): δ 9.42 (s, 1H, H₆), 8.63 – 8.59 (m, 2H, H_{1/2}), 8.50 – 8.49 (d, 1H, *J*₁ = 7.38 Hz, H₅), 8.37 – 8.35 (d, 1H, *J*₁ = 8.22 Hz, H₃), 7.81 – 7.86 (t, 1H, *J*₁ = 7.80 Hz, H₄), 7.39 – 7.37 (dd, 2H, *J*₁ = 5.46 Hz, *J*₂ = 8.58 Hz, H₁₁), 7.00 – 6.97 (t, 1H, *J*₁ = 8.73 Hz, H₁₂), 6.76 – 6.74 (d, 1H, *J*₁ = 15.84 Hz, H₁₀), 6.20 – 6.15 (dt, 1H, *J*₁ = 7.74 Hz, *J*₂ = 15.84 Hz, H₁₃), 3.81 – 3.80 (d, 2H, *J*₁ = 7.26 Hz, H₉), 3.21 – 3.15 (m, 4H); ¹³C-NMR (MeOD): δ 169.88, 167.42, 156.13, 143.83, 143.16, 141.85, 140.69, 140.43, 138.57, 137.61, 134.68, 134.24, 133.89, 125.96, 123.43, 121.85, 121.05, 120.84, 54.88, 54.34, 51.9, 44.57; MS: *m/z* = 386.13 (M+H)⁺. HRMS: calcd for [C₂₀H₂₀FN₃O₂S + H]⁺ = 386.13330, found 386.13377.



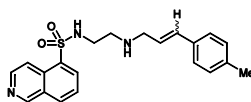
(E)-N-(2-(3-(4-chlorophenyl)allylamino)ethyl)isoquinoline-5-sulfonamide **55c**. Yield: 93.7 mg, 182 μmol, 18.2 %. ¹H-NMR (MeOD): δ 9.70 (bs, 1H, H₆), 8.87 – 8.86 (d, 1H,

H₂, $J_1 = 5.94$ Hz), 8.70 (bs, 1H, H₁), 8.65 – 8.64 (d, 1H, H₅, $J_1 = 7.32$ Hz), 8.58 – 8.56 (d, 1H, H₃, $J_1 = 8.28$ Hz), 7.98 – 7.96 (t, 1H, H₄, $J_1 = J_2 = 7.80$ Hz), 7.40 – 7.38 (d, 2H, H₁₁, $J_1 = 8.40$ Hz), 7.30 – 7.28 (d, 2H, H₁₂, $J_1 = 8.40$ Hz), 6.80 – 6.77 (d, 1H, H₁₀, $J_1 = 15.84$ Hz), 6.28 – 6.24 (dt, 1H, H₁₃, $J_1 = 7.20$ Hz, $J_2 = 7.80$ Hz, $J_3 = 15.84$ Hz), 3.82 – 3.81 (d, 2H, H₉, $J_1 = 7.14$ Hz), 3.19 – 3.14 (m, 4H, H_{7/8}); ¹³C-NMR (MeOD): δ 151.43, 138.61, 137.8, 136.43, 136.15, 135.54, 135.44, 129.87, 129.75, 129.33, 121.93, 120.02, 50.35, 47.55, 40.12; MS: $m/z = 402.27, 404.07$ 3:1 (M+H)⁺; HRMS: calcd for [C₂₀H₂₀ClN₃O₂S + H]⁺ = 402.10375, found 402.10349



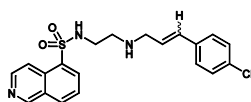
(E)-N-(2-(3-(4-bromophenyl)allylamino)ethyl)isoquinoline-5-sulfonamide, **55d**. Yield: 108.5 mg, 194 μmol, 18%

¹H NMR (600 MHz, MeOD) δ 9.67 (s, 1H), 8.83 (d, $J = 5.8$, 1H), 8.69 (s, 1H), 8.63 (d, $J = 6.6$, 1H), 8.54 (d, $J = 8.2$, 1H), 7.95 (t, $J = 7.8$, 1H), 7.44 (d, $J = 8.5$, 2H), 7.32 (d, $J = 8.5$, 2H), 6.77 (d, $J = 15.9$, 1H), 6.27 (dt, $J = 7.2, 7.2, 14.7$, 1H), 3.81 (d, $J = 7.2$, 2H), 3.22 – 3.15 (m, 4H). ¹³C NMR (150 MHz, MeOD) δ 152.21, 140.42, 138.68, 136.96, 136.08, 135.93, 135.91, 133.88, 132.87, 129.58, 129.16, 123.53, 120.12, 50.35, 49.85, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 47.57, 40.11. HRMS: calcd for [C₂₀H₂₀BrN₃O₂S + H]⁺ = 446.05324, found 446.05330.



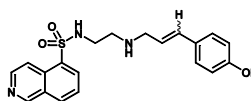
(E)-N-(2-(3-(p-tolyl)allylamino)ethyl)isoquinoline-5-sulfonamide **55e**. Yield: 189 mg, 382 μmol, 38%. ¹H NMR (600 MHz, MeOD) δ 9.43 (s, 1H), 8.62 (s, 2H), 8.49 (d, $J = 7.3$, 1H),

8.35 (d, $J = 8.2$, 1H), 7.79 (t, $J = 7.8$, 1H), 7.26 (d, $J = 8.0$, 2H), 7.08 (d, $J = 7.9$, 2H), 6.73 (d, $J = 15.8$, 1H), 6.17 (dt, 1H), 3.79 (d, $J = 7.2$, 2H), 3.19 (d, $J = 4.6$, 2H), 3.17 (d, $J = 4.6$, 2H). ¹³C NMR (100 MHz, MeOH) δ 139.97, 139.92, 137.27, 136.17, 135.94, 134.01, 133.89, 130.31, 129.34, 127.76, 117.80, 50.54, 49.43, 49.21, 49.00, 48.79, 48.57, 48.36, 47.38, 40.07, 21.23. HRMS: calcd for [C₂₁H₂₃N₃O₂S + H]⁺ = 382.15837, found 382.15876.



(E)-N-(2-(3-(4-(trifluoromethyl)phenyl)allylamino)ethyl)isoquinoline-5-sulfonamide **55f**. Yield: 77mg, 140 μmol, 13% ¹H NMR (600 MHz, MeOD) δ 9.66 (s, 1H), 8.82 (d, $J =$

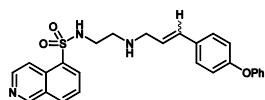
5.6, 1H), 8.70 (s, 1H), 8.62 (d, $J = 7.4$, 1H), 8.54 (d, $J = 8.3$, 1H), 7.95 (t, $J = 7.8$, 1H), 7.61 (s, 4H), 6.90 (d, $J = 15.9$, 1H), 6.45 – 6.38 (m, 1H), 3.87 (d, $J = 7.1$, 2H), 3.20 (s, 4H). ¹³C NMR (150 MHz, MeOD) δ 152.52, 141.04, 140.63, 138.27, 136.73, 135.99, 135.85, 133.74, 131.68, 131.47, 131.25, 131.04, 130.03, 128.99, 128.39, 128.21, 126.67, 126.64, 126.62, 126.41, 124.61, 122.30, 120.88, 50.24, 49.85, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 47.71, 40.13. HRMS: calcd for [C₂₁H₂₀F₃N₃O₂S + H]⁺ = 436.13011, found 436.13001.



(E)-N-(2-(3-(4-methoxyphenyl)allylamino)ethyl)isoquinoline-5-sulfonamide **55g**.

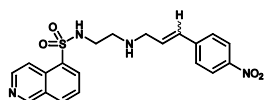
Yield: 234.5 mg, 459 μmol, 46% ¹H NMR (600 MHz, MeOD) δ 9.32 (s, 1H), 8.58 (s, 1H), 8.54 (d, $J = 5.3$, 1H), 8.45 (d, $J = 7.3$, 1H), 8.29 (d, $J = 8.2$, 1H), 7.74 (t, $J = 7.8$, 1H), 7.29 (d, $J = 8.6$, 2H), 6.80 (d, $J = 8.6$, 2H), 6.69 (d, $J = 15.8$, 1H), 6.10 – 6.03 (m, 1H), 3.78 (d, $J = 7.3$, 2H), 3.72 (s, 3H), 3.20 (t, $J = 9.2$, 2H), 3.17 (d, $J = 4.8$, 2H). ¹³C NMR (100 MHz, MeOH) δ 161.48, 152.91, 142.13, 139.66, 136.05, 135.64, 135.50, 133.16, 129.28, 129.17, 128.45, 120.18, 116.32, 115.02, 55.69, 50.61, 49.85, 49.63,

49.43, 49.21, 49.00, 48.79, 48.57, 48.36, 47.30, 40.04. HRMS: calcd for $[C_{21}H_{24}N_3O_3S + H]^+ = 398.15329$, found 398.15289.



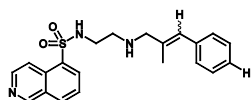
(E)-N-(2-(3-(4-phenoxyphenyl)allylamino)ethyl)isoquinoline-5-sulfonamide **55h**.

Yield: 133 mg, 232 μ mol, 21% ^1H NMR (600 MHz, MeOD) δ 9.28 (s, 1H), 8.49 (s, 2H), 8.37 (d, $J = 7.3$, 1H), 8.24 (d, $J = 8.2$, 1H), 7.67 (t, $J = 7.8$, 1H), 7.26 (d, $J = 8.7$, 2H), 7.19 (t, $J = 8.0$, 2H), 6.97 (t, $J = 7.4$, 1H), 6.85 – 6.80 (m, 2H), 6.76 (d, $J = 8.7$, 2H), 6.65 (d, $J = 15.8$, 1H), 6.08 – 6.01 (m, 1H), 3.69 (d, $J = 7.3$, 2H), 3.07 (s, 4H). ^{13}C NMR (150 MHz, MeOD) δ 159.29, 157.86, 153.45, 143.26, 139.23, 135.62, 135.47, 135.36, 132.89, 131.75, 130.91, 130.80, 130.38, 129.44, 128.14, 124.79, 120.19, 119.69, 119.46, 117.86, 50.48, 49.85, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 47.40, 40.04. HRMS: calcd for $[C_{26}H_{25}N_3O_3S + H]^+ = 460.16894$, found 460.16891.



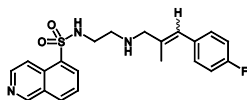
(E)-N-(2-(3-(4-nitrophenyl)allylamino)ethyl)isoquinoline-5-sulfonamide **55i**. Yield:

146 mg, 278 μ mol, 28% ^1H NMR (600 MHz, MeOD) δ 9.53 (s, 2H), 8.72 – 8.63 (m, 5H), 8.56 (d, $J = 7.4$, 1H), 8.52 – 8.46 (m, 3H), 8.24 (dd, $J = 8.8$, 17.3, 4H), 7.90 (dt, $J = 7.8$, 11.5, 2H), 7.70 (d, $J = 8.8$, 2H), 7.51 (d, $J = 8.6$, 2H), 6.99 (d, $J = 4.8$, 1H), 6.96 (d, $J = 9.1$, 1H), 6.51 (dt, $J = 7.1$, 15.8, 1H), 5.99 – 5.93 (m, 1H), 4.03 (dd, $J = 1.7$, 6.7, 2H), 3.90 (d, $J = 7.1$, 2H), 3.21 (d, $J = 4.9$, 2H), 3.19 (d, $J = 4.7$, 2H), 3.17 – 3.14 (m, 3H), 3.09 (t, $J = 5.8$, 3H). ^{13}C NMR (100 MHz, MeOH) δ 151.34, 143.23, 138.31, 137.95, 137.48, 136.51, 136.25, 134.99, 134.63, 130.89, 129.87, 128.79, 124.95, 124.73, 124.18, 50.16, 49.64, 49.43, 49.21, 49.00, 48.79, 48.57, 48.36, 47.81, 46.24, 40.17, 40.04. HRMS: calcd for $[C_{20}H_{20}N_4O_4S + H]^+ = 413.12780$, found 413.12771.

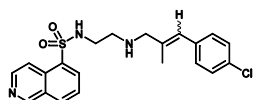


(E)-N-(2-(2-methyl-3-phenylallylamino)ethyl)isoquinoline-5-sulfonamide **55j**.

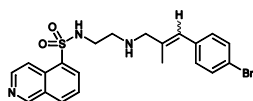
Data for the E-isomer Yield: 45.2 mg, 91 μ mol, 9.1 %. ^1H NMR (600 MHz, $CDCl_3$): δ 9.60 (s, 1H, H6), 8.74 (m, 2H, H1, H2), 8.64 – 8.46 (m, 2H, H3, H5), 7.99 – 7.91 (dd, 1H, H4, $J_1 = 7.31$ Hz, $J_2 = 8.04$ Hz), 7.42 – 7.17 (m, 5H, H_{phenyl}), 6.71 (s, 1H, $CMe=CHPh$), 3.80 (s, 2H, NCH_2), 3.21 (s, 4H, H7, H8), 1.98 (s, 3H, CH_3). ^{13}C -NMR ($CDCl_3$): δ 152.9 (C6), 144.4 (C1), 137.2 (Cq_{phenyl}), 135.5 ($MeC=CH$), 134.2 (C9), 133.3 (C3), 133.0 (C5), 130.9 (C10), 128.7 (C11), 128.5 – 129.9 (CH_{phenyl} , $HC=CMe$), 125.8 (C4), 117.2 (C2), 57.0 (C8), 47.1 (NCH_2), 42.1 (C7), 16.1 (CH_3). HRMS: calcd for $[C_{21}H_{23}N_3O_2S + H]^+ = 382.15837$, found 382.15874. **Data for the Z-isomer:** Yield: 12.2 mg, 24.6 μ mol, 2.5 %. ^1H NMR (600 MHz, MeOD) δ 9.68 (s, 1H), 8.76 (s, 2H), 8.59 (d, $J = 8.2$, 1H), 8.52 (d, $J = 7.3$, 1H), 7.98 (t, $J = 7.8$, 1H), 7.40 (t, $J = 7.5$, 2H), 7.31 (t, $J = 7.3$, 1H), 7.21 (d, $J = 7.5$, 2H), 6.84 (s, 1H), 3.93 (s, 2H), 3.05 (s, 5H). ^{13}C NMR (150 MHz, MeOD) δ 161.83, 161.59, 152.65, 141.10, 137.33, 136.74, 136.08, 135.78, 135.59, 133.76, 130.87, 130.78, 130.03, 129.78, 129.71, 129.41, 129.03, 129.02, 128.63, 120.81, 66.91, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.68, 48.57, 47.98, 47.62, 39.63, 36.53, 30.82, 30.45, 30.32, 30.23, 28.11, 26.92, 21.41, 15.44. HRMS: calcd for $[C_{21}H_{23}N_3O_2S + H]^+ = 382.15837$, found 382.15861.

(E)-N-(2-(3-(4-fluorophenyl)-2-methylallylamino)ethyl)isoquinoline-5-sulfonamide **55k**.

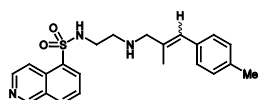
Data for the E-isomer: Yield: 50.5 mg, 111.5 μmol , 11.2 ^1H -NMR (MeOD): δ 9.09 (bs, 1H, H1), 8.75 – 8.74 (d, 1H, H5, $J_1 = 7.26 \text{ Hz}$), 8.67 – 8.66 (d, 1H, H3, $J_1 = 8.10 \text{ Hz}$), 8.08 – 8.05 (t, 1H, H4, $J_1 = 7.62 \text{ Hz}$, $J_2 = 7.74 \text{ Hz}$), 7.35 – 7.33 (m, 2H, H11), 7.13 – 7.10 (t, 2H, H12, $J_1 = 8.60 \text{ Hz}$), 6.94 (s, 1H, H10), 3.82 (s, 2H, H9), 3.28 – 3.24 (m, 4H, H7/8), 1.99 (s, 3H, H13); ^{13}C -NMR (MeOD): δ 1647.23, 162.60, 137.89, 136.64, 136.42, 134.30, 133.81, 133.78, 133.36, 132.04, 131.99, 130.07, 129.60, 116.27, 116.13, 66.93, 56.44, 47.86, 39.96, 16.57; HRMS: calcd for $[\text{C}_{21}\text{H}_{22}\text{FN}_3\text{O}_2\text{S} + \text{H}]^+ = 400.14895$, found 400.14842. **Data for the Z-isomer:** Yield: 19.0 mg, 41.9 μmol , 4.2 %. ^1H NMR (600 MHz, MeOD) δ 9.87 (s, 1H), 8.89 (s, 2H), 8.64 (d, $J = 8.2$, 1H), 8.59 (d, $J = 7.3$, 1H), 8.03 (t, $J = 7.8$, 1H), 7.28 – 7.20 (m, 2H), 7.12 (t, $J = 8.6$, 2H), 6.79 (s, 1H), 3.91 (s, 2H), 3.08 (s, 4H). ^{13}C NMR (150 MHz, MeOD) δ 164.26, 162.63, 137.32, 136.37, 136.05, 134.30, 134.12, 133.55, 133.53, 131.74, 131.69, 129.52, 129.43, 116.59, 116.44, 66.90, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.63, 48.57, 47.70, 39.68, 21.38, 15.44. HRMS: calcd for $[\text{C}_{21}\text{H}_{22}\text{FN}_3\text{O}_2\text{S} + \text{H}]^+ = 400.14895$, found 400.14851.

(E)-N-(2-(3-(4-chlorophenyl)-2-methylallylamino)ethyl)isoquinoline-5-sulfonamide **55l**.

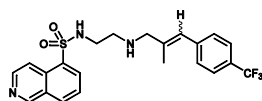
Data for E-isomer: Yield: 55.5 mg, 105 μmol , 10.5 %. ^1H -NMR (MeOD): δ 9.65 (bs, 1H, H6), 8.81 – 8.80 (d, 1H, H2, $J_1 = 6.48 \text{ Hz}$), 8.70 – 8.69 (d, 1H, H1, $J_1 = 6.18 \text{ Hz}$), 8.64 – 8.63 (d, 1H, H5, $J_1 = 7.26 \text{ Hz}$), 8.57 – 8.55 (d, 1H, H3, $J_1 = 8.22 \text{ Hz}$), 7.97 – 7.95 (t, 1H, H4, $J_1 = 7.80 \text{ Hz}$, $J_2 = 7.86 \text{ Hz}$), 7.36 – 7.34 (d, 2H, H11, $J_1 = 8.40 \text{ Hz}$), 7.28 – 7.26 (d, 2H, H12, $J_1 = 8.46 \text{ Hz}$), 6.64 (s, 1H, H10), 3.78 (s, 2H, H9), 3.22 – 3.18 (m, 4H, H7/8), 1.95 (s, 3H, H13); ^{13}C NMR (150 MHz, MeOD) δ 162.27, 162.03, 152.22, 140.19, 137.17, 136.21, 136.16, 136.01, 134.35, 134.08, 133.07, 131.57, 131.36, 130.42, 129.84, 129.52, 129.30, 121.24, 61.53, 56.29, 49.57, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 47.86, 39.90, 16.61, 14.45. HRMS: calcd for $[\text{C}_{21}\text{H}_{22}\text{ClN}_3\text{O}_2\text{S} + \text{H}]^+ = 416.11940$, found 416.11928. The HPLC fractions of the Z-isomers were contaminated with the 1,2,3,4-tetrahydroisoquinoline based sideproduct.

(E)-N-(2-(3-(4-bromophenyl)-2-methylallylamino)ethyl)isoquinoline-5-sulfonamide **55m**.

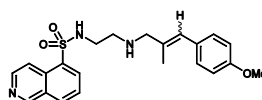
Data for E isomer: Yield: 71.2 mg, 124.2 μmol , 12.4 %. ^1H NMR (600 MHz, MeOD) δ 9.70 (s, 1H), 8.86 (d, $J = 6.4$, 1H), 8.72 (s, 1H), 8.67 (d, $J = 7.4$, 1H), 8.59 (d, $J = 8.3$, 1H), 7.99 (t, $J = 7.8$, 1H), 7.50 (d, $J = 8.4$, 2H), 7.21 (d, $J = 8.4$, 2H), 6.63 (s, 1H), 3.97 (s, 2H), 3.78 (s, 2H), 3.24 (d, $J = 4.9$, 2H), 3.21 (d, $J = 5.1$, 2H), 1.95 (s, 3H). **Data for 3/5 EZ mixture:** ^1H NMR (600 MHz, MeOD) δ 9.66 (s, 1H), 8.72 (s, 2H), 8.61 (d, $J = 7.3$, 1H), 8.54 (d, $J = 8.2$, 1H), 8.48 (d, $J = 7.3$, 1H), 7.94 (t, $J = 6.5$, 3H), 7.57 – 7.45 (m, 9H), 7.24 (d, $J = 8.2$, 4H), 7.12 (d, $J = 8.1$, 2H), 6.73 (s, 1H), 6.66 (s, 1H), 3.88 (s, 2H), 3.80 (s, 2H), 3.54 (d, $J = 5.3$, 2H), 3.52 (d, $J = 5.4$, 4H), 3.25 (d, $J = 5.0$, 2H), 3.22 (d, $J = 5.2$, 4H), 2.03 (s, 3H), 1.97 (s, 3H). ^{13}C NMR (150 MHz, MeOD) δ 153.49, 143.06, 135.74, 134.16, 133.08, 132.88, 132.56, 132.34, 131.86, 131.64, 130.56, 130.09, 128.36, 56.29, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 48.03, 47.64, 45.79, 42.04, 39.84, 39.58, 23.96, 16.63, 15.44. HRMS: calcd for $[\text{C}_{21}\text{H}_{22}\text{BrN}_3\text{O}_2\text{S} + \text{H}]^+ = 460.06889$, found 460.06910.

(E)-N-(2-(2-methyl-3-p-tolylallylamino)ethyl)isoquinoline-5-sulfonamide **55n**.

Data for the E-isomer: Yield: 53.4 mg, 104.9 μmol , 10.5 %. ^1H NMR (600 MHz, MeOD) δ 9.58 (s, 1H), 8.71 (s, 2H), 8.58 (d, J = 6.6, 1H), 8.48 (d, J = 8.2, 1H), 7.90 (t, J = 7.8, 1H), 7.23 – 7.17 (m, 4H), 6.66 (s, 1H), 3.79 (s, 2H), 3.25 (t, J = 5.6, 2H), 3.21 (t, J = 5.6, 2H), 2.34 (d, J = 12.7, 3H), 1.98 (d, J = 1.0, 3H). ^{13}C NMR (150 MHz, MeOD) δ 158.77, 153.29, 142.73, 138.57, 135.98, 135.71, 135.63, 135.47, 134.56, 134.55, 133.18, 130.84, 130.75, 130.32, 129.98, 129.97, 129.60, 128.76, 128.47, 128.46, 116.95, 115.06, 56.62, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 47.69, 39.86, 31.11, 30.79, 30.29, 28.09, 26.90, 21.22, 16.63. HRMS: calcd for $[\text{C}_{22}\text{H}_{25}\text{N}_3\text{O}_2\text{S} + \text{H}]^+$ = 396.17402, found 396.17351. **Data for the Z-isomer:** Yield: 68.7 mg, 135.0 μmol , 13.5 %. ^1H NMR (600 MHz, MeOD) δ 9.60 (s, 1H), 8.70 (s, 2H), 8.52 (d, J = 8.2, 1H), 8.49 (d, J = 7.3, 1H), 7.91 (t, J = 7.8, 1H), 7.18 (d, J = 7.8, 2H), 7.07 (d, J = 7.9, 2H), 6.75 (s, 1H), 3.91 (s, 2H), 3.09 – 3.01 (m, 4H), 2.33 (s, 3H), 2.03 (d, J = 1.1, 3H). ^{13}C NMR (150 MHz, MeOD) δ 152.97, 142.03, 138.49, 136.23, 135.83, 135.60, 135.44, 134.35, 133.36, 130.32, 129.96, 129.60, 128.63, 128.45, 49.43, 49.28, 49.14, 49.00, 48.86, 48.71, 48.57, 47.57, 39.63, 21.44, 21.19. HRMS: calcd for $[\text{C}_{22}\text{H}_{25}\text{N}_3\text{O}_2\text{S} + \text{H}]^+$ = 396.17402, found 396.17341.

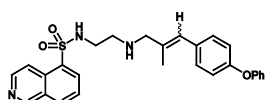
(E)-N-(2-(2-methyl-3-(4-(trifluoromethyl)phenyl)allylamino)ethyl)isoquinoline-5-sulfonamide **55o**.

Data for the E-isomer: Yield: 48.1 mg, 85.4 μmol , 8.5%. ^1H NMR (600 MHz, MeOD) δ 9.63 (s, 1H), 8.75 (s, 2H), 8.61 (d, J = 6.8, 1H), 8.52 (d, J = 8.1, 1H), 7.94 (t, J = 7.8, 1H), 7.69 (d, J = 8.2, 2H), 7.51 (d, J = 8.1, 2H), 6.78 (s, 1H), 3.86 (s, 2H), 3.26 (s, 4H), 2.02 (s, 3H). ^{13}C NMR (150 MHz, MeOD) δ 153.13, 142.36, 141.47, 136.18, 135.81, 135.68, 133.32, 132.65, 132.15, 130.57, 130.43, 130.21, 128.61, 126.27, 126.24, 56.04, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 47.98, 39.89, 16.63. HRMS: calcd for $[\text{C}_{22}\text{H}_{22}\text{F}_3\text{N}_3\text{O}_2\text{S} + \text{H}]^+$ = 450.14576, found 450.14559. **Data for 1/4 E/Z mix:** Yield: 39.2 mg, 69.6 μmol , 7.0 %. ^1H NMR (600 MHz, MeOD) δ 9.64 (s, 1H), 8.70 (s, 2H), 8.51 (d, J = 8.3, 1H), 8.48 (d, J = 7.3, 1H), 7.95 (d, J = 7.5, 1H), 7.90 (t, J = 7.8, 1H), 7.67 (d, J = 7.9, 2H), 7.55 – 7.46 (m, 1H), 7.39 (d, J = 8.0, 2H), 6.82 (s, 1H), 6.76 (s, 1H), 3.89 (s, 2H), 3.84 (s, 1H), 3.25 (dd, J = 4.1, 7.8, 1H), 3.06 (s, 4H), 2.07 (s, 3H), 2.00 (s, 1H). ^{13}C NMR (150 MHz, MeOD) δ 152.99, 142.05, 141.31, 139.18, 136.22, 135.89, 135.63, 133.72, 133.37, 133.08, 132.64, 132.34, 132.20, 132.04, 131.44, 130.59, 130.53, 130.42, 130.32, 130.14, 128.66, 128.33, 126.61, 126.59, 126.25, 66.89, 56.06, 49.43, 49.28, 49.14, 49.00, 48.86, 48.81, 48.72, 48.57, 48.12, 47.96, 45.78, 42.02, 39.84, 39.74, 23.94, 21.52, 16.65, 15.43. HRMS: calcd for $[\text{C}_{22}\text{H}_{22}\text{F}_3\text{N}_3\text{O}_2\text{S} + \text{H}]^+$ = 450.14576, found 450.14574.

(E)-N-(2-(3-(4-methoxyphenyl)-2-methylallylamino)ethyl)isoquinoline-5-sulfonamide **55p**.

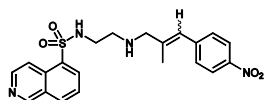
Data for the E-isomer: Yield: 115.2 mg, 219.4 μmol , 21.9 %. ^1H NMR (600 MHz, MeOD) δ 9.56 (s, 1H), 8.73 (s, 1H), 8.60 (s, 1H), 8.52 (d, J = 7.3, 1H), 8.42 (d, J = 8.3, 1H), 7.83 (t, J = 7.8, 1H), 7.08 (d, J = 8.7, 2H), 6.74 (d, J = 8.8, 2H), 6.46 (s, 1H), 3.64 (s, 3H), 3.13 (t, J = 6.1, 2H), 3.06 (t, J = 6.1, 2H), 1.83 (s, 3H). ^1H NMR (600 MHz, MeOD) δ 9.56 (s, 1H), 8.73 (s, 1H), 8.60 (s, 1H), 8.52 (d, J = 7.3, 1H), 8.42 (d, J = 8.3, 1H), 7.83 (t, J = 7.8, 1H), 7.08 (d, J = 8.7, 2H), 6.74 (d, J = 8.8, 2H), 6.46 (s, 1H), 3.64 (s, 3H), 3.13 (t, J = 6.1, 2H), 3.06 (t, J = 6.1, 2H), 1.83 (s, 3H). HRMS: calcd for $[\text{C}_{22}\text{H}_{25}\text{N}_3\text{O}_3\text{S} + \text{H}]^+$ = 412.16894, found 412.16883

Data for the Z-isomer: Yield: 27.6 mg, 52.6 μmol , 5.7 %. ^1H NMR (600 MHz, MeOD) δ 9.63 (s, 1H), 8.76 (s, 2H), 8.71 (s, 1H), 8.60 (s, 1H), 8.52 (d, J = 8.3, 2H), 7.93 (t, J = 6.9, 4H), 7.51 – 7.43 (m, 6H), 7.37 (d, J = 8.7, 4H), 7.25 (d, J = 8.7, 5H), 6.90 (dd, J = 8.7, 12.2, 9H), 6.62 (s, 2H), 3.78 (d, J = 5.6, 17H), 3.76 (s, 6H), 3.52 (d, J = 5.3, 5H), 3.50 (d, J = 5.2, 6H), 3.23 (t, J = 5.9, 5H), 3.21 – 3.13 (m, 12H), 1.97 (s, 8H). ^{13}C NMR (150 MHz, MeOD) δ 161.79, 160.57, 139.96, 139.24, 136.45, 135.92, 135.86, 134.39, 133.58, 133.08, 132.32, 132.04, 131.43, 130.11, 129.94, 129.44, 129.30, 128.82, 128.35, 127.35, 116.29, 115.19, 114.79, 56.88, 55.77, 55.72, 50.70, 49.85, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 47.82, 47.39, 45.78, 42.03, 40.13, 39.84, 23.94, 16.67. HRMS: calcd for $[\text{C}_{22}\text{H}_{25}\text{N}_3\text{O}_3\text{S} + \text{H}]^+ = 412.16894$, found 412.16883.



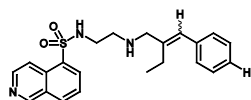
(E)-N-(2-(2-methyl-3-(4-phenoxyphenyl)allylamino)ethyl)isoquinoline-5-sulfonamide **55q**.

Data for the E-isomer: Yield: ^1H NMR (600 MHz, MeOD) δ 9.64 (s, 1H), 8.79 (s, 1H), 8.64 (s, 1H), 8.57 (d, J = 7.3, 1H), 8.49 (d, J = 8.2, 1H), 7.88 (t, J = 7.8, 1H), 7.22 (dd, J = 7.5, 8.5, 2H), 7.17 (d, J = 8.6, 2H), 6.99 (t, J = 7.4, 1H), 6.88 – 6.85 (m, 2H), 6.83 (d, J = 8.7, 2H), 6.54 (s, 1H), 3.66 (s, 2H), 3.12 (d, J = 5.2, 2H), 3.09 (d, J = 5.3, 2H), 1.86 (s, 3H). ^{13}C NMR (150 MHz, MeOD) δ 158.21, 158.18, 151.51, 138.74, 137.77, 136.44, 136.20, 134.44, 133.84, 132.44, 131.66, 130.94, 129.75, 128.64, 124.72, 120.15, 119.28, 56.62, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 47.74, 39.90, 16.65. HRMS: calcd for $[\text{C}_{27}\text{H}_{27}\text{N}_3\text{O}_3\text{S} + \text{H}]^+ = 474.18459$, found 474.18443. The HPLC fractions of the Z-isomers were contaminated with the 1,2,3,4-tetrahydroisoquinoline based sideproduct.

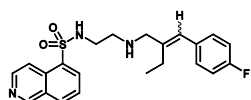


(E)-N-(2-(2-methyl-3-(4-nitrophenyl)allylamino)ethyl)isoquinoline-5-sulfonamide **55r**.

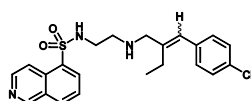
Data for 6/1 E/Z mix: Yield: 40.1, 74.3 μmol , 7.4%. ^1H NMR (600 MHz, MeOD) δ 9.63 (s, 1H), 8.77 (d, J = 4.8, 1H), 8.70 (s, 1H), 8.64 (s, 1H), 8.59 – 8.55 (m, 1H), 8.53 (d, J = 7.4, 1H), 8.49 (d, J = 8.3, 1H), 8.47 (s, 1H), 8.44 (d, J = 7.4, 1H), 8.10 (d, J = 8.8, 2H), 8.08 (d, J = 8.7, 1H), 8.04 (d, J = 8.7, 1H), 7.89 (t, J = 7.8, 1H), 7.86 (t, J = 7.8, 1H), 7.41 (d, J = 8.7, 2H), 7.35 – 7.29 (m, 1H), 6.72 (s, 1H), 6.66 (s, 1H), 3.80 (s, 1H), 3.73 (s, 2H), 3.13 (s, 4H), 3.00 – 2.95 (m, 1H), 1.97 (s, 1H), 1.89 (d, J = 1.0, 3H). ^{13}C NMR (150 MHz, MeOD) δ 151.73, 148.14, 144.23, 144.07, 139.14, 137.63, 137.39, 136.40, 136.14, 134.36, 133.63, 133.09, 132.71, 131.95, 131.31, 131.03, 130.91, 129.65, 129.57, 124.79, 124.61, 124.52, 55.95, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 48.07, 47.96, 39.92, 39.73, 33.80, 21.67, 16.80. HRMS: calcd for $[\text{C}_{21}\text{H}_{23}\text{N}_4\text{O}_4\text{S} + \text{H}]^+ = 427.14345$, found 427.14322. **Data for the Z-isomer:** Yield: 38.4 mg, 71.1 μmol , 7.1%. ^1H NMR (600 MHz, MeOD) δ 9.67 (s, 1H), 8.75 (d, J = 5.3, 1H), 8.70 (s, 1H), 8.55 (d, J = 8.3, 1H), 8.51 (d, J = 7.3, 1H), 8.18 (d, J = 8.7, 2H), 7.93 (t, J = 7.8, 1H), 7.52 (d, J = 8.6, 1H), 7.42 (d, J = 8.6, 2H), 6.83 (s, 1H), 6.76 (s, 1H), 3.96 (s, 1H), 3.90 (s, 2H), 3.10 – 3.03 (m, 4H), 2.07 (s, 3H), 2.00 (s, 1H). ^{13}C NMR (150 MHz, MeOD) δ 152.56, 148.28, 144.06, 141.00, 136.55, 136.05, 135.80, 133.74, 133.14, 132.67, 131.98, 131.03, 130.90, 128.96, 124.80, 124.52, 55.96, 49.85, 49.43, 49.28, 49.14, 49.00, 48.86, 48.71, 48.57, 47.98, 39.73, 21.66. HRMS: calcd for $[\text{C}_{21}\text{H}_{23}\text{N}_4\text{O}_4\text{S} + \text{H}]^+ = 427.14345$, found 427.14334.

(E)-N-(2-(2-benzylidenebutylamino)ethyl)isoquinoline-5-sulfonamide **55s**.

Data for the E-isomer: Yield: 71.9 mg, 142.3 μmol , 14.2 %. $^1\text{H-NMR}$ (MeOD): δ 9.57 (bs, 1H, H6), 8.78 – 8.71 (m, 2H, H1/2), 8.58 – 8.56 (dd, 1H, H5, $J_1 = 0.80 \text{ Hz}$, $J_2 = 7.30 \text{ Hz}$), 8.47 – 8.46 (d, 1H, H3, $J_1 = 8.16 \text{ Hz}$), 7.90 – 7.87 (t, 1H, H4, $J_1 = 7.68 \text{ Hz}$, $J_2 = 7.92 \text{ Hz}$), 7.35 – 7.24 (m, 5H, H11/12/15), 6.65 (s, 1H, H10), 3.80 (s, 1H, H9), 3.25 – 3.21 (m, 4H, H7/8), 2.35 – 2.31 (dd, 2H, H13, $J_1 = 7.48 \text{ Hz}$, $J_2 = 15.00 \text{ Hz}$), 1.11 – 1.08 (t, 3H, H14, $J_1 = 7.50 \text{ Hz}$, $J_2 = 7.62 \text{ Hz}$); $^{13}\text{C-NMR}$ (MeOD): δ 152.24, 140.53, 137.84, 137.62, 136.66, 136.51, 136.13, 134.47, 134.25, 130.04, 139.90, 129.80, 129.02, 53.29, 50.32, 48.48, 40.35, 23.57, 13.45; HRMS: calcd for $[\text{C}_{22}\text{H}_{25}\text{N}_3\text{O}_2\text{S} + \text{H}]^+ = 396.16402$, found 396.17339. **Data for the 1/5 E/Z mix:** Yield: 232. mg, 45.8 μmol , 4.6 %. $^1\text{H NMR}$ (400 MHz, MeOH) δ 10.50 – 9.17 (m, 1H), 8.74 (s, 1H), 8.62 (d, $J = 7.3$, 1H), 8.53 (d, $J = 8.2$, 1H), 8.48 (d, $J = 6.8$, 1H), 7.93 (t, $J = 7.8$, 1H), 7.38 (t, $J = 7.4$, 2H), 7.30 (d, $J = 7.4$, 2H), 7.20 (d, $J = 7.5$, 2H), 6.83 (s, 1H), 6.70 (s, 1H), 3.92 (s, 2H), 3.83 (s, 1H), 3.01 (s, 4H), 2.39 – 2.30 (m, 2H), 1.24 (t, $J = 7.4$, 3H), 1.14 (dd, $J = 5.7$, 9.3, 1H). $^{13}\text{C NMR}$ (100 MHz, MeOH) δ 137.42, 136.16, 135.87, 135.66, 134.75, 134.13, 133.79, 129.80, 129.71, 129.60, 129.48, 128.70, 128.60, 49.64, 49.43, 49.21, 49.00, 48.79, 48.57, 48.36, 48.06, 47.59, 47.22, 39.56, 27.99, 13.01, 12.67. HRMS: calcd for $[\text{C}_{22}\text{H}_{25}\text{N}_3\text{O}_2\text{S} + \text{H}]^+ = 396.16402$, found 396.17340.

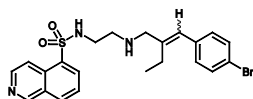
(E)-N-(2-(2-(4-fluorobenzylidene)butylamino)ethyl)isoquinoline-5-sulfonamide **55t**.

Data for the E-isomer: Yield: 44.8 mg, 85.0 μmol , 8.5 %. $^1\text{H-NMR}$ (MeOD): δ 9.65 (bs, 1H, H6), 8.77 (bs, 1H, H2), 8.71 (bs, 1H, H1), 8.62 – 8.60 (dd, 1H, H5, $J_1 = 0.84 \text{ Hz}$, $J_2 = 7.32 \text{ Hz}$), 8.57 – 8.52 (d, 1H, H3, $J_1 = 8.22 \text{ Hz}$), 7.95 – 7.92 (t, 1H, H4, $J_1 = 7.74 \text{ Hz}$, $J_2 = 7.86 \text{ Hz}$), 7.29 – 7.26 (dd, 1H, H11, $J_1 = 5.46 \text{ Hz}$, $J_2 = 8.46 \text{ Hz}$), 7.09 – 7.06 (t, 1H, H12, $J_1 = J_2 = 8.76 \text{ Hz}$), 6.62 (s, 1H, H10), 3.79 (s, 2H, H9), 3.23 – 3.20 (m, 4H, H7/8), 2.33 – 2.30 (dd, 2H, H13, $J_1 = 7.50 \text{ Hz}$, $J_2 = 15.06 \text{ Hz}$), 1.11 – 1.08 (t, 3H, H14, $J_1 = 7.50 \text{ Hz}$, $J_2 = 7.62 \text{ Hz}$); $^{13}\text{C-NMR}$ (MeOD): δ 164.27, 162.64, 152.78, 141.55, 136.52, 135.95, 135.87, 133.62, 133.61, 132.61, 131.60, 131.54, 128.87, 116.32, 116.17, 53.05, 49.85, 48.07, 39.88, 23.09, 12.91; HRMS: calcd for $[\text{C}_{22}\text{H}_{24}\text{FN}_3\text{O}_2\text{S} + \text{H}]^+ = 414.16460$, found 414.16438. **Data for the 1/4 E/Z mix:** Yield: 31.2 mg, 59.2 μmol , 5.9 %. $^1\text{H NMR}$ (600 MHz, MeOD) δ 9.63 (s, 3H), 8.76 (s, 1H), 8.70 (s, 6H), 8.53 (d, $J = 7.9$, 4H), 8.48 (d, $J = 7.3$, 3H), 7.96 – 7.87 (m, 5H), 7.52 – 7.43 (m, 3H), 7.29 (dd, $J = 5.6$, 8.4, 3H), 7.22 – 7.15 (m, 7H), 7.13 – 7.04 (m, 10H), 6.75 (s, 3H), 6.65 (s, 1H), 3.87 (s, 7H), 3.80 (s, 2H), 3.53 (d, $J = 5.3$, 2H), 3.50 (d, $J = 5.3$, 3H), 3.26 – 3.19 (m, 6H), 3.00 (s, 14H), 2.38 – 2.24 (m, 10H), 1.19 (t, $J = 7.4$, 11H), 1.11 (t, $J = 7.5$, 4H). $^{13}\text{C NMR}$ (150 MHz, MeOD) δ 164.28, 162.65, 152.94, 141.88, 139.23, 136.29, 135.91, 135.68, 135.12, 133.61, 133.59, 133.47, 133.07, 132.91, 132.58, 132.33, 132.05, 131.75, 131.70, 131.61, 131.55, 130.12, 128.72, 128.34, 116.63, 116.48, 116.31, 116.17, 53.08, 49.85, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 48.22, 47.67, 47.17, 45.79, 42.04, 39.82, 39.60, 27.98, 23.94, 23.12, 12.93, 12.64. HRMS: calcd for $[\text{C}_{22}\text{H}_{24}\text{FN}_3\text{O}_2\text{S} + \text{H}]^+ = 414.16460$, found 414.16458.

(E)-N-(2-(2-(4-chlorobenzylidene)butylamino)ethyl)isoquinoline-5-sulfonamide **55u**.

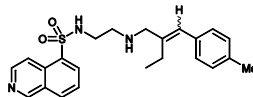
Data for the E-isomer: Yield: 13.1 mg, 24.1 μmol , 2.4%. $^1\text{H-NMR}$ (MeOD): δ 9.68 (bs, 1H, H6), 8.85 – 8.84 (d, 1H, H2, $J_1 = 6.18 \text{ Hz}$), 8.71 (bs, 1H, H1), 8.66 – 8.65 (d, 1H, H5, $J_1 = 7.38 \text{ Hz}$), 8.58 – 8.57 (d, 1H, H3, $J_1 = 8.22 \text{ Hz}$), 7.99 – 7.96 (t, 1H, H4, $J_1 = J_2 = 7.73 \text{ Hz}$), 7.35 – 7.33 (d, 2H,

H11, $J_1 = 8.28$ Hz), 7.24 – 7.23 (d, 2H, H12, $J_1 = 8.28$ Hz), 6.62 (s, 1H, H10), 3.79 (s, 2H, H9), 3.23 – 3.21 (m, 4H, H7/8), 2.34 – 2.30 (dd, 2H, H13, $J_1 = 7.56$ Hz, $J_2 = 15.12$ Hz), 1.11 – 1.08 (t, 3H, H14, $J_1 = 7.44$ Hz, $J_2 = 7.56$ Hz); ^{13}C -NMR (MeOD): δ 151.85, 139.41, 137.50, 136.67, 136.33, 136.13, 136.06, 134.35, 134.29, 132.29, 131.19, 129.59, 129.55, 121.61, 52.95, 48.09, 39.88, 23.17, 12.92; HRMS: calcd for $[\text{C}_{22}\text{H}_{24}\text{ClN}_3\text{O}_2\text{S} + \text{H}]^+ = 430.13505$, found 430.13503. The HPLC fractions of the Z-isomer were contaminated with the 1,2,3,4-tetrahydroisoquinoline based sideproduct.



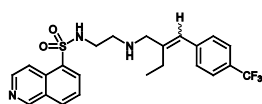
(E)-N-(2-(2-(4-bromobenzylidene)butylamino)ethyl)isoquinoline-5-sulfonamide **55v**.

Data for the E-isomer: Yield: 38.3 mg, 65.1 μmol , 6.5 %. ^1H -NMR (MeOD): δ 9.43 (bs, 1H, H6), 8.94 (bs, 1H, H2), 8.57 (bs, 1H, H1), 8.50 – 8.49 (d, 1H, H5, $J_1 = 7.20$ Hz), 8.41 – 8.40 (d, 1H, H3, $J_1 = 7.98$ Hz), 7.84 – 7.81 (t, 1H, H4, $J_1 = J_2 = 7.61$ Hz), 7.50 – 7.49 (d, 2H, H1, $J_1 = 8.34$ Hz), 7.18 – 1.17 (d, 2H, H12, $J_1 = 8.22$ Hz), 6.59 (s, 1H, H10), 3.78 (s, 2H, H9), 3.20 (m, 4H, H7/8), 2.32 – 2.29 (dd, 2H, H13, $J_1 = 7.44$ Hz, $J_2 = 15.00$ Hz), 1.10 – 1.08 (t, 3H, H14, $J_1 = 7.44$ Hz, $J_2 = 7.50$ Hz); ^{13}C -NMR (MeOD): δ 154.05, 144.37, 136.73, 136.46, 135.44, 135.39, 132.89, 132.72, 132.69, 132.62, 132.31, 131.67, 131.47, 127.93, 122.39, 122.05, 121.44, 52.92, 49.85, 48.08, 26.47, 23.19, 12.92; HRMS: calcd for $[\text{C}_{22}\text{H}_{24}\text{BrN}_3\text{O}_2\text{S} + \text{H}]^+ = 474.08454$, found 474.08461. **Data for the Z-isomer:** Yield: 15.1 mg, 25.7 μmol , 2.6 %. ^1H NMR (600 MHz, MeOD) δ 9.32 (s, 11H), 8.53 (s, 12H), 8.40 (s, 13H), 8.31 (d, $J = 8.2$, 14H), 8.25 (d, $J = 7.3$, 12H), 7.73 (t, $J = 7.8$, 13H), 7.40 (d, $J = 8.3$, 28H), 7.33 (d, $J = 7.1$, 8H), 7.09 (d, $J = 8.3$, 3H), 7.00 (d, $J = 8.2$, 23H), 6.62 (s, 11H), 6.51 (s, 1H), 3.76 (s, 22H), 2.91 – 2.84 (m, 52H), 2.53 (s, 39H), 2.26 – 2.14 (m, 27H), 1.09 (dd, $J = 6.1$, 8.6, 38H), 1.00 (t, $J = 7.5$, 5H). ^{13}C NMR (150 MHz, MeOD) δ 154.27, 144.84, 136.51, 135.70, 135.40, 135.20, 135.05, 132.92, 132.72, 132.58, 131.71, 130.96, 130.28, 127.84, 122.47, 49.85, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 47.63, 47.10, 40.42, 39.52, 28.07, 12.61. HRMS: calcd for $[\text{C}_{22}\text{H}_{24}\text{BrN}_3\text{O}_2\text{S} + \text{H}]^+ = 474.08454$, found 474.08451.



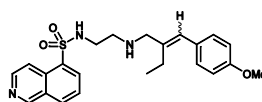
(E)-N-(2-(2-(4-methylbenzylidene)butylamino)ethyl)isoquinoline-5-sulfonamide **55w**.

Data for the E-isomer: Yield: 55.7 mg, 106.5 μmol , 10.7 %. ^1H NMR (400 MHz, MeOH) δ 9.64 (s, 1H), 8.81 (s, 1H), 8.72 (s, 1H), 8.63 (d, $J = 7.2$, 1H), 8.52 (d, $J = 8.2$, 1H), 7.94 (t, $J = 7.8$, 1H), 7.14 (s, 4H), 6.62 (s, 1H), 3.80 (s, 2H), 3.27 (d, $J = 5.0$, 2H), 3.24 (d, $J = 5.0$, 2H), 2.35 (dd, $J = 5.7$, 13.3, 2H), 2.31 (s, 3H), 1.11 (t, $J = 7.5$, 3H). ^{13}C NMR (100 MHz, MeOH) δ 152.09, 140.21, 138.50, 137.02, 136.09, 135.94, 134.79, 134.37, 133.87, 133.83, 130.01, 129.51, 129.19, 53.28, 49.64, 49.43, 49.21, 49.00, 48.79, 48.57, 48.36, 47.91, 39.85, 23.06, 21.18, 12.95. HRMS: calcd for $[\text{C}_{23}\text{H}_{27}\text{N}_3\text{O}_2\text{S} + \text{H}]^+ = 410.18967$, found 410.18936. **Data for the 1/2 E/Z mix:** Yield: 99.6 mg, 190.4 μmol , 19.0 %. ^1H NMR (400 MHz, MeOH) δ 9.70 (s, 3H), 8.85 (s, 2H), 8.79 (s, 3H), 8.77 – 8.70 (m, 3H), 8.67 (d, $J = 7.4$, 2H), 8.59 (d, $J = 8.3$, 4H), 8.55 (d, $J = 7.4$, 3H), 7.99 (dd, $J = 7.3$, 15.3, 4H), 7.19 (d, $J = 6.8$, 11H), 7.08 (d, $J = 8.0$, 6H), 6.78 (s, 3H), 6.64 (s, 1H), 3.92 (s, 6H), 3.81 (s, 3H), 3.26 (d, $J = 4.5$, 2H), 3.24 (d, $J = 4.6$, 2H), 3.09 – 2.95 (m, 12H), 2.39 – 2.20 (m, 21H), 1.22 (t, $J = 7.4$, 9H), 1.13 (t, $J = 7.5$, 4H). ^{13}C NMR (150 MHz, MeOD) δ 152.66, 140.72, 139.01, 137.59, 137.45, 136.62, 136.38, 135.31, 134.90, 134.64, 134.53, 134.43, 130.84, 130.52, 130.09, 130.02, 129.77, 129.67, 53.76, 48.05, 47.73, 40.33, 40.06, 28.49, 23.58, 21.63, 13.16, 13.42. HRMS: calcd for $[\text{C}_{23}\text{H}_{27}\text{N}_3\text{O}_2\text{S} + \text{H}]^+ = 410.18967$, found 410.18942.



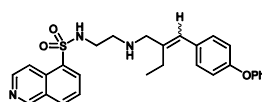
(E)-N-(2-(2-(4-(trifluoromethyl)benzylidene)butylamino)ethyl)isoquinoline-5-sulfonamide **55x**.

Data for the E-isomer: Yield: 4.9 mg, 8.5 μmol , 0.9%. ^1H NMR (600 MHz, MeOD) δ 9.64 (s, 1H), 8.79 (d, J = 5.0, 1H), 8.71 (s, 1H), 8.62 (d, J = 6.6, 1H), 8.53 (d, J = 8.2, 1H), 7.94 (t, J = 7.8, 1H), 7.64 (d, J = 8.2, 2H), 7.44 (d, J = 8.1, 2H), 6.71 (s, 1H), 3.83 (s, 2H), 3.24 (s, 4H), 2.33 (q, J = 7.5, 2H), 1.11 (t, J = 7.5, 3H). ^{13}C NMR (150 MHz, MeOD) δ 152.80, 141.62, 141.44, 138.21, 136.50, 135.92, 135.84, 133.60, 131.89, 130.84, 130.71, 130.50, 130.29, 130.21, 130.07, 128.84, 128.30, 126.50, 126.38, 126.35, 126.33, 126.30, 124.70, 122.91, 120.70, 52.77, 49.85, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 48.22, 39.89, 30.28, 23.30, 12.93. HRMS: calcd for $[\text{C}_{23}\text{H}_{24}\text{F}_3\text{N}_3\text{O}_2\text{S} + \text{H}]^+ = 464.16141$, found 464.16121. **Data for the Z-isomer:** Yield: 42.6 mg, 73.8 μmol , 7.4 %. ^1H NMR (600 MHz, MeOD) δ 9.59 (s, 1H), 8.69 (s, 2H), 8.51 (d, J = 8.2, 1H), 8.49 (dd, J = 0.9, 7.4, 1H), 7.90 (t, J = 7.8, 1H), 7.66 (d, J = 8.1, 2H), 7.38 (d, J = 8.1, 2H), 6.82 (s, 1H), 3.88 (s, 2H), 3.03 (t, J = 4.0, 4H), 2.34 (q, J = 7.3, 2H), 1.22 (t, J = 7.4, 3H). ^{13}C NMR (150 MHz, MeOD) δ 153.03, 142.09, 141.49, 136.95, 136.20, 135.86, 135.63, 133.43, 132.35, 130.79, 130.57, 130.46, 130.36, 130.23, 130.14, 130.12, 128.63, 128.34, 128.29, 126.67, 126.64, 126.62, 126.59, 126.49, 126.35, 124.70, 122.90, 120.37, 49.85, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 48.38, 47.96, 47.46, 39.70, 28.03, 12.94, 12.53. HRMS: calcd for $[\text{C}_{23}\text{H}_{24}\text{F}_3\text{N}_3\text{O}_2\text{S} + \text{H}]^+ = 464.16141$, found 464.16121.



(E)-N-(2-(2-(4-methoxybenzylidene)butylamino)ethyl)isoquinoline-5-sulfonamide **55y**.

Data for the 5/1 E/Z mix: Yield: 30.2 mg, 56.0 μmol , 5.6 %. ^1H NMR (600 MHz, MeOD) δ 9.34 (s, 1H), 8.59 (d, J = 6.1, 1H), 8.52 (d, J = 6.1, 1H), 8.46 (d, J = 7.4, 1H), 8.34 (d, J = 8.2, 1H), 8.31 (d, J = 7.3, 1H), 7.82 – 7.73 (m, 1H), 7.65 (d, J = 8.6, 1H), 7.27 (d, J = 8.6, 1H), 7.18 (d, J = 8.6, 2H), 7.06 (d, J = 8.6, 1H), 6.92 (d, J = 8.8, 1H), 6.88 (t, J = 8.8, 3H), 6.68 (s, 1H), 6.55 (s, 1H), 3.81 – 3.72 (m, 7H), 3.19 (d, J = 5.0, 2H), 3.16 (t, J = 11.4, 2H), 2.96 (dd, J = 3.9, 6.7, 1H), 2.32 (q, J = 7.5, 2H), 2.28 – 2.22 (m, 1H), 1.09 (t, J = 7.5, 3H). ^{13}C NMR (150 MHz, MeOD) δ 160.50, 160.42, 154.15, 144.65, 143.47, 142.42, 135.29, 135.09, 135.04, 133.75, 133.70, 133.65, 133.41, 132.56, 132.50, 131.96, 131.34, 130.98, 130.56, 129.63, 129.53, 127.78, 127.73, 119.14, 115.17, 115.11, 115.05, 114.81, 55.80, 55.68, 53.42, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 47.84, 47.33, 46.93, 39.80, 39.44, 35.97, 28.56, 28.03, 23.03, 21.96, 21.74, 12.95, 12.77. HRMS: calcd for $[\text{C}_{23}\text{H}_{27}\text{N}_3\text{O}_3\text{S} + \text{H}]^+ = 426.18459$, found 426.18456.

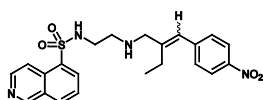


(E)-N-(2-(2-(4-phenoxybenzylidene)butylamino)ethyl)isoquinoline-5-sulfonamide **55z**.

Data for the E-isomer: Yield: 85.3 mg, 141.9 μmol , 14.2 %. ^1H NMR (600 MHz, MeOD) δ 9.38 (s, 1H), 8.60 (s, 1H), 8.55 (s, 1H), 8.48 (d, J = 7.2, 1H), 8.37 (d, J = 8.1, 1H), 7.80 (t, J = 7.8, 1H), 7.32 (dd, J = 7.6, 8.3, 2H), 7.24 (d, J = 8.5, 2H), 7.09 (t, J = 7.4, 1H), 6.96 (d, J = 7.8, 2H), 6.93 (d, J = 8.6, 2H), 6.60 (s, 1H), 3.77 (s, 2H), 3.19 (dd, J = 4.0, 5.9, 4H), 2.34 (q, J = 7.4, 2H), 1.09 (t, J = 7.5, 3H). ^{13}C NMR (150 MHz, MeOD) δ 158.22, 158.15, 154.02, 144.37, 135.37, 135.34, 135.24, 134.98, 133.08, 132.67, 132.26, 131.25, 130.93, 130.57, 127.89, 124.70, 120.13, 119.67, 119.37, 119.29, 53.19, 49.85, 49.43, 49.28, 49.14, 49.00, 48.86,

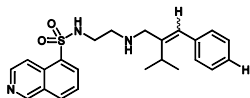
48.72, 48.57, 47.93, 39.81, 23.09, 12.96. HRMS: calcd for $[C_{28}H_{29}N_3O_3S + H]^+ = 488.20024$, found 488.20013.

Data for the Z-isomer: Yield: 28.9 mg, 48.1 μ mol, 4.8 %. ^1H NMR (600 MHz, MeOD) δ 9.37 (s, 1H), 8.60 (d, $J = 6.0$, 1H), 8.47 (d, $J = 5.9$, 1H), 8.38 (d, $J = 8.3$, 1H), 8.36 (d, $J = 7.4$, 1H), 7.80 (t, $J = 7.8$, 1H), 7.32 (dd, $J = 9.0$, 17.3, 3H), 7.16 (d, $J = 8.4$, 2H), 7.10 (t, $J = 7.4$, 1H), 7.01 – 6.93 (m, 5H), 6.75 (s, 1H), 3.90 (s, 2H), 2.98 (d, $J = 4.2$, 2H), 2.97 (d, $J = 4.2$, 2H), 2.29 (q, $J = 7.0$, 2H), 1.19 (t, $J = 7.4$, 3H). ^{13}C NMR (150 MHz, MeOD) δ 158.27, 158.14, 154.36, 145.00, 135.34, 135.16, 134.99, 134.41, 133.39, 132.51, 132.24, 131.37, 130.97, 130.66, 127.73, 124.78, 120.16, 119.71, 119.41, 118.98, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 47.49, 47.05, 39.51, 28.06, 12.73. HRMS: calcd for $[C_{28}H_{29}N_3O_3S + H]^+ = 488.20024$, found 488.20006.



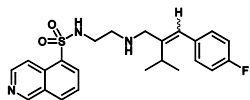
(E)-N-(2-(2-(4-nitrobenzylidene)butylamino)ethyl)isoquinoline-5-sulfonamide **55aa**.

Data for the 3/2 E/Z mix: Yield: 55.9 mg, 100.9 μ mol, 10.1 %. ^1H NMR (600 MHz, MeOD) δ 9.40 (s, 2H), 8.63 (bs, 2H), 8.54 (d, $J = 5.8$, 1H), 8.49 (d, $J = 7.2$, 1H), 8.47 (d, $J = 5.8$, 1H), 8.44 – 8.38 (m, 2H), 8.35 (d, $J = 7.2$, 1H), 8.26 – 8.16 (m, 4H), 7.83 (t, $J = 7.8$, 1H), 7.79 (t, $J = 7.8$, 1H), 7.56 (d, $J = 8.6$, 1H), 7.50 (d, $J = 8.6$, 2H), 7.43 (d, $J = 8.5$, 1H), 6.84 (s, 1H), 6.73 (s, 1H), 3.90 (s, 1H), 3.85 (s, 2H), 3.22 (d, $J = 4.3$, 2H), 3.20 (d, $J = 4.2$, 2H), 3.02 (d, $J = 5.0$, 1H), 3.00 (d, $J = 5.0$, 1H), 2.39 – 2.31 (m, 4H), 1.23 (t, $J = 7.3$, 3H), 1.13 (t, $J = 7.5$, 4H). ^{13}C NMR (150 MHz, MeOD) δ 154.25, 148.26, 144.76, 144.27, 144.16, 139.54, 138.08, 135.39, 135.31, 135.15, 134.93, 132.65, 131.74, 131.07, 130.98, 130.73, 127.84, 127.75, 124.85, 124.65, 119.16, 52.62, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 48.28, 47.87, 47.26, 39.83, 39.59, 28.17, 23.48, 12.92, 12.48. HRMS: calcd for $[C_{22}H_{24}N_4O_4S + H]^+ = 441.15910$, found 441.15893.



(E)-N-(2-(2-benzylidene-3-methylbutylamino)ethyl)isoquinoline-5-sulfonamide **55ab**.

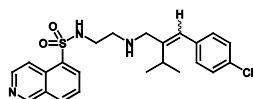
Data for the E-isomer: Yield: 16.2 mg, 31.0 μ mol, 3.1 %. ^1H -NMR (MeOD): δ 8.76 (m, 2H, H1/2), 8.60 – 8.59 (d, 1H, H5, $J_1 = 7.20$ Hz), 8.52– 8.51 (d, 1H, H3, $J_1 = 8.16$ Hz), 7.94 – 7.91 (t, 1H, H4, $J_1 = J_2 = 7.80$ Hz), 7.37 – 7.23 (m, 5H, H11/12/15), 6.56 (s, 1H, H10), 3.83 (s, 2H, H9), 3.30 – 3.29 (m, 4H, H7/8), 3.16 – 3.11 (m, 1H, H13), 1.10 – 1.09 (d, 6H, H14, $J_1 = 6.96$ Hz); ^{13}C -NMR (MeOD): δ 143.08, 140.48, 139.89, 139.08, 138.97, 132.43, 132.28, 132.20, 131.59, 131.18, 42.78, 32.60, 23.79; HRMS: calcd for $[C_{23}H_{27}N_3O_2S + H]^+ = 410.18967$, found 410.18936. **Data for the 1/10 EZ-mix:** Yield: 17.3 mg, 33.1 μ mol, 3.3 %. ^1H NMR (600 MHz, MeOD) δ 10.08 – 9.51 (m, 1H), 8.76 (bs, 2H), 8.66 – 8.61 (m, 1H), 8.55 (d, $J = 8.1$, 1H), 8.49 (d, $J = 7.2$, 1H), 7.99 – 7.92 (m, 1H), 7.36 (t, $J = 7.6$, 2H), 7.27 (t, $J = 7.4$, 1H), 7.25 – 7.21 (m, 1H), 7.18 (d, $J = 7.6$, 2H), 6.87 (s, 1H), 6.58 – 6.54 (m, 1H), 3.93 (s, 2H), 3.83 (s, 1H), 3.28 – 3.23 (m, 1H), 2.97 (bs, 4H), 2.55 – 2.46 (m, 1H), 1.63 – 1.56 (m, 1H), 1.24 (s, 3H), 1.23 (s, 3H), 1.10 (s, 1H), 1.09 (s, 1H). ^{13}C NMR (100 MHz, MeOH) δ 139.70, 137.73, 137.55, 136.52, 136.19, 134.42, 132.88, 129.87, 129.82, 129.72, 129.60, 129.45, 128.64, 87.53, 49.64, 49.43, 49.21, 49.00, 48.79, 48.57, 48.36, 47.72, 46.90, 39.56, 32.01, 22.22, 20.97. HRMS: calcd for $[C_{23}H_{27}N_3O_2S + H]^+ = 410.18967$, found 410.18939.



(E)-N-(2-(2-(4-fluorobenzylidene)-3-methylbutylamino)ethyl)isoquinoline-5-sulfonamide **55ac**.

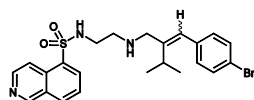
Data for the E-isomer: Yield: 24.9 mg, 46.0 μ mol, 4.6 %. ^1H -NMR (MeOD): δ 9.58 (bs,

1H, H6), 8.73 – 8.72 (d, 1H, H2, $J_1 = 6.00$ Hz), 8.68 (bs, 1H, H1), 8.60 – 8.59 (dd, 1H, H5, $J_1 = 0.78$ Hz, $J_2 = 7.38$ Hz), 8.53 – 8.51 (d, 1H, H3, $J_1 = 8.16$ Hz), 7.94 – 7.91 (t, 1H, H4, $J_1 = J_2 = 7.76$ Hz), 7.25 – 7.07 (m, 5H, H11/12/15), 6.50 (s, 1H, H10), 3.80 (s, 2H, H9), 3.29 – 3.27 (m, 4H, H7/8), 3.09 – 3.05 (m, 1H, H13), 1.08 – 1.07 (d, 6H, H14, $J_1 = 7.02$ Hz); ^{13}C -NMR (MeOD): δ 164.16, 162.53, 152.94, 141.77, 140.60, 136.48, 135.95, 135.81, 133.82, 133.79, 133.60, 131.58, 131.52, 128.82, 127.41, 120.55, 116.30, 116.1548.28, 39.90, 29.76, 20.91; HRMS: calcd for $[\text{C}_{23}\text{H}_{26}\text{FN}_3\text{O}_2\text{S} + \text{H}]^+ = 428.18025$, found 428.12813. **Data for the 1/3 EZ mix:** Yield: 22.7 mg, 42.0 μmol , 4.2 %. ^1H NMR (600 MHz, MeOD) δ 9.44 (s, 1H), 8.63 – 8.50 (m, $J = 13.2, 26.1$, 3H), 8.48 (d, $J = 7.3$, 1H), 8.42 – 8.38 (m, 1H), 8.34 (dd, $J = 1.0, 7.3$, 1H), 7.84 – 7.76 (m, 1H), 7.16 – 7.12 (m, $J = 8.3$, 1H), 7.11 – 7.07 (m, 2H), 7.02 – 6.95 (m, 3H), 6.71 (s, 1H), 6.40 (s, 1H), 3.79 (s, 2H), 3.71 (s, 1H), 3.13 (t, $J = 5.6$, 1H), 2.97 (dt, $J = 7.0, 13.9$, 1H), 2.92 – 2.83 (m, 4H), 2.44 – 2.32 (m, 1H), 1.12 (s, 3H), 1.11 (s, 3H), 0.98 (s, 1H), 0.97 (s, 1H). ^{13}C NMR (150 MHz, MeOD) δ 164.29, 164.16, 162.66, 162.53, 162.29, 153.14, 142.22, 140.60, 139.99, 136.28, 136.18, 135.87, 135.74, 135.56, 133.79, 133.77, 133.69, 133.67, 133.46, 133.39, 131.77, 131.72, 131.70, 131.57, 131.52, 130.86, 130.77, 130.58, 128.67, 128.61, 127.38, 120.26, 116.70, 116.56, 116.31, 116.16, 49.85, 49.43, 49.28, 49.14, 49.00, 48.94, 48.86, 48.72, 48.57, 48.28, 47.69, 46.71, 39.90, 39.51, 31.97, 30.82, 30.31, 29.77, 26.93, 22.20, 20.92. HRMS: calcd for $[\text{C}_{23}\text{H}_{26}\text{FN}_3\text{O}_2\text{S} + \text{H}]^+ = 428.18025$, found 428.18016.



(E)-N-(2-(2-(4-chlorobenzylidene)-3-methylbutylamino)ethyl)isoquinoline-5-sulfonamide **55ad**.

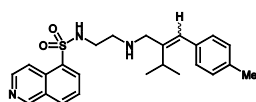
Data for the E-isomer: Yield: 74.6 mg, 133.9 μmol , 13.4 %. ^1H -NMR (MeOD): δ 9.68 (bs, 1H, H6), 8.84 – 8.83 (d, 1H, H2, $J_1 = 6.06$ Hz), 8.71 (bs, 1H, H1), 8.66 – 8.65 (d, 1H, H5, $J_1 = 7.32$ Hz), 8.59 – 8.57 (d, 1H, H3, $J_1 = 8.22$ Hz), 7.99 – 7.97 (t, 1H, H4, $J_1 = 7.14$ Hz, $J_1 = 7.80$ Hz), 7.35 – 7.34 (d, 2H, H11, $J_1 = 8.28$ Hz), 7.21 – 7.19 (d, 2H, H12, $J_1 = 8.22$ Hz), 6.49 (s, 1H, H10), 3.81 (s, 2H, H9), 3.29 – 3.26 (m, 4H, H7/8), 3.08 – 3.00 (m, 1H, H13), 1.08 – 1.07 (d, 6H, H14, $J_1 = 7.02$ Hz); ^{13}C -NMR (MeOD): δ 152.02, 141.22, 139.76, 137.35, 136.31, 136.29, 136.10, 134.20, 134.14, 131.22, 129.56, 129.45, 127.16, 121.47, 48.93, 48.26, 39.93, 29.84, 20.88; HRMS: calcd for $[\text{C}_{23}\text{H}_{26}\text{ClN}_3\text{O}_2\text{S} + \text{H}]^+ = 444.15070$, found 444.15066. **Data for the Z-isomer:** Yield: 41.8 mg, 75.0 μmol , 7.5 %. ^1H NMR (600 MHz, MeOD) δ 9.51 (s, 1H), 8.69 (s, 1H), 8.57 (d, $J = 6.1$, 1H), 8.50 (d, $J = 8.2$, 1H), 8.42 (d, $J = 7.3$, 1H), 7.91 (t, $J = 7.8$, 1H), 7.42 (d, $J = 8.3$, 2H), 7.22 (d, $J = 8.3$, 2H), 6.87 (s, 1H), 3.96 (s, 2H), 3.02 (d, $J = 5.3$, 2H), 2.99 (d, $J = 5.3$, 2H), 2.52 (dt, $J = 6.7, 13.4$, 1H), 1.28 (d, $J = 6.7$, 6H). ^{13}C NMR (150 MHz, MeOD) δ 154.01, 144.13, 140.43, 136.17, 135.60, 135.33, 135.28, 134.59, 132.68, 131.68, 131.42, 131.42, 131.05, 130.00, 130.00, 129.99, 129.63, 128.01, 60.79, 49.43, 49.43, 49.42, 49.29, 49.28, 49.28, 49.15, 49.14, 49.14, 49.01, 49.00, 49.00, 48.86, 48.86, 48.85, 48.72, 48.72, 48.71, 48.58, 48.57, 48.57, 47.67, 46.68, 39.45, 32.06, 22.20. HRMS: calcd for $[\text{C}_{23}\text{H}_{26}\text{ClN}_3\text{O}_2\text{S} + \text{H}]^+ = 444.15070$, found 444.15069.



(E)-N-(2-(2-(4-bromobenzylidene)-3-methylbutylamino)ethyl)isoquinoline-5-sulfonamide **55ae**.

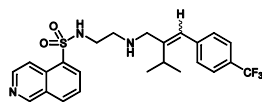
Data for the E-isomer: Yield: 50 mg, 83.2 μmol , 8.3 %. ^1H -NMR (MeOD): δ 9.69 (bs, 1H, H6), 8.80 (m, 2H, H1/2), 8.66 – 8.64 (d, 1H, H5, $J_1 = 7.30$ Hz), 8.58 – 8.56 (d, 1H, H3, $J_1 = 8.20$ Hz), 7.99 – 7.96 (t, 1H, H4, $J_1 = J_2 = 7.80$ Hz), 7.55 – 7.53 (d, 2H, H11, $J_1 = 8.30$ Hz), 7.19 – 7.17 (d, 2H, H12, $J_1 = 8.30$ Hz), 6.52 (s, 1H, H10), 3.85 (s, 2H, H9),

3.33 – 3.29 (m, 4H, H7/8), 3.12 – 3.07 (m, 1H, H13), 1.12 – 1.11 (d, 6H, H14, $J_1 = 6.95$ Hz); ^{13}C -NMR (MeOD): δ 154.50, 145.19, 141.40, 136.69, 135.43, 135.32, 134.98, 132.69, 132.60, 131.54, 127.74, 127.13, 122.27, 118.95, 39.91, 29.90, 20.92; HRMS: calcd for $[\text{C}_{23}\text{H}_{26}\text{BrN}_3\text{O}_2\text{S} + \text{H}]^+ = 488.10019$, found 488.10000. **Data for the Z-isomer:** yield: 100.0 mg, 166.4 μmol , 16.6 %. ^1H NMR (500 MHz, MeOD) δ 10.28 – 9.33 (m, 1H), 8.84 (s, 2H), 8.58 (d, $J = 8.2$, 1H), 8.54 (d, $J = 7.3$, 1H), 7.98 (t, $J = 7.8$, 1H), 7.48 (d, $J = 8.3$, 3H), 7.10 (d, $J = 8.2$, 3H), 6.76 (s, 1H), 3.89 (s, 3H), 3.02 (s, 6H), 2.56 – 2.44 (m, 2H), 1.21 (d, $J = 6.7$, 9H). ^{13}C NMR (MeOD) δ 140.64, 137.40, 136.55, 136.37, 136.03, 134.12, 132.88, 131.61, 131.45, 129.63, 122.42, 47.79, 46.82, 39.54, 31.96, 22.13.



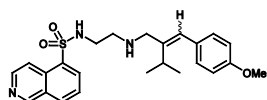
(E)-N-(2-(3-methyl-2-(4-methylbenzylidene)butylamino)ethyl)isoquinoline-5-sulfonamide **55af**.

Data for the E-isomer: Yield: 8.7 mg, 16.2 μmol , 1.6%. ^1H NMR (400 MHz, MeOH) δ 9.65 (bs, 1H), 8.73 (s, 2H), 8.61 (d, $J = 6.4$, 1H), 8.54 (d, $J = 8.2$, 1H), 8.00 – 7.90 (m, 1H), 7.21 (d, $J = 8.0$, 2H), 7.16 (d, $J = 8.1$, 2H), 6.54 (s, 1H), 3.84 (s, 2H), 3.24 – 3.11 (m, 2H), 2.36 (s, 3H), 1.13 (s, 3H), 1.11 (s, 3H). HRMS: calcd for $[\text{C}_{24}\text{H}_{29}\text{N}_3\text{O}_2\text{S} + \text{H}]^+ = 424.20532$, found 424.20530. **Data for the Z-isomer:** Yield: 42.0 mg, 78.2 μmol , 7.8 %. ^1H NMR (600 MHz, MeOD) δ 9.53 (s, 1H), 8.67 (d, $J = 22.1$, 1H), 8.62 (d, $J = 6.2$, 1H), 8.49 (d, $J = 8.2$, 1H), 8.45 (dd, $J = 0.9$, 7.4, 1H), 7.88 (t, $J = 7.8$, 1H), 7.17 (d, $J = 7.8$, 2H), 7.05 (d, $J = 7.9$, 2H), 6.81 (s, 1H), 3.92 (s, 2H), 2.98 (s, 4H), 2.48 (dt, $J = 6.7$, 13.3, 1H), 2.30 (s, 3H), 1.22 (s, 3H), 1.21 (s, 3H). ^{13}C NMR (100 MHz, MeOH) δ 151.92, 139.60, 139.17, 138.60, 137.31, 136.29, 136.00, 134.52, 134.20, 132.73, 130.47, 129.62, 129.44, 121.44, 49.64, 49.43, 49.21, 49.00, 48.79, 48.57, 48.36, 47.73, 46.97, 39.58, 32.07, 22.22, 21.20. HRMS: calcd for $[\text{C}_{24}\text{H}_{29}\text{N}_3\text{O}_2\text{S} + \text{H}]^+ = 424.20532$, found 424.20514.



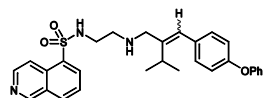
(E)-N-(2-(3-methyl-2-(4-(trifluoromethyl)benzylidene)butylamino)ethyl)isoquinoline-5-sulfonamide **55ag**.

Data for the E-isomer: Yield: 89.8 mg, 151.9 μmol , 15.2 %. ^1H NMR (600 MHz, MeOD) δ 9.53 (s, 1H), 8.66 (s, 1H), 8.61 (s, 1H), 8.52 (dd, $J = 1.0$, 7.4, 1H), 8.44 (d, $J = 8.2$, 1H), 7.84 (t, $J = 7.8$, 1H), 7.55 (d, $J = 8.1$, 2H), 7.31 (d, $J = 8.1$, 2H), 6.47 (s, 1H), 3.75 (s, 2H), 3.20 (d, $J = 6.0$, 1H), 3.15 (d, $J = 5.5$, 2H), 2.94 (dt, $J = 6.9$, 13.9, 1H), 0.99 (s, 3H), 0.98 (s, 3H). ^{13}C NMR (150 MHz, MeOD) δ 152.63, 142.54, 141.81, 141.13, 136.77, 136.06, 135.91, 133.78, 130.36, 130.29, 130.14, 129.03, 128.35, 126.69, 126.55, 126.36, 126.34, 124.76, 122.96, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 48.13, 39.93, 30.01, 20.87. HRMS: calcd for $[\text{C}_{24}\text{H}_{26}\text{F}_3\text{N}_3\text{O}_2\text{S} + \text{H}]^+ = 478.17706$, found 478.17681. **Data for the Z-isomer:** Yield: 78.6 mg, 133.0 μmol , 13.3%. ^1H NMR (600 MHz, MeOD) δ 9.75 (s, 1H), 8.86 (d, $J = 6.0$, 1H), 8.72 (s, 1H), 8.61 (d, $J = 8.3$, 1H), 8.59 (d, $J = 7.3$, 1H), 7.99 (t, $J = 7.8$, 1H), 7.66 (d, $J = 8.1$, 2H), 7.38 (d, $J = 8.1$, 2H), 6.87 (s, 1H), 3.90 (s, 2H), 3.05 (d, $J = 4.6$, 2H), 3.04 (d, $J = 4.7$, 2H), 2.54 (dt, $J = 6.6$, 13.2, 1H), 1.24 (s, 3H), 1.23 (s, 3H). ^{13}C NMR (150 MHz, MeOD) δ 151.95, 141.83, 141.64, 139.70, 137.26, 136.29, 135.97, 134.17, 131.27, 130.81, 130.60, 130.45, 130.38, 130.24, 130.17, 129.40, 128.28, 126.68, 126.65, 126.63, 126.48, 126.29, 125.89, 124.69, 122.83, 121.47, 116.95, 115.07, 49.85, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 48.07, 47.08, 39.69, 32.02, 29.88, 22.17, 20.90. HRMS: calcd for $[\text{C}_{24}\text{H}_{26}\text{F}_3\text{N}_3\text{O}_2\text{S} + \text{H}]^+ = 478.17706$, found 478.17683.



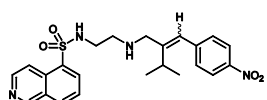
(E)-N-(2-(2-(4-methoxybenzylidene)-3-methylbutylamino)ethyl)isoquinoline-5-sulfonamide **55ah**.

Data for the 1/4 EZ mix: Yield: 36.5 mg, 66.0 μmol , 6.6 %. ^1H NMR (600 MHz, MeOD) δ 9.64 (s, 4H), 8.78 (s, 1H), 8.71 (s, 4H), 8.62 (s, 3H), 8.58 (d, J = 7.4, 2H), 8.50 (t, J = 7.5, 5H), 8.44 – 8.41 (m, 4H), 7.89 (dt, J = 7.9, 15.7, 5H), 7.05 (d, J = 8.5, 3H), 6.99 (d, J = 8.4, 8H), 6.83 – 6.77 (m, 10H), 6.67 (s, 4H), 6.38 (s, 1H), 3.83 (s, 7H), 3.67 (s, 4H), 3.66 (s, 11H), 3.18 – 3.15 (m, 4H), 3.08 – 2.99 (m, 1H), 2.87 (p, J = 5.6, 15H), 2.36 (dt, J = 6.6, 13.4, 4H), 1.10 (s, 12H), 1.09 (s, 11H), 0.98 (s, 4H), 0.97 (s, 3H). ^{13}C NMR (150 MHz, MeOD) δ 160.54, 160.39, 151.68, 139.02, 138.80, 138.45, 137.68, 137.57, 136.42, 136.25, 136.07, 134.43, 134.34, 132.45, 131.02, 130.92, 129.83, 129.71, 129.66, 129.64, 128.73, 115.25, 114.83, 55.74, 55.70, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 47.51, 46.74, 39.93, 39.49, 32.10, 29.65, 22.24, 20.99. HRMS: calcd for $[\text{C}_{24}\text{H}_{29}\text{N}_3\text{O}_3\text{S} + \text{H}]^+ = 440.20024$, found 440.20019.



(E)-N-(2-(3-methyl-2-(4-phenoxybenzylidene)butylamino)ethyl)isoquinoline-5-sulfonamide **55ai**.

Data for the 1/1 EZ mix: Yield: 59.0 mg, 95.9 μmol , 9.6%. ^1H NMR (600 MHz, MeOD) δ 9.27 (s, 2H), 8.51 (s, 2H), 8.44 (d, J = 5.8, 1H), 8.41 – 8.36 (m, 2H), 8.31 – 8.25 (m, 3H), 7.75 – 7.67 (m, 2H), 7.25 – 7.18 (m, 4H), 7.11 (d, J = 8.5, 2H), 7.05 (d, J = 8.4, 2H), 6.99 (t, J = 7.4, 2H), 6.89 – 6.81 (m, 8H), 6.69 (s, 1H), 6.41 (s, 1H), 3.83 (s, 2H), 3.70 (s, 2H), 3.16 (d, J = 5.5, 2H), 3.12 (d, J = 5.4, 1H), 3.07 – 2.97 (m, 1H), 2.88 (d, J = 4.1, 2H), 2.86 (d, J = 4.2, 2H), 2.42 – 2.32 (m, 1H), 1.11 (s, 3H), 1.09 (s, 3H), 0.98 (s, 3H), 0.97 (s, 3H). ^{13}C NMR (150 MHz, MeOD) δ 158.28, 158.10, 158.04, 154.22, 144.74, 139.86, 139.34, 135.36, 135.32, 135.17, 135.12, 135.07, 132.62, 132.56, 132.51, 132.28, 132.07, 131.35, 131.24, 130.96, 130.94, 130.66, 128.05, 127.82, 127.80, 124.78, 124.65, 120.17, 120.07, 119.73, 119.46, 119.11, 49.85, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 48.39, 47.79, 47.60, 46.72, 39.87, 39.47, 32.05, 29.74, 22.23, 20.98. HRMS: calcd for $[\text{C}_{29}\text{H}_{31}\text{N}_3\text{O}_4\text{S} + \text{H}]^+ = 502.21589$, found 502.21576.



(E)-N-(2-(3-methyl-2-(4-nitrobenzylidene)butylamino)ethyl)isoquinoline-5-sulfonamide **55aj**.

Data for the 10/1 E/Z mix: yield: 117.0 mg, 206.0 μmol , 20.6%. ^1H NMR (600 MHz, MeOD) δ 9.71 (s, 1H), 8.81 (s, 1H), 8.74 (s, 1H), 8.64 (d, J = 7.4, 1H), 8.57 (d, J = 8.2, 1H), 8.56 – 8.53 (m, 1H), 8.49 (d, J = 7.3, 1H), 8.24 (d, J = 8.7, 2H), 8.21 (d, J = 8.8, 1H), 7.97 (t, J = 7.8, 1H), 7.93 (t, J = 7.8, 1H), 7.47 (d, J = 8.4, 2H), 7.44 (d, J = 8.5, 1H), 6.91 (s, 1H), 6.59 (s, 1H), 3.93 (s, 1H), 3.88 (s, 2H), 3.31 (d, J = 5.6, 2H), 3.26 (d, J = 5.5, 2H), 3.11 – 3.00 (m, 1H), 2.59 – 2.51 (m, 1H), 1.11 (s, 3H), 1.10 (s, 3H). ^{13}C NMR (150 MHz, MeOD) δ 152.42, 148.23, 144.57, 143.73, 140.64, 136.95, 136.18, 135.98, 133.90, 131.00, 130.79, 129.21, 125.97, 124.90, 124.64, 49.43, 49.28, 49.14, 49.06, 49.00, 48.86, 48.72, 48.57, 48.09, 39.95, 30.18, 20.84. HRMS: calcd for $[\text{C}_{23}\text{H}_{26}\text{N}_4\text{O}_4\text{S} + \text{H}]^+ = 455.17475$, found 455.17455. **Data for the 1/10 E/Z mix:** Yield: 60.8 mg, 107.0 μmol , 10.7 %. ^1H NMR (600 MHz, MeOD) δ 9.60 (s, 1H), 8.69 (s, 1H), 8.65 (s, 1H), 8.60 (d, J = 7.4, 1H), 8.53 (d, J = 9.4, 1H), 8.51 (d, J = 8.2, 1H), 8.45 (dd, J = 1.0, 7.4, 1H), 8.28 – 8.23 (m, 1H), 8.22 (d, J = 8.7, 2H), 7.97 – 7.92 (m, 1H), 7.89 (t, J = 7.8, 1H), 7.49 – 7.46 (m, 1H), 7.44 (d, J = 8.4, 2H), 6.92 (s, 1H), 6.58 (s, 1H), 3.93 (s, 2H), 3.03 (t, J = 5.4, 2H), 2.99 (t, J = 5.4, 2H), 2.57 –

2.50 (m, 1H), 1.26 (s, 4H), 1.25 (s, 3H), 1.12 (s, 1H), 1.11 (s, 1H). ^{13}C NMR (150 MHz, MeOD) δ 153.00, 144.43, 142.81, 141.87, 136.16, 135.97, 135.65, 133.49, 131.00, 130.84, 128.72, 124.91, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 47.96, 46.80, 39.56, 32.25, 22.15. HRMS: calcd for $[\text{C}_{23}\text{H}_{26}\text{N}_4\text{O}_4\text{S} + \text{H}]^+$ =455.17475, found 455.17463.

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Chapter 4 | Synthesis of New H-89 Analogues

Introduction

Protein kinase B (PKB/Akt) is a regulator of many physiological processes and overstimulated PKB/Akt activity has been implicated in many types of diseases.¹ Inhibitors of PKB/Akt are currently being developed as anti tumour agents.² Recently, a novel application of inhibitors of one of the PKB/Akt isoforms (PKB/Akt1) was discovered.³ In Chapters 2 and 3, the involvement of activated PKB/Akt1 in bacterial infection and the development of inhibitors against PKB/Akt1 are described. In short, *Salmonella* bacteria are internalized by means of phagocytosis into the host cell where they reside in a membrane enclosed "Salmonella containing vacuole" (SCV). From these SCVs, proteins are excreted into the host cell cytoplasm. These proteins interfere with several biochemical pathways resulting,

amongst others, in the circumvention of the host immune system. One such pathway is the regulation of the fusion between SCVs and lysosomes. Preventing lysosomal fusion with SCVs, and thereby prohibiting degradation of the contents of SCVs, allows *Salmonella* to survive and reproduce in an environment protected from the immune system. A central role in this process is played by PKB/Akt1. Upon excretion of SopB into the host cell cytosol by *Salmonella*, a signaling pathway centered around PKB/Akt1 is activated, blocking lysosomal fusion.

In chapter 3, the synthesis and inhibitory potency against PKB/Akt1 of several new isoquinolinesulfonamides is described. Chemical modification of a known,⁴ moderately selective,⁵ inhibitor of protein kinase A (PKA), H-89 (**1**, Figure 1), resulted in the identification of **2** as a potent and selective inhibitor for PKB/Akt1. The biological results identified the styrene moiety in H-89 as suitable candidate for modification towards inhibitors with improved biological properties. Halogen replacement resulted in modified activity, altering substituents on the double bond influenced selectivity. These findings identified regions on the H-89 core structure that can be exploited to obtain more potent, and also, more selective inhibitors (highlighted in Figure 1). This chapter describes the synthesis of a library of compounds (general structures **A**, **B** and **C** in Figure 1) based on H-89, as potential inhibitors of PKB/Akt1.

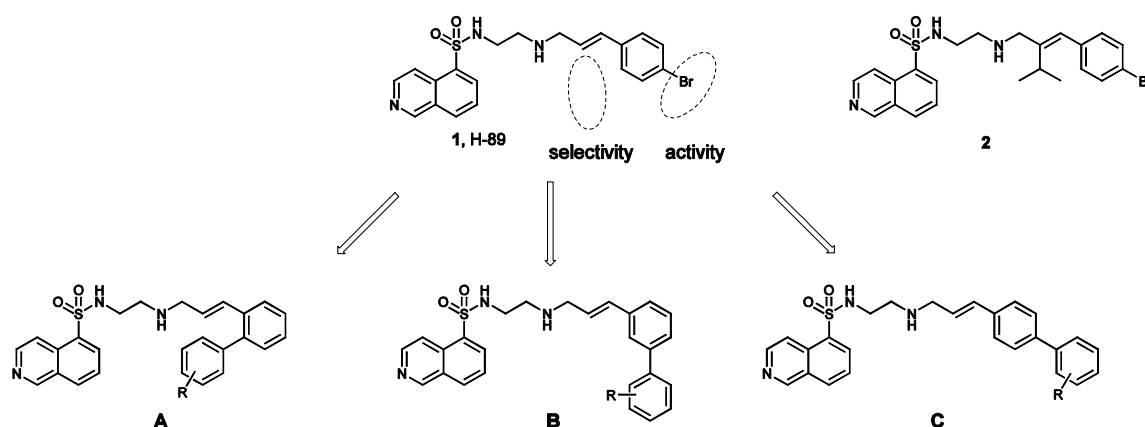
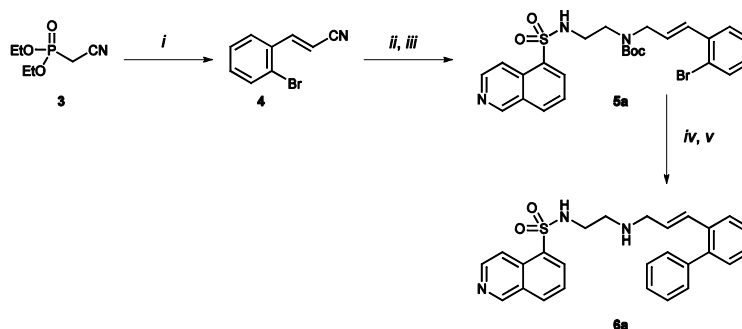


Figure 1; Target structures of the novel isoquinolinesulfonamide based library

Results and discussion

The construction of the library of compounds was accomplished as illustrated for compound **6a** (Scheme 1). Similarly to the synthesis of H-89 described in chapter 3, diethyl cyanomethylphosphonate **3** is treated with NaH and *o*-bromobenzaldehyde in a Horner-Wadsworth-Emmons reaction in DMF to afford *ortho*-bromocinnamonitril **4** in 89% yield with an E/Z ratio of 3/1. The thus obtained nitril **4** was subjected to DiBAL-H, MeOH, *N*-(2-aminoethyl)isoquinoline-5-sulfonamide·TFA and NaBH₄ respectively in the reduction-transimination-reduction sequence, followed by treatment of the crude reaction mixture with Boc₂O in THF yielding nitrogen-protected aryl-bromide **5a** in 32% after silica column chromatography. Coupling of the aryl bromide with phenylboronic acid was performed under the agency of Pd(PPh₃)₄ and K₂CO₃ in toluene at 85°C for 16h. affording biarylated isoquinolinesulfonamide **6a** with >95% conversion according to LCMS analysis. Extraction of the reaction mixture and crude deprotection using 50% TFA/DCM, followed by HPLC purification resulted in the isolation of the target compound in a yield 25.5% over two steps. For the yields of the complete library of isoquinolinesulfonamides see Table 1 and the experimental section.



Scheme 1; Example of the synthesis of biarylated isoquinolinesulfonamides. *Reagents and conditions:* (i) 1.1 eq. NaH, 1.2 eq. *ortho*-Bromobenzaldehyde, DMF, 0°C – r.t., 89% E/Z = 3/1. (ii) a: 2.0 eq. DiBAL-H, Et₂O, -78°C to 0°C, 30 min. b: MeOH, -100°C c: 1.5 eq. *N*-(2-aminoethyl)isoquinoline-5-sulfonamide·TFA, r.t., 3h. d: 2.0 eq. NaBH₄, -18°C to r.t., 16h. (iii) 2.5 eq. Boc₂O, THF, 0°C to r.t. 32% over two steps. (iv) 2.0 eq. phenylboronic acid, 2.5 eq. K₂CO₃, 0.05 eq. Pd(PPh₃)₄. (v) 50% TFA/DCM, 25.5% after two steps.

Conclusion

The synthesis of 63 novel analogues of H89 was performed successfully in a parallel fashion by Suzuki coupling of 21 boronic acids to 3 suitably protected aryl bromides. The deprotection of the Suzuki products could be performed on the crude reaction mixtures

after extraction only. Reaction progress of both the Suzuki reaction and the deprotection were conveniently monitored by means of LCMS. HPLC purification allowed the isolation of the final compounds in yields ranging from 6.1 – 47.5 %. Their biological evaluation will be performed in the near future.

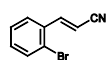
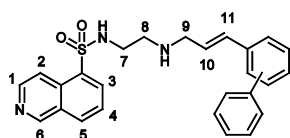
	R =	6a-u, (%)	7a-u, (%)	8a-u, (%)		R =	6a-u, (%)	7a-u, (%)	8a-u, (%)
a		25.5	17.3	13.4	l		16.3	13.6	47.5
b		29.4	19.3	15.4	m		27.2	30.3	28.2
c		30.6	10.2	17.7	n		23.7	13.1	22.1
d		21.8	18.0	17.2	o		25.0	14.0	27.7
e		24.7	17.5	12.7	p		25.2	22.3	28.3
f		22.0	19.0	9.1	q		25.5	12.2	26.3
g		35.8	10.2	9.2	r		9.3	33.1	33.1
h		14.0	7.6	27.7	s		15.1	6.9	13.5
i		19.2	11.0	36.8	t		22.7	44.5	44.3
J		12.6	10.2	20.7	u		22.1	29.4	31.1
k		19.6	6.1	42.7					

Table 1; Yields of the Suzuki cross coupling

Experimental Section

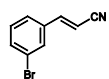
General: PE with a boiling range of 40 - 60°C was used. THF and Et₂O were distilled over LiAlH₄ prior to use. DCM was distilled over CaH₂ prior to use. All other solvents used under anhydrous conditions were stored over molecular sieves (4Å) except for methanol which was stored over 3Å molecular sieves. Solvents used for work-up and column chromatography were of technical grade and distilled before use. Unless stated otherwise, solvents were removed by rotary evaporation under reduced pressure at 40°C. Reactions were monitored by TLC-analysis using Merck 25 DC plastikfolien 60 F₂₅₄ with detection by spraying with 20% H₂SO₄ in EtOH, (NH₄)₆Mo₇O₂₄·4H₂O (25 g/L) and (NH₄)₄Ce(SO₄)₄·2H₂O (10 g/L) in 10% sulfuric acid or by spraying with a solution of ninhydrin (3 g/L) in EtOH / AcOH (20/1 v/v), followed by charring at approx. 150°C. Column chromatography was performed on Fluka silicagel (0.04 – 0.063 mm). For LC/MS analysis, an JASCO HPLC-system (detection simultaneously at 214 and 254 nm) equipped with an analytical C₁₈ column (4.6 mmD × 250 mmL, 5μ particle size) in combination with buffers A: H₂O, B: MeCN and C: 0.5% aq. TFA and coupled to a mass instrument with a custom-made Electrospray Interface (ESI) was used. For reversed-phase HPLC purification of the final compounds, an automated HPLC system supplied with a semi-preparative C₁₈ column (10.0 mmD × 250 mmL, 5μ particle size) was used. The applied buffers were A: H₂O, B: MeCN and C: 1.0% aq. TFA. High resolution mass spectra were recorded by direct injection (2 μL of a 2 μM solution in water/acetonitrile; 50/50; v/v and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150-2000) and dioctylphthalate (m/z = 391.28428) as a "lock mass".⁶ The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). ¹H- en ¹³C-NMR spectra were measured on a Joel JNM-FX-200 (200/50 Mhz), a Brüker AV-400 (400/100 MHz), a Brüker AV-500 (500/125 MHz) or a Brüker DMX-600 (600/125 MHz). Chemical shifts are given in ppm (δ) relative to TMS (0 ppm) or MeOD (3.30 ppm) and coupling constants are given in Hz.

The numbering of isoquinolinesulfonamides is generally as follows:



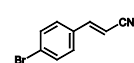
(E/Z)-3-(2-bromophenyl)acrylonitrile: ortho-4: To an ice-cold solution of NaH (5.16g, 129 mmol, 60% in mineral oil) and in DMF (400mL) diethyl cyanomethylphosphonate (22.7g, 128 mmol) was slowly added and allowed to stir for 25 minutes before addition of the aldehyde (15.8 mL, 135 mmol). The reaction was allowed to stir until completion (TLC 10% EtOAc/PE) and quenched by addition of freshly prepared sat. aq. Na₂HSO₃ (500 mL). The mixture was diluted with H₂O (500 mL) and Et₂O (500 mL), the layers were separated, the aqueous phase washed with Et₂O (3 x 400 mL) and the combined organic phase was washed with sat. aq. bicarb. (1 x 250mL) and brine (1 x 250 mL), dried over Na₂SO₄, filtered and concentrated. The residue was further purified by silica column chromatography (0-3% Et₂O/PE) to afford the title compound as

yellow oil (22.1 g, 107 mmol, 84%, E/Z = 3/1): ^1H NMR (400 MHz, CDCl_3) δ 8.06 (d, J = 7.8, 1H), 7.83 (d, J = 16.6, 3H), 7.72 – 7.64 (m, 4H), 7.54 (t, J = 9.3, 4H), 7.45 (t, J = 7.6, 1H), 7.38 (t, J = 7.5, 3H), 7.35 – 7.29 (m, 4H), 5.89 (d, J = 16.6, 3H), 5.62 (d, J = 12.0, 1H). ^{13}C NMR (101 MHz, CDCl_3) δ 149.03, 147.85, 133.61, 133.49, 133.13, 132.09, 131.81, 129.24, 127.90, 127.83, 126.97, 124.74, 117.55, 99.09, 98.28.



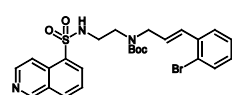
(E/Z)-3-(3-bromophenyl)acrylonitrile meta-4: Prepared according to the same procedure as described for ortho-4 yielding the title compound E/Z = 7/1 (24.7 g, 119 mmol, 93%) as yellow oil.

^1H NMR (400 MHz, CDCl_3) δ 7.83 – 7.74 (m, 1H), 7.61 – 7.45 (m, 4H), 7.40 – 7.30 (m, 2H), 7.30 (m, 2H), 7.03 (d, J = 12.1, 1H), 5.87 (d, J = 16.7, 1H), 5.51 (d, J = 12.1, 1H). ^{13}C NMR (101 MHz, CDCl_3) δ 148.45, 146.61, 135.22, 133.62, 133.43, 131.68, 130.34, 130.18, 129.78, 126.86, 125.76, 122.91, 117.31, 97.83, 96.59.



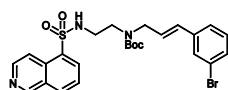
(E)-3-(4-bromophenyl)acrylonitrile para-4: Prepared according to the same procedure as described for ortho-4 yielding the title compound E/Z = 4/1 (25.5 g, 123 mmol, 96%) as of white solid.

^1H NMR (400 MHz, CDCl_3) δ 7.68 (d, J = 8.5, 2H), 7.61 – 7.52 (m, 3H), 7.38 – 7.24 (m, 4H), 7.07 (d, J = 12.1, 1H), 5.88 (d, J = 16.7, 1H), 5.49 (d, J = 12.1, 1H). ^{13}C NMR (101 MHz, CDCl_3) δ 149.23, 147.37, 132.43, 132.39, 132.24, 130.41, 128.72, 125.69, 117.81, 97.13, 95.86.



(E)-tert-butyl 3-(2-bromophenyl)allyl(2-(isoquinoline-5-sulfonamido)ethyl)carbamate, ortho-5. A solution of ortho-4 (21 g, 100 mmol) in anhydrous Et_2O (500 mL) is cooled to

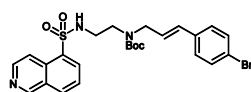
-78°C before dropwise addition of DiBAL-H (200 mL, 200 mmol, 1M soln. in hexanes) and the reaction mixture was allowed to warm to 0°C in 60 min. under stirring. The reaction mixture was cooled to -100°C followed by rapid addition of MeOH (50 mL). After 5 min., a soln. of amine **45** (200 mmol) in MeOH (150 mL) was slowly added and the reaction mixture was allowed to stir at r.t. for 3 h. Then, the reaction mixture was cooled to -18°C and NaBH_4 (200 mmol) was added and the reaction mixture was allowed to stir for 16 h. at r.t.. The reaction mixture was diluted with 0.5M aq. NaOH (500 mL), the layers were separated and the aqueous phase was washed with CHCl_3 (3 x 250 mL). The combined organic phase was washed with H_2O (3 x 250 mL) and brine (50 mL), dried, filtered and concentrated. The residue was dissolved in anhydrous THF (500 mL), cooled to 0°C and charged with Boc_2O (250 mmol) and allowed to stir for 16h at r.t. The reaction mixture was diluted with H_2O (500 mL) and EtOAc (500 mL). The organic phase was separated; the aqueous phase was washed with EtOAc (3 x 150 mL) and the combined organic phase was washed with sat. aq. bicarb. (2 x 100 mL) and brine (1 x 100 mL), dried, filtered and concentrated. The residue was purified by silica column chromatography (0 – 20% EtOAc/PE) to afford the title compound (17.8 g, 32 mmol, 32%) as white solid. ^1H NMR (400 MHz, CDCl_3) δ 9.23 (s, 1H), 8.54 (d, J = 6.0, 1H), 8.38 (d, J = 6.0, 1H), 8.33 (d, J = 7.3, 1H), 8.03 (d, J = 8.1, 1H), 7.52 (t, J = 7.8, 1H), 7.44 (d, J = 7.9, 1H), 7.32 (d, J = 7.6, 1H), 7.16 (d, J = 7.3, 2H), 7.09 (d, J = 7.5, 2H), 7.02 (t, J = 7.5, 1H), 6.61 (d, J = 15.7, 1H), 6.57 – 6.25 (m, 1H), 5.93 – 5.83 (m, 1H), 3.83 (d, J = 5.8, 2H), 3.10 (s, 2H), 1.38 (s, 9H). ^{13}C NMR (101 MHz, CDCl_3) δ 152.99, 144.73, 136.15, 134.43, 133.19, 132.83, 132.66, 131.05, 130.63, 128.86, 128.81, 128.00, 127.93, 127.37, 126.98, 125.68, 125.09, 123.25, 117.20, 80.49, 53.31, 46.61, 28.16, 28.10. HRMS: calcd for $[\text{C}_{25}\text{H}_{28}\text{BrN}_3\text{O}_4\text{S} + \text{H}]^+$ = 546.10567, found 546.10583.



(E)-tert-butyl 3-(3-bromophenyl)allyl(2-(isoquinoline-5-

sulfonamido)ethyl)carbamate, meta-5. Prepared according to the same procedure as

described for ortho-5. Starting from meta-4 (2.1 g, 10 mmol) the title compound was obtained (2.2 g, 4.1 mmol, 41%) as off white solid. ^1H NMR (400 MHz, CDCl_3) δ 9.34 (s, 1H), 8.64 (d, J = 6.1, 1H), 8.41 (d, J = 5.8, 1H), 8.38 (dd, J = 1.2, 7.4, 1H), 8.17 (d, J = 8.2, 1H), 7.65 – 7.61 (m, 1H), 7.45 (d, J = 1.6, 1H), 7.38 (d, J = 7.4, 1H), 7.20 (dt, J = 7.7, 18.1, 2H), 6.35 (bs, 1H), 6.31 (d, J = 15.9, 1H), 6.03 (d, J = 16.0, 1H), 5.91 (bs, 1H), 3.89 (d, J = 5.9, 2H), 3.35 (s, 2H), 3.11 (s, 2H), 1.45 (s, 9H). ^{13}C NMR (101 MHz, CDCl_3) δ 153.16, 145.04, 138.42, 134.29, 133.40, 133.01, 131.13, 130.57, 130.09, 129.11, 128.95, 126.48, 125.76, 125.04, 122.71, 117.21, 80.78, 50.32, 46.49, 42.68, 28.28. $[\text{C}_{25}\text{H}_{28}\text{BrN}_3\text{O}_4\text{S} + \text{H}]^+ = 546.10567$, found 546.10583.



(E)-tert-butyl 3-(4-bromophenyl)allyl(2-(isoquinoline-5-sulfonamido)ethyl)carbamate,

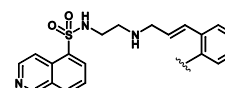
para-5. Prepared according to the same procedure as described for ortho-5. Starting

from meta-4 (2.1 g, 10 mmol) the title compound was obtained (2.1 g, 3.9 mmol, 39%) as off white solid. ^1H NMR (400 MHz, CDCl_3) δ 9.24 (s, 1H), 8.50 (d, J = 5.4, 1H), 8.40 (d, J = 5.9, 1H), 8.30 (d, J = 7.2, 1H), 7.52 (t, J = 7.5, 1H), 7.31 (s, 3H), 7.08 (s, 3H), 6.24 (d, J = 15.8, 1H), 5.97 (d, J = 15.6, 1H), 3.82 (s, 2H), 3.29 (s, 3H), 3.07 (s, 3H), 1.35 (s, 9H). ^{13}C NMR (101 MHz, CDCl_3) δ 152.72, 144.21, 135.05, 134.27, 133.11, 132.70, 132.03, 131.28, 131.04, 130.86, 130.63, 130.02, 128.66, 127.59, 125.67, 125.57, 121.04, 117.25, 113.80, 80.16, 77.32, 77.00, 76.68, 50.03, 46.37, 41.77, 29.33, 28.01. $[\text{C}_{25}\text{H}_{28}\text{BrN}_3\text{O}_4\text{S} + \text{H}]^+ = 546.10567$, found 546.10590.

General procedure for the Suzuki cross coupling:

Stock solutions were prepared of arylbromide **5** (0.20 M in toluene), K_2CO_3 (1.25 M in H_2O) and $\text{Pd}(\text{PPh}_3)_4$ (0.017 M in toluene) and thoroughly degassed in a sonication bath under an atmosphere of argon for 15 min. Arylbromide **5** (109 mg, 200 μmol , 1 mL stock), K_2CO_3 (95 mg, 500 μmol , 0.4 mL stock) and $\text{Pd}(\text{PPh}_3)_4$ (12 mg, 10 μmol , 0.6 mL stock) were added to the appropriate boronic acid (0.4 mmol). The reaction mixture was allowed to stir for 16h. at 85°C under an atmosphere of argon. The reaction progress was monitored by LCMS analysis. The reaction mixture was diluted with H_2O (1 mL), the organic layer was separated and the aqueous phase was washed with toluene (3 x 0.5 mL). The combined organic phase was washed with brine (1 x 1 mL), dried, filtered and concentrated and used in the following reaction without further purification. The crude product was dissolved DCM (0.5 mL) and TFA (0.5 mL) is added and the reaction mixture is allowed to stir for 1 h. before it is concentrated. The residue is purified by HPLC.

(E)-N-(2-(3-biphenyl-2-yl-allylamino)ethyl)isoquinoline-5-sulfonamides 6a-u



6a: Prepared according to the general procedure. Yield 28.4 mg, 51.0 μmol , 25.5%. ^1H NMR (400 MHz, MeOD) δ 9.44 (bs, 1H, H6), 8.64 (d, J = 6.1, 1H, H1), 8.59 (d, J = 6.2, 1H, H2), 8.49 (d, J = 7.3, 1H, H3), 8.43 (d, J = 8.2, 1H, H5), 7.84 (t, J = 7.8, 1H, H4), 7.69 – 7.64 (m, 1H, H_{Ar}), 7.44 – 7.31 (m, 5H, H_{Ar}), 7.31 – 7.25 (m, 3H, H_{Ar}), 6.78 (d, J = 15.8, 1H, H11), 6.23 – 6.13 (m, 1H, H10), 3.72 (d, J = 7.2, 2H, H9), 3.12 (m, 4H, H7+H8). ^{13}C

NMR (101 MHz, MeOD) δ 153.81, 143.80, 142.92, 141.83, 139.05, 135.52, 135.49, 134.64, 132.94, 131.34, 130.75, 129.80, 129.37, 128.81, 128.42, 128.10, 127.40, 119.98, 119.54, 50.51, 47.47, 40.02. HRMS: calcd for $[\text{C}_{26}\text{H}_{25}\text{N}_3\text{O}_2\text{S} + \text{H}]^+ = 444.17402$, found 444.17394.



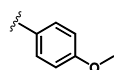
6b: Prepared according to the general procedure. Yield: 33.6 mg, 58.8 μmol , 29.4%. ^1H NMR (400 MHz, MeOD) δ 9.45 (bs, 1H, H6), 8.63 (bs, 1H, H1), 8.60 (d, $J = 6.0$, 1H, H2), 8.49 (d, $J = 7.3$, 1H, H3), 8.42 (d, $J = 8.3$, 1H, H5), 7.83 (t, $J = 7.8$, 1H, H4), 7.67 – 7.62 (m, 1H, H_{Ar}), 7.39 – 7.32 (m, 2H, H_{Ar}), 7.28 – 7.24 (m, 1H, H_{Ar}), 7.22 (d, $J = 7.9$, 2H, H_{Ar}), 7.15 (d, $J = 8.0$, 2H, H_{Ar}), 6.78 (d, $J = 15.8$, 1H, H11), 6.22 – 6.12 (m, 1H, H10), 3.72 (d, $J = 7.2$, 2H, H9), 3.17 – 3.08 (m, 4H, H7+H8), 2.35 (s, 3H, CH_3). ^{13}C NMR (101 MHz, MeOD) δ 153.75, 143.67, 142.92, 139.23, 138.85, 138.29, 135.53, 134.65, 132.97, 131.32, 130.65, 129.98, 129.75, 128.59, 128.13, 127.37, 119.73, 119.61, 50.55, 47.48, 40.02, 21.17. HRMS: calcd for $[\text{C}_{27}\text{H}_{27}\text{N}_3\text{O}_2\text{S} + \text{H}]^+ = 458.18967$, found 458.18958.



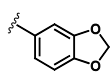
6c: Prepared according to the general procedure. Yield: 35.0 mg, 61.3 μmol , 30.6%. ^1H NMR (400 MHz, MeOD) δ 9.43 (bs, 1H, H6), 8.63 (d, $J = 6.0$, 1H, H1), 8.58 (d, $J = 6.2$, 1H, H2), 8.48 (d, $J = 7.3$, 1H, H3), 8.42 (d, $J = 8.3$, 1H, H5), 7.83 (t, $J = 7.8$, 1H, H4), 7.69 – 7.63 (m, 1H, H_{Ar}), 7.40 – 7.32 (m, 2H, H_{Ar}), 7.30 – 7.23 (m, 2H, H_{Ar}), 7.16 (d, $J = 7.5$, 1H, H_{Ar}), 7.08 (s, 1H, H_{Ar}), 7.04 (d, $J = 7.6$, 1H, H_{Ar}), 6.78 (d, $J = 15.8$, 1H, H11), 6.23 – 6.12 (m, 1H, H10), 3.72 (d, $J = 7.3$, 2H, H9), 3.15 – 3.08 (m, 4H, H7+H8), 2.35 (s, 3H, CH_3). ^{13}C NMR (101 MHz, MeOD) δ 153.81, 143.81, 143.11, 141.81, 139.18, 139.09, 135.51, 135.47, 134.58, 132.92, 131.31, 131.24, 130.61, 129.73, 129.21, 129.07, 128.70, 128.08, 127.92, 127.28, 119.78, 119.53, 50.51, 47.43, 40.01, 21.50. HRMS: calcd for $[\text{C}_{27}\text{H}_{27}\text{N}_3\text{O}_2\text{S} + \text{H}]^+ = 458.18967$, found 458.18964.



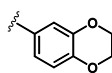
6d: Prepared according to the general procedure. Yield: 28.4 mg, 51.0 μmol , 25.5%. ^1H NMR (400 MHz, MeOD) δ 9.47 (bs, 1H, H6), 8.65 (bs, 1H, H1), 8.59 (d, $J = 6.0$, 1H, H2), 8.48 (d, $J = 7.4$, 1H, H3), 8.45 (d, $J = 8.2$, 1H, H5), 7.85 (t, $J = 7.8$, 1H, H4), 7.74 – 7.67 (m, 1H, H_{Ar}), 7.40 – 7.36 (m, 2H, H_{Ar}), 7.25 (d, $J = 4.1$, 2H, H_{Ar}), 7.23 – 7.17 (m, 1H, H_{Ar}), 7.15 – 7.11 (m, 1H, H_{Ar}), 7.05 (d, $J = 7.0$, 1H, H_{Ar}), 6.51 (d, $J = 15.8$, 1H, H11), 6.21 – 6.11 (m, 1H, H10), 3.68 – 3.62 (m, 2H, H9), 3.06 (m, 4H, H7+H8), 2.00 (s, 3H, CH_3). ^{13}C NMR (101 MHz, MeOD) δ 153.78, 143.73, 142.66, 141.49, 138.27, 137.19, 135.54, 134.91, 132.96, 131.18, 131.07, 130.76, 129.71, 128.85, 128.83, 128.13, 126.79, 126.74, 119.96, 119.60, 50.48, 47.45, 39.97, 20.21. HRMS: calcd for $[\text{C}_{27}\text{H}_{27}\text{N}_3\text{O}_2\text{S} + \text{H}]^+ = 458.18967$, found 458.18958.



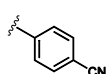
6e: Prepared according to the general procedure. Yield: 29.0 mg, 49.4 μmol , 24.7%. ^1H NMR (400 MHz, MeOD) δ 9.43 (bs, 1H, H6), 8.64 (d, $J = 6.2$, 1H, H1), 8.58 (d, $J = 6.3$, 1H, H2), 8.48 (d, $J = 7.2$, 1H, H3), 8.42 (d, $J = 8.2$, 1H, H5), 7.83 (t, $J = 7.8$, 1H, H4), 7.66 – 7.61 (m, 1H, H_{Ar}), 7.37 – 7.32 (m, 2H, H_{Ar}), 7.28 – 7.25 (m, 1H, H_{Ar}), 7.20 (d, $J = 8.6$, 2H, H_{Ar}), 6.97 (d, $J = 8.6$, 2H, H_{Ar}), 6.80 (d, $J = 15.8$, 1H, H11), 6.21 – 6.12 (m, 1H, H10), 3.81 (s, 3H, OCH_3), 3.73 (d, $J = 7.2$, 2H, H9), 3.13 (m, 4H, H7+H8). ^{13}C NMR (101 MHz, MeOD) δ 160.60, 153.84, 143.87, 142.63, 139.40, 135.51, 135.44, 134.72, 134.01, 132.92, 131.88, 131.36, 130.62, 129.77, 128.43, 128.07, 127.44, 119.65, 119.50, 114.80, 55.78, 50.53, 47.46, 40.01. HRMS: calcd for $[\text{C}_{27}\text{H}_{27}\text{N}_3\text{O}_3\text{S} + \text{H}]^+ = 474.18459$, found 474.18445.



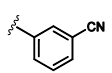
6f: Prepared according to the general procedure. Yield: 26.4 mg, 43.9 μmol , 22.0 %. ^1H NMR (400 MHz, MeOD) δ 9.44 (bs, 1H, H6), 8.64 (bs, 1H, H1), 8.59 (d, J = 6.2, 1H, H2), 8.49 (d, J = 7.4, 1H, H3), 8.43 (d, J = 8.2, 1H, H5), 7.84 (t, J = 7.8, 1H, H4), 7.63 (dd, J = 3.1, 6.0, 1H, H_{Ar}), 7.35 (dd, J = 3.2, 5.7, 2H, H_{Ar}), 7.26 (dd, J = 3.2, 5.8, 1H, H_{Ar}), 6.86 (d, J = 7.9, 1H, H_{Ar}), 6.81 (d, J = 15.8, 1H, H11), 6.75 (d, J = 1.5, 1H, H_{Ar}), 6.73 (dd, J = 1.7, 7.9, 1H, H_{Ar}), 6.21 – 6.12 (m, 1H, H10), 5.95 (s, 2H, OCH_2O), 3.75 (d, J = 7.2, 2H, H9), 3.13 (m, 4H, H7+H8). ^{13}C NMR (101 MHz, MeOD) δ 153.86, 149.00, 148.51, 143.90, 142.58, 139.19, 135.64, 135.53, 135.46, 134.80, 132.92, 131.34, 129.77, 128.65, 128.07, 127.45, 124.33, 119.84, 119.51, 111.11, 109.10, 102.55, 50.50, 47.46, 40.01. HRMS: calcd for $[\text{C}_{27}\text{H}_{25}\text{N}_3\text{O}_4\text{S} + \text{H}]^+ = 488.16385$, found 488.16372.



6g: Prepared according to the general procedure. Yield: 44.0 mg, 71.5 μmol , 35.8 %. ^1H NMR (400 MHz, MeOD) δ 9.43 (bs, 1H, H6), 8.63 (bs, 1H, H1), 8.59 (d, J = 6.1, 1H, H2), 8.49 (d, J = 7.3, 1H, H3), 8.41 (d, J = 8.2, 1H, H5), 7.83 (t, J = 7.8, 1H, H4), 7.63 – 7.58 (m, 1H, H_{Ar}), 7.35 – 7.30 (m, 2H, H_{Ar}), 7.24 (dt, J = 3.0, 6.1, 1H, H_{Ar}), 6.85 (d, J = 7.9, 1H, H_{Ar}), 6.80 (d, J = 15.8, 1H, H11), 6.72 – 6.70 (m, 2H, H_{Ar}), 6.20 – 6.10 (m, 1H, H10), 4.24 – 4.18 (m, 4H, $\text{OCH}_2\text{CH}_2\text{O}$), 3.74 (d, J = 7.2, 2H, H9), 3.13 (m, 4H, H7+H8). ^{13}C NMR (101 MHz, MeOD) δ 153.78, 144.64, 144.58, 143.77, 142.37, 139.40, 135.51, 134.92, 134.71, 132.92, 131.22, 130.61, 129.71, 128.53, 128.10, 127.43, 123.76, 119.68, 119.50, 118.08, 65.64, 50.47, 47.39, 40.00. HRMS: calcd for $[\text{C}_{28}\text{H}_{27}\text{N}_3\text{O}_4\text{S} + \text{H}]^+ = 502.17950$, found 502.17935.



6h: Prepared according to the general procedure. Yield: 16.3 mg, 28.0 μmol , 14.0 %. ^1H NMR (400 MHz, MeOD) δ 9.46 (bs, 1H, H6), 8.65 (bs, 1H, H1), 8.59 (d, J = 6.2, 1H, H2), 8.49 (d, J = 7.2, 1H, H3), 8.45 (d, J = 8.2, 1H, H5), 7.86 (t, J = 7.8, 1H, H4), 7.80 (d, J = 8.2, 2H, H_{Ar}), 7.73 – 7.69 (m, 1H, H_{Ar}), 7.50 (d, J = 8.2, 2H, H_{Ar}), 7.46 – 7.42 (m, 2H, H_{Ar}), 7.35 – 7.31 (m, 1H, H_{Ar}), 6.73 (d, J = 15.7, 1H, H11), 6.28 – 6.18 (m, 1H, H10), 3.76 (d, J = 7.2, 2H, H9), 3.13 (m, 4H, H7+H8). ^{13}C NMR (101 MHz, MeOD) δ 154.12, 146.77, 144.44, 140.92, 138.14, 135.47, 135.40, 135.20, 134.77, 133.33, 132.78, 131.82, 131.13, 130.69, 130.10, 129.88, 127.93, 127.85, 121.32, 119.64, 119.67, 112.27, 50.33, 47.63, 40.03. HRMS: calcd for $[\text{C}_{27}\text{H}_{24}\text{N}_4\text{O}_2\text{S} + \text{H}]^+ = 469.16927$, found 469.16911.



6i: Prepared according to the general procedure. Yield: 22.3 mg, 38.3 μmol , 19.2 %. ^1H NMR (400 MHz, MeOD) δ 9.46 (bs, 1H, H6), 8.64 (bs, 1H, H1), 8.60 (d, J = 6.0, 1H, H2), 8.50 (d, J = 7.3, 1H, H3), 8.44 (d, J = 8.2, 1H, H5), 7.85 (t, J = 7.8, 1H, H4), 7.76 – 7.72 (m, 1H, H_{Ar}), 7.72 – 7.68 (m, 1H, H_{Ar}), 7.66 (s, 1H, H_{Ar}), 7.64 – 7.61 (m, 2H, H_{Ar}), 7.46 – 7.41 (m, 2H, H_{Ar}), 7.32 (dd, J = 3.0, 6.0, 1H, H_{Ar}), 6.71 (d, J = 15.7, 1H, H11), 6.28 – 6.18 (m, 1H, H10), 3.76 (d, J = 7.2, 2H, H9), 3.14 (m, 4H, H7+H8). ^{13}C NMR (101 MHz, MeOD) δ 153.76, 143.70, 143.20, 140.46, 138.17, 135.61, 135.57, 135.48, 134.90, 134.15, 132.98, 132.18, 131.27, 130.70, 130.11, 129.78, 128.16, 127.83, 121.37, 119.63, 113.54, 50.36, 47.56, 40.08. HRMS: calcd for $[\text{C}_{27}\text{H}_{24}\text{N}_4\text{O}_2\text{S} + \text{H}]^+ = 469.16927$, found 469.16913.



6j: Prepared according to the general procedure. Yield: 14.7 mg, 25.2 μmol , 12.6 %. ^1H NMR (400

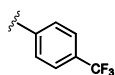
MHz, MeOD) δ 9.46 (bs, 1H, H₆), 8.65 (s, 1H, H₁), 8.58 (d, J = 6.1, 1H, H₂), 8.48 (d, J = 7.3, 1H, H₃), 8.45 (d, J = 8.3, 1H, H₅), 7.84 (M, 2H, H_{Ar}), 7.78 – 7.72 (m, 2H, H_{Ar}), 7.56 (t, J = 7.3, 1H, H₄), 7.53 – 7.38 (m, 4H, H_{Ar}), 7.30 (d, J = 7.4, 1H, H_{Ar}), 6.58 (d, J = 15.8, 1H, H₁₁), 6.28 – 6.19 (m, 1H, H₁₀), 3.72 (d, J = 7.1, 2H, H₉), 3.10 (m, 4H, H₇+H₈). ¹³C NMR (101 *MHz*, MeOD) δ 153.96, 145.57, 144.08, 139.10, 137.37, 135.52, 135.39, 135.31, 134.25, 134.17, 132.88, 132.26, 131.42, 130.32, 129.90, 129.57, 128.05, 127.38, 121.64, 119.44, 118.95, 50.28, 47.48, 40.03. HRMS: calcd for [C₂₇H₂₄N₄O₂S + H]⁺ = 469.16927, found 469.16911.



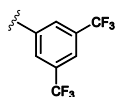
6k: Prepared according to the general procedure. Yield: 22.6 mg, 39.3 μ mol, 19.6 %. ¹H NMR (400 *MHz*, MeOD) δ 9.46 (bs, 1H, H₆), 8.65 (bs, 1H, H₁), 8.59 (d, J = 5.8, 1H, H₂), 8.49 (d, J = 7.2, 1H, H₃), 8.44 (d, J = 8.2, 1H, H₅), 7.85 (t, J = 7.8, 1H, H₄), 7.69 – 7.65 (m, 1H, H_{Ar}), 7.38 (dd, J = 3.6, 5.5, 2H, H_{Ar}), 7.30 (M, 3H, H_{Ar}), 7.15 (t, J = 8.8, 2H, H_{Ar}), 6.77 (d, J = 15.8, 1H, H₁₁), 6.24 – 6.15 (m, 1H, H₁₀), 3.74 (d, J = 7.2, 2H, H₉), 3.13 (m, 4H, H₇+H₈). ¹³C NMR (101 *MHz*, MeOD) δ 164.93, 162.49, 153.87, 143.91, 141.78, 138.82, 137.97, 135.53, 135.43, 134.79, 132.92, 132.65, 132.56, 131.37, 129.88, 129.00, 128.08, 127.53, 120.33, 119.55, 116.24, 116.03, 50.48, 47.54, 40.03. HRMS: calcd for [C₂₆H₂₄FN₃O₂S + H]⁺ = 462.16460, found 462.16445.



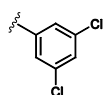
6l: Prepared according to the general procedure. Yield: 18.8 mg, 32.7 μ mol, 16.3 %. ¹H NMR (400 *MHz*, MeOD) δ 9.45 (bs, 1H, H₆), 8.64 (bs, 1H, H₁), 8.58 (d, J = 6.1, 1H, H₂), 8.48 (d, J = 7.4, 1H, H₃), 8.44 (d, J = 8.2, 1H, H₅), 7.84 (t, J = 7.8, 1H, H₄), 7.71 (m, 1H, H_{Ar}), 7.46 – 7.36 (m, 3H, H_{Ar}), 7.25 (m, 3H, H_{Ar}), 7.19 – 7.12 (m, 1H, H_{Ar}), 6.67 (d, J = 15.7, 1H, H₁₁), 6.25 – 6.14 (m, 1H, H₁₀), 3.71 (d, J = 7.2, 2H, H₉), 3.11 – 3.07 (m, 4H, H₇+H₈). ¹³C NMR (101 *MHz*, MeOD) δ 153.93, 144.05, 138.32, 136.63, 135.50, 135.38, 133.14, 133.11, 132.88, 131.88, 131.07, 130.99, 129.74, 129.52, 128.04, 126.94, 125.51, 125.47, 120.48, 119.46, 116.78, 116.56, 50.43, 47.40, 40.01. HRMS: calcd for [C₂₆H₂₄FN₃O₂S + H]⁺ = 462.16460, found 462.16446.



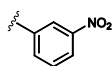
6m: Prepared according to the general procedure. Yield: 34.0 mg, 54.4 μ mol, 27.2 %. ¹H NMR (400 *MHz*, MeOD) δ 9.56 (bs, 1H, H₆), 8.71 (d, J = 6.3, 1H, H₂), 8.68 (bs, 1H, H₁), 8.56 (d, J = 7.4, 1H, H₃), 8.50 (d, J = 8.2, 1H, H₅), 7.91 (t, J = 7.8, 1H, H₄), 7.74 (d, J = 8.2, 2H, H_{Ar}), 7.72 – 7.68 (m, 1H, H_{Ar}), 7.50 (d, J = 8.1, 2H, H_{Ar}), 7.45 – 7.41 (m, 2H, H_{Ar}), 7.33 (dd, J = 3.2, 5.8, 1H, H_{Ar}), 6.75 (d, J = 15.8, 1H, H₁₁), 6.29 – 6.20 (m, 1H, H₁₀), 3.75 (d, J = 7.2, 2H, H₉), 3.14 (m, 4H, H₇+H₈). ¹³C NMR (101 *MHz*, MeOD) δ 152.95, 145.83, 141.86, 141.26, 138.30, 136.34, 135.88, 135.79, 134.77, 133.56, 131.46, 131.25, 130.00, 129.60, 128.73, 127.68, 126.33, 126.29, 121.02, 120.45, 50.43, 47.64, 40.07. HRMS: calcd for [C₂₇H₂₄F₃N₃O₂S + H]⁺ = 512.16141, found 512.16122.



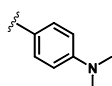
6n: Prepared according to the general procedure. Yield: 32.8 mg, 47.3 μ mol, 23.7 %. ¹H NMR (400 *MHz*, MeOD) δ 9.45 (bs, 1H, H₆), 8.63 (bs, 1H, H₁), 8.59 (d, J = 6.1, 1H, H₂), 8.49 (d, J = 7.3, 1H, H₃), 8.44 (d, J = 8.2, 1H, H₅), 8.00 (s, 1H, H_{Ar}), 7.88 (s, 2H, H_{Ar}), 7.85 (t, J = 7.8, 1H, H₄), 7.75 (m, 1H, H_{Ar}), 7.48 (M, 2H, H_{Ar}), 7.36 (m, 1H, H_{Ar}), 6.68 (d, J = 15.7, 1H, H₁₁), 6.32 – 6.22 (m, 1H, H₁₀), 3.76 (d, J = 7.2, 2H, H₉), 3.12 (m, 4H, H₇+H₈). ¹³C NMR (101 *MHz*, MeOD) δ 153.78, 144.43, 143.74, 139.50, 137.67, 135.55, 135.44, 135.01, 132.97, 132.64, 131.33, 131.14, 130.32, 130.28, 128.09, 128.02, 126.11, 123.41, 122.26, 122.07, 120.71, 119.58, 50.27, 47.50, 40.10. HRMS: calcd for [C₂₈H₂₃F₃N₃O₂S + H]⁺ = 580.14879, found 580.14882.



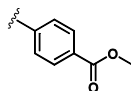
6o: Prepared according to the general procedure. Yield: 31.3 mg, 50.1 μmol , 25.0 %. ^1H NMR (400 MHz, MeOD) δ 9.45 (bs, 1H, H6), 8.64 (bs, 1H, H1), 8.60 (d, J = 6.2, 1H, H2), 8.50 (dd, J = 1.1, 7.4, 1H, H3), 8.44 (d, J = 8.2, 1H, H5), 7.85 (t, J = 7.8, 1H, H4), 7.69 (m, 1H, H_{Ar}), 7.47 – 7.39 (m, 3H, H_{Ar}), 7.30 – 7.26 (m, 1H, H_{Ar}), 7.24 (d, J = 1.9, 2H, H_{Ar}), 6.72 (d, J = 15.8, 1H, H11), 6.26 – 6.17 (m, 1H, H10), 3.78 (d, J = 6.8, 2H, H9), 3.14 (m, 4H, H7+H8). ^{13}C NMR (101 MHz, MeOD) δ 153.73, 145.20, 143.65, 139.90, 138.08, 136.02, 135.60, 135.57, 135.47, 134.82, 133.00, 131.07, 130.07, 129.91, 129.31, 128.39, 128.15, 127.78, 121.38, 119.63, 50.29, 47.47, 40.05. HRMS: calcd for $[\text{C}_{26}\text{H}_{23}\text{Cl}_2\text{N}_3\text{O}_2\text{S} + \text{H}]^+ = 512.09608$, found 512.09600.



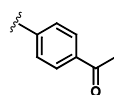
6p: Prepared according to the general procedure. Yield: 30.3 mg, 50.3 μmol , 25.2 %. ^1H NMR (400 MHz, MeOD) δ 9.56 (bs, 1H, H6), 8.73 (bs, 2H, H1+H2), 8.55 (d, J = 7.4, 1H, H3), 8.48 (m, 2H, H5+H_{Ar}), 8.27 – 8.19 (m, 1H), 8.11 (t, J = 1.9, 1H, H4), 7.90 (t, J = 7.8, 2H, H_{Ar}), 7.77 – 7.65 (m, 4H, H_{Ar}), 7.50 – 7.42 (m, 3H, H_{Ar}), 7.39 – 7.32 (m, 1H, H_{Ar}), 6.74 (d, J = 15.8, 1H, H11), 6.28 – 6.19 (m, 1H, H10), 3.76 (d, J = 6.6, 2H, H9), 3.15 (m, 4H, H7+H8). ^{13}C NMR (101 MHz, MeOD) δ 143.41, 140.22, 138.28, 136.95, 136.33, 135.88, 135.00, 131.26, 130.90, 130.76, 130.18, 129.92, 129.61, 128.72, 128.00, 125.37, 123.31, 122.65, 121.56, 50.32, 47.51, 40.13. HRMS: calcd for $[\text{C}_{26}\text{H}_{24}\text{N}_4\text{O}_4\text{S} + \text{H}]^+ = 489.15910$, found 489.15885.



6q: Prepared according to the general procedure. Yield: 30.6 mg, 51.0 μmol , 25.5 %. ^1H NMR (400 MHz, MeOD) δ 9.58 (bs, 1H), 8.74 (d, J = 6.3, 1H, H2), 8.69 (bs, 1H, H1), 8.58 (dd, J = 1.2, 7.4, 1H, H3), 8.52 (d, J = 8.3, 1H, H5), 7.95 – 7.89 (t, J = 7.8, 1H, H4), 7.71 – 7.65 (m, 1H, H_{Ar}), 7.47 – 7.37 (m, 6H, H_{Ar}), 7.29 (dd, J = 3.3, 5.8, 1H, H_{Ar}), 6.79 (d, J = 15.8, 1H, H11), 6.27 – 6.17 (m, 1H, H10), 3.76 (d, J = 7.2, 2H, H9), 3.21 (s, 6H, 2xNCH₃), 3.16 (m, 4H, H7+H8). ^{13}C NMR (101 MHz, MeOD) δ 152.75, 146.04, 141.52, 141.41, 138.76, 136.61, 136.00, 135.85, 134.80, 133.70, 132.45, 131.49, 131.28, 129.93, 129.09, 128.91, 127.70, 120.67, 120.55, 119.01, 50.45, 47.57, 45.05, 40.07. HRMS: calcd for $[\text{C}_{28}\text{H}_{30}\text{N}_4\text{O}_2\text{S} + \text{H}]^+ = 487.21622$, found 487.21600.



6r: Prepared according to the general procedure. Yield: 11.5 mg, 18.7 μmol , 9.3 %. ^1H NMR (400 MHz, MeOD) δ 9.51 (bs, 1H, H6), 8.66 (bs, 1H, H1), 8.64 (d, J = 6.0, 1H, H2), 8.52 (d, J = 7.4, 1H, H3), 8.48 (d, J = 8.2, 1H, H5), 8.08 (d, J = 8.3, 2H, H_{Ar}), 7.88 (t, J = 7.8, 1H, H4), 7.73 – 7.68 (m, 1H, H_{Ar}), 7.46 – 7.40 (m, 4H, H_{Ar}), 7.36 – 7.31 (m, 1H, H_{Ar}), 6.77 (d, J = 15.8, 1H, H11), 6.26 – 6.17 (m, 1H, H10), 3.91 (s, 3H, OCH₃), 3.74 (d, J = 7.2, 2H, H9), 3.12 (m, 4H, H7+H8). ^{13}C NMR (101 MHz, MeOD) δ 168.29, 153.54, 146.77, 143.11, 141.70, 138.53, 135.81, 135.70, 134.72, 133.20, 131.18, 131.04, 130.58, 130.00, 129.52, 128.36, 127.67, 120.78, 119.88, 52.71, 50.43, 47.61, 40.05. HRMS: calcd for $[\text{C}_{28}\text{H}_{27}\text{N}_3\text{O}_4\text{S} + \text{H}]^+ = 502.17950$, found 502.17923.



6s: Prepared according to the general procedure. Yield: 18.1 mg, 30.2 μmol , 15.1 %. ^1H NMR (400 MHz, MeOD) δ 9.53 (bs, 1H, H₆), 8.67 (bs, 2H, H₁+H₂), 8.53 (d, J = 6.7, 1H, H₃), 8.49 (d, J = 8.1, 1H, H₅), 8.06 (d, J = 8.2, 2H, H_{Ar}), 7.89 (t, J = 7.8, 1H, H₄), 7.73 – 7.68 (m, 1H, H_{Ar}), 7.54 (d, J = 8.2, 1H, H_{Ar}), 7.45 (d, J = 8.4, 2H, H_{Ar}), 7.44 – 7.41 (m, 1H, H_{Ar}), 7.36 – 7.29 (m, 1H, H_{Ar}), 6.77 (d, J = 15.8, 1H, H₁₁), 6.27 – 6.17 (m, 1H, H₁₀), 3.75 (d, J = 7.2, 2H, H₉), 3.13 (m, 4H, H₇+H₈), 2.62 (s, 3H, C(O)CH₃). ^{13}C NMR (101 MHz, MeOD) δ 200.08, 153.32, 146.90, 142.64, 141.66, 139.05, 138.55, 137.25, 136.01, 135.78, 134.76, 133.32, 131.45, 131.17, 130.55, 129.98, 129.56, 128.86, 128.51, 127.72, 127.40, 120.83, 120.03, 50.41, 47.60, 40.04, 26.78. HRMS: calcd for [C₂₈H₂₇N₃O₃S + H]⁺ = 486.18459, found 486.18444.

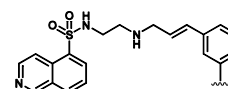


6t: Prepared according to the general procedure. Yield: 27.6 mg, 45.5 μmol , 22.7 %. ^1H NMR (400 MHz, MeOD) δ 9.50 (bs, 1H, H₆), 8.66 (bs, 1H, H₁), 8.58 (d, J = 5.6, 1H, H₂), 8.46 (m, 2H, H₃+5), 7.90 – 7.83 (m, 3H, H₄+H_{Ar}), 7.78 (d, J = 7.5, 1H, H_{Ar}), 7.55 – 7.38 (m, 5H, H_{Ar}), 7.34 – 7.30 (m, 3H, H_{Ar}), 7.30 – 7.25 (m, 1H, H_{Ar}), 6.38 (d, J = 15.8, 1H, H₁₁), 6.18 – 6.08 (m, 1H, H₁₀), 3.49 (qd, J = 7.3, 13.6, 2H, H₉), 2.89 (m, 4H, H₇+H₈). ^{13}C NMR (101 MHz, MeOD) δ 153.65, 143.44, 141.19, 139.51, 138.66, 135.94, 135.62, 135.47, 135.04, 133.51, 133.04, 132.14, 129.75, 129.38, 129.29, 129.10, 128.49, 128.24, 127.33, 127.00, 126.82, 126.45, 120.00, 50.11, 46.99, 39.75. HRMS: calcd for [C₃₀H₂₇N₃O₂S + H]⁺ = 494.18967, found 494.18950.



6u: Prepared according to the general procedure. Yield: 26.8 mg, 44.1 μmol , 22.1%. ^1H NMR (400 MHz, MeOD) δ 9.71 – 9.33 (m, 1H), 8.64 (s, 1H), 8.61 (s, 1H), 8.45 (d, J = 7.4, 1H), 8.42 (d, J = 8.3, 1H), 7.90 (d, J = 8.5, 1H), 7.88 – 7.83 (m, 3H), 7.81 (d, J = 8.0, 1H), 7.74 (s, 1H), 7.72 – 7.69 (m, 1H), 7.51 – 7.44 (m, 3H), 7.41 (dt, J = 3.5, 6.1, 4H), 6.80 (d, J = 15.8, 1H), 6.24 – 6.15 (m, 1H), 3.69 (d, J = 7.1, 2H), 3.07 (s, 4H). ^{13}C NMR (101 MHz, MeOD) δ 153.58, 143.32, 142.88, 139.34, 139.13, 135.66, 135.61, 135.52, 134.91, 134.66, 133.95, 133.03, 131.57, 129.85, 129.50, 129.07, 128.92, 128.69, 128.25, 127.53, 127.30, 120.16, 50.43, 49.64, 49.43, 49.21, 49.00, 48.79, 48.57, 48.36, 47.39, 39.96. HRMS: calcd for [C₃₀H₂₇N₃O₂S + H]⁺ = 494.18967, found 494.18949.

(E)-N-(2-(3-biphenyl-3-yl-allylamino)ethyl)isoquinoline-5-sulfonamides 7a-u



7a: Prepared according to the general procedure. Yield: 19.3 mg, 34.6 μmol , 17.3 %. ^1H NMR (400 MHz, MeOD) δ 9.43 (bs, 1H, H₆), 8.63 (d, J = 5.8, 1H, H₁), 8.59 (d, J = 6.2, 1H, H₂), 8.50 (dd, J = 1.1, 7.4, 1H, H₃), 8.41 (d, J = 8.2, 1H, H₅), 7.83 (dd, J = 7.6, 8.1, 1H, H₄), 7.68 (s, 1H, H_{Ar}), 7.63 – 7.58 (m, 2H, H_{Ar}), 7.58 – 7.54 (m, 1H, H_{Ar}), 7.47 – 7.40 (m, 5H, H_{Ar}), 7.34 (t, J = 7.4, 1H, H_{Ar}), 6.92 (d, J = 15.9, 1H, H₁₁), 6.39 – 6.30 (m, 1H, H₁₀), 3.86 (d, J = 7.2, 2H, H₉), 3.18 (m, 4H, H₇+H₈). ^{13}C NMR (101 MHz, MeOD) δ 153.93, 144.08, 143.13, 141.92, 140.13, 137.38, 135.48, 135.39, 132.87, 130.36, 129.92, 128.64, 128.57, 128.01, 126.72, 126.63, 119.41, 50.50, 47.55, 40.13. HRMS: calcd for [C₂₆H₂₅N₃O₂S + H]⁺ = 444.17402, found 444.17396.



7b: Prepared according to the general procedure. Yield: 22.1 mg, 38.7 μmol , 19.3 %. ^1H NMR (400 MHz, MeOD) δ 9.42 (bs, 1H, H₆), 8.63 (d, J = 5.9, 1H, H₁), 8.59 (d, J = 6.2, 1H, H₂), 8.50 (dd, J = 1.1,

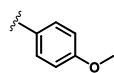
7.4, 1H, H3), 8.40 (d, $J = 8.2$, 1H, H5), 7.85 – 7.80 (t, $J = 7.6$, 1H, H4), 7.65 (s, 1H, H_{Ar}), 7.56 – 7.52 (m, 1H, H_{Ar}), 7.52 – 7.47 (m, 2H, H_{Ar}), 7.41 (m, 2H, H_{Ar}), 7.24 (d, $J = 7.9$, 2H, H_{Ar}), 6.91 (d, $J = 15.8$, 1H, H11), 6.38 – 6.28 (m, 1H, H10), 3.85 (d, $J = 7.1$, 2H, H9), 3.17 (m, 4H, H7+H8), 2.36 (s, 3H, CH₃). ¹³C NMR (101 MHz, MeOD) δ 153.92, 144.06, 143.02, 140.22, 138.98, 138.58, 137.30, 135.48, 135.39, 132.87, 130.55, 130.30, 128.35, 128.01, 127.83, 126.43, 126.40, 119.43, 119.28, 50.51, 47.53, 40.12, 21.11. HRMS: calcd for [C₂₇H₂₇N₃O₂S + H]⁺ = 458.18967, found 458.18964.



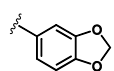
7c: Prepared according to the general procedure. Yield: 11.7 mg, 20.5 μ mol, 10.2 %. ¹H NMR (400 MHz, MeOD) δ 9.41 (s, 1H, H6), 8.64 (d, $J = 6.2$, 1H, H1), 8.56 (d, $J = 6.2$, 1H, H2), 8.49 (dd, $J = 1.2$, 7.4, 1H, H3), 8.41 (d, $J = 8.3$, 1H, H5), 7.82 (dd, $J = 7.5$, 8.2, 1H, H4), 7.67 (s, 1H, H_{Ar}), 7.57 – 7.53 (m, 1H, H_{Ar}), 7.46 – 7.41 (m, 3H, H_{Ar}), 7.39 (d, $J = 8.9$, 1H, H_{Ar}), 7.31 (t, $J = 7.6$, 1H, H_{Ar}), 7.17 (d, $J = 7.5$, 1H, H_{Ar}), 6.93 (d, $J = 15.9$, 1H, H11), 6.38 – 6.29 (m, 1H, H10), 3.86 (d, $J = 7.2$, 2H, H9), 3.19 – 3.16 (m, 4H, H7+H8), 2.40 (s, 3H, CH₃). ¹³C NMR (101 MHz, MeOD) δ 154.15, 144.53, 143.31, 140.22, 137.31, 135.43, 135.21, 132.76, 130.31, 129.83, 129.32, 128.66, 128.62, 127.88, 126.64, 125.15, 119.32, 119.22, 50.51, 47.55, 40.13, 21.55. HRMS: calcd for [C₂₇H₂₇N₃O₂S + H]⁺ = 458.18967, found 458.18962.



7d: Prepared according to the general procedure. Yield: 20.6 mg, 36.1 μ mol, 18.0 %. ¹H NMR (400 MHz, MeOD) δ 9.42 (bs, 1H, H6), 8.63 (bs, 1H, H1), 8.57 (d, $J = 6.2$, 1H, H2), 8.49 (dd, $J = 1.1$, 7.4, 1H, H3), 8.41 (d, $J = 8.3$, 1H, H5), 7.85 – 7.80 (dd, $J = 7.2$, 8.0, 1H, H4), 7.47 – 7.37 (m, 3H, H_{Ar}), 7.30 – 7.13 (m, 6H, H_{Ar}), 6.90 (d, $J = 15.9$, 1H, H11), 6.35 – 6.25 (m, 1H, H10), 3.85 (d, $J = 6.6$, 2H, H9), 3.17 (m, 4H, H7+H8), 2.22 (s, 3H, CH₃). ¹³C NMR (101 MHz, MeOD) δ 154.06, 144.35, 144.04, 142.73, 140.12, 136.78, 136.24, 135.43, 135.28, 132.80, 131.35, 130.74, 130.53, 129.71, 128.67, 128.59, 127.94, 126.90, 126.43, 119.33, 50.49, 47.55, 40.12, 20.51. HRMS: calcd for [C₂₇H₂₇N₃O₂S + H]⁺ = 458.18967, found 458.18967.

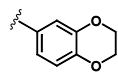


7e: Prepared according to the general procedure. Yield: 20.5 mg, 34.9 μ mol, 17.5 %. ¹H NMR (400 MHz, MeOD) δ 9.41 (s, 1H, H6), 8.64 (d, $J = 6.0$, 1H, H1), 8.56 (d, $J = 6.2$, 1H, H2), 8.49 (dd, $J = 1.1$, 7.4, 1H, H3), 8.40 (d, $J = 8.2$, 1H, H5), 7.85 – 7.79 (dd, $J = 7.6$, 8.1, 1H, H4), 7.64 (s, 1H, H_{Ar}), 7.57 – 7.50 (m, 3H, H_{Ar}), 7.41 – 7.38 (m, 2H, H_{Ar}), 7.02 – 6.97 (m, 2H, H_{Ar}), 6.91 (d, $J = 15.9$, 1H, H11), 6.37 – 6.28 (m, 1H, H10), 3.86 (d, $J = 6.8$, 2H, H9), 3.82 (s, 3H, OCH₃), 3.17 (m, 4H, H7+H8). ¹³C NMR (101 MHz, MeOD) δ 160.99, 154.13, 144.49, 142.77, 140.30, 137.28, 135.42, 135.22, 134.28, 132.75, 130.67, 130.29, 129.04, 128.15, 127.89, 126.18, 126.07, 119.19, 115.34, 55.77, 50.52, 47.53, 40.13. HRMS: calcd for [C₂₇H₂₇N₃O₃S + H]⁺ = 474.18459, found 474.18440.

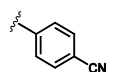


7f: Prepared according to the general procedure. Yield: 22.9 mg, 38.1 μ mol, 19.0 %. ¹H NMR (400 MHz, MeOD) δ 9.41 (bs, 1H, H6), 8.63 (d, $J = 6.1$, 1H, H1), 8.57 (d, $J = 6.2$, 1H, H2), 8.49 (dd, $J = 1.1$, 7.4, 1H, H3), 8.40 (d, $J = 8.2$, 1H, H5), 7.86 – 7.79 (t, $J = 7.6$, 8.1, 1H, H4), 7.60 (s, 1H, H_{Ar}), 7.51 – 7.47 (m, 1H, H_{Ar}), 7.42 – 7.37 (m, 2H, H_{Ar}), 7.09 (s, 1H, H_{Ar}), 7.08 (dd, $J = 1.7$, 7.2, 2H, H_{Ar}), 6.90 – 6.86 (m, 2H, H_{Ar}+H11), 6.37 – 6.27 (m, 1H, H10), 5.97 (s, 2H, OCH₂O), 3.85 (d, $J = 7.1$, 2H, H9), 3.17 (s, 4H, H7+H8). ¹³C NMR (101 MHz, MeOD) δ 154.03, 149.73, 148.82, 144.30, 142.82, 140.16, 137.30, 136.20, 135.44, 135.29, 132.80, 130.65,

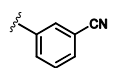
130.29, 128.32, 127.94, 126.36, 121.62, 119.33, 109.52, 108.32, 102.58, 50.49, 47.53, 40.12. HRMS: calcd for $[C_{27}H_{25}N_3O_4S + H]^+ = 488.16385$, found 488.16376.



7g: Prepared according to the general procedure. Yield: 12.6 mg, 20.5 μ mol, 10.2 %. ^1H NMR (400 MHz, MeOD) δ 9.42 (bs, 1H, H6), 8.64 (bs, 1H, H1), 8.56 (d, $J = 6.0$, 1H, H2), 8.49 (dd, $J = 1.0$, 7.4, 1H, H3), 8.41 (d, $J = 8.2$, 1H, H5), 7.85 – 7.79 (t, $J = 7.8$, 1H, H4), 7.61 (s, 1H, H_{Ar}), 7.52 – 7.47 (m, 1H, H_{Ar}), 7.41 – 7.37 (m, 2H, H_{Ar}), 7.09 (s, 1H, H_{Ar}), 7.07 (d, $J = 2.2$, 1H, H_{Ar}), 6.90 – 6.88 (m, 2H, $H_{Ar}+H11$), 6.36 – 6.27 (m, 1H, H10), 4.26 (m, 4H, OCH_2CH_2O), 3.85 (d, $J = 7.0$, 2H, H9), 3.17 (m, 4H, H7+H8). ^{13}C NMR (101 MHz, MeOD) δ 154.15, 145.31, 144.98, 144.53, 142.57, 140.27, 137.26, 135.43, 135.20, 132.74, 130.28, 128.18, 127.88, 126.27, 126.18, 120.88, 119.22, 118.61, 116.61, 65.73, 65.70, 50.51, 47.53, 40.13. HRMS: calcd for $[C_{28}H_{27}N_3O_4S + H]^+ = 502.17950$, found 502.17922.



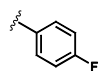
7h: Prepared according to the general procedure. Yield: 8.9 mg, 15.3 μ mol, 7.6 %. ^1H NMR (400 MHz, MeOD) δ 9.41 (bs, 1H, H6), 8.64 (d, $J = 6.0$, 1H, H1), 8.55 (d, $J = 6.2$, 1H, H2), 8.49 (dd, $J = 1.1$, 7.4, 1H, H3), 8.42 (d, $J = 8.3$, 1H, H5), 7.86 – 7.79 (m, 6H, $H_{Ar}+H4$), 7.75 (s, 1H, H_{Ar}), 7.64 (d, $J = 7.5$, 1H, H_{Ar}), 7.52 (m, 2H, H_{Ar}), 6.95 (d, $J = 15.9$, 1H, H11), 6.43 – 6.33 (m, 1H, H10), 3.88 (d, $J = 7.1$, 2H, H9), 3.22 – 3.14 (m, 4H, H7+H8). ^{13}C NMR (101 MHz, MeOD) δ 154.25, 146.53, 144.73, 141.11, 139.67, 137.80, 135.40, 135.13, 133.84, 132.70, 130.73, 128.95, 128.73, 127.99, 127.83, 126.86, 120.06, 119.69, 119.13, 112.26, 50.44, 47.62, 40.13. HRMS: calcd for $[C_{27}H_{24}N_4O_2S + H]^+ = 469.16927$, found 469.16910.



7i: Prepared according to the general procedure. Yield: 12.8 mg, 22.0 μ mol, 11.0 %. ^1H NMR (400 MHz, MeOD) δ 9.42 (bs, 1H, H6), 8.64 (d, $J = 6.0$, 1H, H1), 8.57 (d, $J = 6.2$, 1H, H2), 8.50 (dd, $J = 1.1$, 7.4, 1H, H3), 8.42 (d, $J = 8.3$, 1H, H5), 7.99 (d, $J = 1.5$, 1H, H_{Ar}), 7.97 – 7.92 (m, 1H, H_{Ar}), 7.87 – 7.81 (t, $J = 7.8$, 1H, H4), 7.75 – 7.70 (m, 2H, H_{Ar}), 7.64 (d, $J = 7.8$, 1H, H_{Ar}), 7.62 (dd, $J = 2.6$, 4.8, 1H, H_{Ar}), 7.56 – 7.46 (m, 2H, H_{Ar}), 6.95 (d, $J = 15.9$, 1H, H11), 6.43 – 6.33 (m, 1H, H10), 3.88 (d, $J = 6.9$, 2H, H9), 3.22 – 3.15 (m, 4H, H7+H8). ^{13}C NMR (101 MHz, MeOD) δ 154.10, 144.42, 143.28, 140.81, 139.70, 137.80, 135.45, 135.27, 132.79, 132.72, 132.19, 131.59, 131.14, 130.73, 128.60, 127.93, 127.76, 126.70, 120.02, 119.65, 119.28, 114.10, 50.44, 47.60, 40.14. HRMS: calcd for $[C_{27}H_{24}N_4O_2S + H]^+ = 469.16927$, found 469.16909.



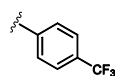
7j: Prepared according to the general procedure. Yield: 11. mg, 20.4 μ mol, 10.2 %. ^1H NMR (400 MHz, MeOD) δ 9.40 (bs, 1H, H6), 8.63 (bs, 1H, H1), 8.55 (d, $J = 6.1$, 1H, H2), 8.49 (d, $J = 7.4$, 1H, H3), 8.41 (d, $J = 8.2$, 1H, H5), 7.82 (t, $J = 7.8$, 1H, H4), 7.68 – 7.60 (m, 3H, H_{Ar}), 7.54 (dt, $J = 1.9$, 6.6, 1H, H_{Ar}), 7.48 – 7.41 (m, 2H, H_{Ar}), 7.21 – 7.14 (m, 2H, H_{Ar}), 6.93 (d, $J = 15.9$, 1H, H11), 6.39 – 6.30 (m, 1H, H10), 3.86 (d, $J = 7.1$, 2H, H9), 3.21 – 3.15 (m, 4H, H7+H8). ^{13}C NMR (101 MHz, MeOD) δ 154.25, 144.75, 142.10, 140.05, 137.46, 135.39, 135.12, 132.69, 130.44, 129.91, 129.83, 128.50, 127.82, 126.74, 126.56, 119.53, 119.13, 116.72, 116.50, 50.49, 49.64, 49.43, 49.21, 49.00, 48.79, 48.58, 48.36, 47.58, 40.13. HRMS: calcd for $[C_{27}H_{24}N_4O_2S + H]^+ = 469.16927$, found 469.16910.



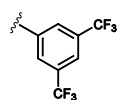
7k: Prepared according to the general procedure. Yield: 7.0 mg, 12.2 μmol , 6.1 %. ^1H NMR (400 MHz, MeOD) δ 9.42 (bs, 1H, H6), 8.64 (bs, 1H, H1), 8.55 (d, J = 6.1, 1H, H2), 8.49 (d, J = 6.4, 1H, H2), 8.42 (d, J = 8.2, 1H, H3), 7.87 – 7.80 (m, 2H, H_{Ar}), 7.75 (dt, J = 1.2, 7.7, 1H, H4), 7.69 (s, 1H, H_{Ar}), 7.60 (d, J = 7.9, 1H, H_{Ar}), 7.56 (dd, J = 1.2, 7.7, 2H, H_{Ar}), 7.54 – 7.50 (m, 2H, H_{Ar}), 6.95 (d, J = 15.8, 1H, H11), 6.41 – 6.31 (m, 1H, H10), 3.87 (d, J = 7.2, 2H, H9), 3.19 – 3.13 (m, 4H, H7+H8). ^{13}C NMR (101 MHz, MeOD) δ 154.27, 146.26, 144.76, 140.34, 139.59, 137.34, 135.41, 135.13, 134.88, 134.46, 132.69, 131.22, 130.38, 130.31, 129.34, 128.78, 128.35, 128.19, 127.84, 120.21, 119.51, 119.13, 112.19, 50.43, 47.61, 40.12. HRMS: calcd for $[\text{C}_{26}\text{H}_{24}\text{FN}_3\text{O}_2\text{S} + \text{H}]^+ = 462.16460$, found 462.16446.



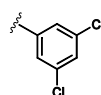
7l: Prepared according to the general procedure. Yield: 15.6 mg, 27.1 μmol , 13.6 %. ^1H NMR (400 MHz, MeOD) δ 9.40 (bs, 1H, H6), 8.64 (d, J = 5.9, 1H, H1), 8.55 (d, J = 6.2, 1H, H2), 8.49 (dd, J = 1.0, 7.4, 1H, H3), 8.40 (d, J = 8.2, 1H, H5), 7.85 – 7.79 (t, J = 7.8, 1H, H4), 7.62 (s, 1H, H_{Ar}), 7.52 – 7.42 (m, 5H, H_{Ar}), 7.41 – 7.34 (m, 1H, H_{Ar}), 7.25 (td, J = 1.1, 7.5, 1H, H_{Ar}), 7.22 – 7.15 (m, 1H, H_{Ar}), 6.92 (d, J = 15.8, 1H, H11), 6.37 – 6.28 (m, 1H, H10), 3.86 (d, J = 6.9, 2H, H9), 3.17 (m, 4H, H7+H8). ^{13}C NMR (101 MHz, MeOD) δ 154.20, 144.65, 139.94, 137.79, 137.08, 135.39, 135.15, 132.70, 131.85, 130.68, 130.59, 130.54, 130.04, 128.60, 127.84, 127.16, 125.74, 119.57, 119.17, 117.14, 116.91, 50.47, 47.56, 40.12. HRMS: calcd for $[\text{C}_{26}\text{H}_{24}\text{FN}_3\text{O}_2\text{S} + \text{H}]^+ = 462.16460$, found 462.16447.



7m: Prepared according to the general procedure. Yield: 37.9 mg, 60.6 μmol , 30.3 %. ^1H NMR (400 MHz, MeOD) δ 9.39 (bs, 1H, H6), 8.62 (d, J = 6.2, 1H, H1), 8.56 (d, J = 6.2, 1H, H2), 8.49 (dd, J = 1.1, 7.4, 1H, H3), 8.39 (d, J = 8.3, 1H, H5), 7.84 – 7.78 (m, 3H, H, H_{Ar}+H4), 7.76 – 7.71 (m, 3H, H_{Ar}), 7.64 – 7.58 (m, 1H, H_{Ar}), 7.54 – 7.44 (m, 2H, H_{Ar}), 6.93 (d, J = 15.9, 1H, H11), 6.42 – 6.32 (m, 1H, H10), 3.87 (d, J = 6.9, 2H, H9), 3.21 – 3.16 (m, 4H, H7+H8). ^{13}C NMR (101 MHz, MeOD) δ 154.07, 145.71, 144.42, 141.43, 139.72, 137.67, 135.39, 135.23, 132.74, 130.72, 130.59, 130.40, 128.69, 128.62, 127.88, 127.65, 127.12, 126.84, 126.77, 124.43, 121.74, 119.93, 119.26, 50.44, 47.58, 40.12. HRMS: calcd for $[\text{C}_{27}\text{H}_{24}\text{F}_3\text{N}_3\text{O}_2\text{S} + \text{H}]^+ = 512.16141$, found 512.16121.

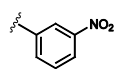


7n: Prepared according to the general procedure. Yield: 18.2 mg, 26.3 μmol , 13.1 %. ^1H NMR (400 MHz, MeOD) δ 9.41 (s, 1H, H6), 8.64 (d, J = 6.1, 1H, H1), 8.55 (d, J = 6.2, 1H, H2), 8.49 (dd, J = 1.1, 7.4, 1H, H3), 8.41 (d, J = 8.3, 1H, H5), 8.18 (s, 2H, H_{Ar}), 7.97 (s, 1H, H_{Ar}), 7.85 – 7.77 (m, 2H, H_{Ar}+H4), 7.68 (d, J = 7.6, 1H, H_{Ar}), 7.62 – 7.51 (m, 2H, H_{Ar}), 6.97 (d, J = 15.9, 1H, H11), 6.46 – 6.36 (m, 1H, H10), 3.88 (d, J = 7.1, 2H, H9), 3.24 – 3.13 (m, 4H, H7+H8). ^{13}C NMR (101 MHz, MeOD) δ 154.21, 144.69, 144.58, 139.94, 139.44, 138.05, 135.39, 135.16, 133.54, 133.21, 132.70, 130.95, 130.70, 128.80, 128.47, 128.29, 127.83, 126.94, 126.20, 123.49, 122.10, 120.79, 120.39, 119.16, 50.40, 47.63, 40.14. HRMS: calcd for $[\text{C}_{28}\text{H}_{23}\text{F}_3\text{N}_3\text{O}_2\text{S} + \text{H}]^+ = 580.14879$, found 580.14870.

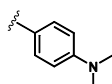


7o: Prepared according to the general procedure. Yield: 17.5 mg, 28.0 μmol , 14.0 %. ^1H NMR (400 MHz, MeOD) δ 9.40 (s, 1H, H6), 8.63 (s, 1H, H1), 8.55 (d, J = 6.2, 1H, H2), 8.49 (dd, J = 1.1, 7.4, 1H,

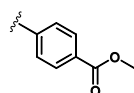
H3), 8.40 (d, $J = 8.3$, 1H, H5), 7.85 – 7.79 (dd, $J = 7.5$, 8.1, 1H, H4), 7.68 (s, 1H, H_{Ar}), 7.60 – 7.44 (m, 5H, H_{Ar}), 7.42 (t, $J = 1.8$, 1H, H_{Ar}), 6.93 (d, $J = 15.9$, 1H, H11), 6.42 – 6.32 (m, 1H, H10), 3.87 (d, $J = 6.8$, 2H, H9), 3.23 – 3.14 (m, 4H, H7+H8). ¹³C NMR (101 MHz, MeOD) δ 154.21, 145.31, 144.70, 140.22, 139.60, 137.79, 136.61, 135.38, 135.13, 132.69, 130.71, 128.55, 128.31, 127.98, 127.82, 126.67, 120.10, 119.16, 50.41, 47.59, 40.13. HRMS: calcd for [C₂₆H₂₃Cl₂N₃O₂S + H]⁺ = 512.09608, found 512.09591.



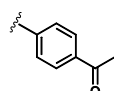
7p: Prepared according to the general procedure. Yield: 26.8 mg, 44.5 μ mol, 22.3 %. ¹H NMR (400 MHz, MeOD) δ 9.40 (s, 1H, H6), 8.63 (d, $J = 5.9$, 1H, H1), 8.56 (d, $J = 6.2$, 1H, H2), 8.49 (dd, $J = 1.1$, 7.4, 1H, H3), 8.45 – 8.35 (m, 3H, H_{Ar}), 8.23 – 8.17 (m, 1H, H_{Ar}), 8.05 – 8.00 (m, 1H, H_{Ar}), 7.86 – 7.79 (dd, $J = 7.5$, 8.1, 1H, H4), 7.75 (s, 1H, H_{Ar}), 7.73 – 7.61 (m, 3H, H_{Ar}), 7.59 – 7.46 (m, 3H, H_{Ar}), 6.95 (d, $J = 15.9$, 1H, H11), 6.44 – 6.34 (m, 1H, H10), 3.88 (d, $J = 7.2$, 2H, H9), 3.23 – 3.17 (m, 4H, H7+H8). ¹³C NMR (101 MHz, MeOD) δ 154.04, 150.16, 144.35, 143.59, 140.61, 139.60, 137.85, 136.98, 135.42, 135.38, 135.27, 134.28, 134.19, 132.77, 131.25, 130.76, 129.79, 128.61, 128.51, 127.92, 127.87, 126.73, 123.27, 122.65, 122.54, 122.32, 120.12, 119.29, 50.42, 47.60, 40.13. HRMS: calcd for [C₂₆H₂₄N₄O₄S + H]⁺ = 489.15910, found 489.15883.



7q: Prepared according to the general procedure. Yield: 14.6 mg, 24.3 μ mol, 12.2 %. ¹H NMR (400 MHz, MeOD) δ 9.44 (s, 1H, H6), 8.64 (d, $J = 6.0$, 1H, H1), 8.60 (d, $J = 6.3$, 1H, H2), 8.51 (dd, $J = 1.1$, 7.4, 1H, H3), 8.42 (d, $J = 8.2$, 1H, H5), 7.87 – 7.81 (m, 1H, H4), 7.69 – 7.61 (m, 3H, H_{Ar}), 7.55 (dt, $J = 1.8$, 4.5, 1H, H_{Ar}), 7.42 (d, $J = 4.9$, 2H, H_{Ar}), 7.20 (d, $J = 8.8$, 2H, H_{Ar}), 6.91 (d, $J = 15.9$, 1H, H11), 6.39 – 6.29 (m, 1H, H10), 3.86 (d, $J = 6.8$, 2H, H9), 3.18 (m, 4H, H7+H8), 3.11 (s, 6H, 2xNCH₃). ¹³C NMR (101 MHz, MeOD) δ 153.83, 148.48, 143.82, 140.19, 137.41, 135.54, 132.96, 130.64, 130.39, 129.12, 128.11, 128.03, 126.30, 126.11, 119.55, 119.36, 117.34, 50.51, 47.55, 43.34, 40.13. HRMS: calcd for [C₂₈H₃₀N₄O₂S + H]⁺ = 487.21622, found 487.21605.



7r: Prepared according to the general procedure. Yield: 40.7 mg, 66.2 μ mol, 33.1 %. ¹H NMR (400 MHz, MeOD) δ 9.54 (bs, 1H, H6), 8.71 (d, $J = 6.1$, 1H, H2), 8.67 (bs, 1H, H1), 8.59 – 8.55 (dd, $J = 1.0$, 7.4, 1H, H3), 8.48 (d, $J = 8.2$, 1H, H5), 8.10 – 8.02 (m, 2H, H_{Ar}+H4), 7.92 – 7.86 (m, 1H, H_{Ar}), 7.76 – 7.70 (m, 4H, H_{Ar}), 7.61 (d, $J = 7.4$, 1H, H_{Ar}), 7.53 – 7.43 (m, 2H, H_{Ar}), 6.93 (d, $J = 15.9$, 1H, H11), 6.42 – 6.32 (m, 1H, H10), 3.91 (s, 3H, OCH₃), 3.87 (d, $J = 7.1$, 2H, H9), 3.20 (m, 4H, H7+H8). ¹³C NMR (101 MHz, MeOD) δ 168.33, 152.99, 146.49, 141.99, 141.71, 139.78, 137.63, 136.30, 135.84, 133.50, 131.12, 130.56, 130.34, 128.68, 128.11, 127.62, 126.80, 120.39, 119.86, 52.66, 50.46, 47.59, 40.16. HRMS: calcd for [C₂₈H₂₇N₃O₄S + H]⁺ = 502.17950, found 502.17926.

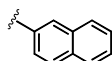


7s: Prepared according to the general procedure. Yield: 8.3 mg, 13.9 μ mol, 6.9 %. ¹H NMR (400 MHz, MeOD) δ 9.56 – 9.41 (bs, 1H, H6), 8.66 – 8.64 (m, 2H, H1+H2), 8.54 (d, $J = 7.4$, 1H, H3), 8.46 (d, $J = 8.1$, 1H, H5), 8.08 (m, H_{Ar}+H4), 7.91 – 7.84 (m, 1H, H_{Ar}), 7.78 (d, $J = 8.4$, 3H, H_{Ar}), 7.69 – 7.60 (m, 2H, H_{Ar}), 7.58 – 7.43 (m, 3H, H_{Ar}), 6.96 (d, $J = 15.9$, 1H, H11), 6.38 (d, $J = 15.8$, 1H, H10), 3.88 (d, $J = 7.0$, 2H, H9), 3.23 – 3.14 (m, 4H, H7+H8), 2.63 (s, 3H, C(O)CH₃). ¹³C NMR (101 MHz, MeOD) δ 153.68, 146.64, 143.44, 141.77, 139.87, 137.65, 137.37, 135.70, 135.65, 133.10, 130.62, 130.41, 130.17, 128.76, 128.59, 128.26, 127.97,

127.72, 127.66, 126.84, 119.83, 50.48, 47.62, 40.16, 26.74. HRMS: calcd for $[C_{28}H_{27}N_3O_3S + H]^+ = 486.18459$, found 486.18442.

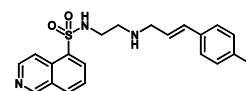


7t: Prepared according to the general procedure. Yield: 54.1 mg, 89.1 μ mol, 44.5 %. ^1H NMR (400 MHz, MeOD) δ 9.40 (s, 1H, H6), 8.62 (d, $J = 6.2$, 1H, H1), 8.58 (d, $J = 6.2$, 1H, H2), 8.48 (d, $J = 7.3$, 1H, H3), 8.37 (d, $J = 8.2$, 1H, H5), 7.89 (d, $J = 8.1$, 1H), 7.86 (d, $J = 8.3$, 1H), 7.80 (t, $J = 7.8$, 1H, H4), 7.78 – 7.73 (m, 1H, H_{Ar}), 7.57 – 7.43 (m, 6H, H_{Ar}), 7.41 – 7.35 (m, 3H, H_{Ar}), 6.91 (d, $J = 15.8$, 1H, H11), 6.36 – 6.26 (m, 1H, H10), 3.84 (d, $J = 7.2$, 2H, H9), 3.16 (m, 4H, H7+H8). ^{13}C NMR (101 MHz, MeOD) δ 153.76, 143.79, 142.67, 140.88, 139.95, 136.96, 135.46, 135.32, 132.90, 132.76, 131.52, 130.57, 129.88, 129.47, 129.42, 128.94, 128.05, 127.87, 127.17, 126.89, 126.52, 126.40, 119.56, 50.45, 47.52, 40.10. HRMS: calcd for $[C_{30}H_{27}N_3O_2S + H]^+ = 494.18967$, found 494.18951.



7u: Prepared according to the general procedure. Yield: 35.7 mg, 58.8 μ mol, 29.4 %. ^1H NMR (400 MHz, MeOD) δ 9.42 (s, 1H, H6), 8.66 – 8.58 (m, 2H, H1+H2), 8.50 (d, $J = 7.3$, 1H, H3), 8.39 (d, $J = 8.2$, 1H, H5), 8.07 (s, 1H, H_{Ar}), 7.91 (t, $J = 7.5$, 2H, H_{Ar}), 7.83 (m, 3H, H_{Ar}+H4), 7.75 (dd, $J = 1.6$, 8.5, 1H, H_{Ar}), 7.71 – 7.67 (m, 1H, H_{Ar}), 7.52 – 7.42 (m, 4H, H_{Ar}), 6.94 (d, $J = 15.8$, 1H, H11), 6.42 – 6.32 (m, 1H, H10), 3.86 (d, $J = 7.2$, 2H, H9), 3.18 (m, 4H, H7+H8). ^{13}C NMR (101 MHz, MeOD) δ 153.69, 143.60, 142.89, 140.10, 139.15, 137.48, 135.57, 135.53, 135.14, 134.23, 132.98, 130.59, 130.45, 129.60, 129.24, 128.82, 128.63, 128.13, 127.43, 127.16, 126.89, 126.83, 126.69, 126.22, 119.63, 119.50, 50.50, 47.54, 40.13. HRMS: calcd for $[C_{30}H_{27}N_3O_2S + H]^+ = 494.18967$, found 494.18948.

(E)-N-(2-(3-biphenyl-4-yl-allylamino)ethyl)isoquinoline-5-sulfonamides 8a-u



8a: Prepared according to the general procedure. Yield: 14.9 mg, 26.7 μ mol, 13.4 %. ^1H NMR (400 MHz, MeOD) δ 9.59 (bs, 1H, H6), 8.73 (bs, 2H, H1+H2), 8.59 (d, $J = 7.4$, 1H, H3), 8.51 (d, $J = 8.1$, 1H, H5), 7.94 – 7.89 (t, $J = 7.8$, 1H, H4), 7.65 – 7.59 (m, 4H, H_{Ar}), 7.54 (d, $J = 8.3$, 2H, H_{Ar}), 7.43 (t, $J = 7.6$, 2H, H_{Ar}), 7.33 (t, $J = 7.4$, 1H, H_{Ar}), 6.89 (d, $J = 15.8$, 1H, H11), 6.36 – 6.26 (m, 1H, H10), 3.86 (d, $J = 7.2$, 2H, H9), 3.20 (m, 4H, H7+H8). ^{13}C NMR (101 MHz, MeOD) δ 152.98, 142.96, 141.94, 141.59, 139.82, 136.35, 135.90, 135.81, 133.57, 129.93, 128.76, 128.66, 128.47, 128.32, 127.83, 118.94, 50.59, 47.59, 40.17. HRMS: calcd for $[C_{26}H_{25}N_3O_2S + H]^+ = 444.17402$, found 444.17397.



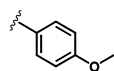
8b: Prepared according to the general procedure. Yield: 17.6 mg, 30.8 μ mol, 15.4 %. ^1H NMR (400 MHz, MeOD) δ 9.56 (bs, 1H, H6), 8.72 (d, $J = 5.9$, 1H, H2), 8.71 – 8.64 (bs, 1H, H1), 8.58 (d, $J = 6.6$, 1H, H3), 8.50 (d, $J = 8.2$, 1H, H5), 7.94 – 7.88 (t, $J = 7.8$, 1H, H4), 7.60 (d, $J = 8.3$, 2H, H_{Ar}), 7.55 – 7.48 (m, 4H, H_{Ar}), 7.24 (d, $J = 8.1$, 2H, H_{Ar}), 6.88 (d, $J = 15.8$, 1H, H11), 6.35 – 6.24 (m, 1H, H10), 3.85 (d, $J = 7.2$, 2H, H9), 3.19 (m, 4H, H7+H8), 2.36 (s, 3H, CH₃). ^{13}C NMR (101 MHz, MeOD) δ 153.02, 142.89, 142.02, 139.89, 138.65, 136.30, 135.87, 135.49, 133.54, 130.57, 129.83, 128.71, 128.43, 128.04, 127.66, 120.42, 118.70, 50.61, 47.57, 40.16, 21.10. HRMS: calcd for $[C_{27}H_{27}N_3O_2S + H]^+ = 458.18967$, found 458.18964.



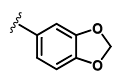
8c: Prepared according to the general procedure. Yield: 20.2 mg, 35.4 μmol , 17.7 %. ^1H NMR (400 MHz, MeOD) δ 9.57 (bs, 1H, H6), 8.74 (d, J = 5.7, 1H, H2), 8.72 – 8.63 (bs, 1H, H1), 8.59 (d, J = 7.4, 1H, H3), 8.50 (d, J = 8.3, 1H, H5), 7.91 (t, J = 7.8, 1H, H4), 7.60 (d, J = 8.2, 2H, H_{Ar}), 7.52 (d, J = 8.3, 2H, H_{Ar}), 7.43 (s, 1H, H_{Ar}), 7.39 (d, J = 8.0, 1H, H_{Ar}), 7.30 (t, J = 7.6, 1H, H_{Ar}), 7.15 (d, J = 7.5, 1H, H_{Ar}), 6.88 (d, J = 15.8, 1H, H11), 6.35 – 6.25 (m, 1H, H10), 3.86 (d, J = 7.2, 2H, H9), 3.20 (m, 4H, H7+H8), 2.39 (s, 3H, CH_3). ^{13}C NMR (101 MHz, MeOD) δ 152.92, 143.10, 141.83, 141.57, 139.85, 139.66, 136.38, 135.91, 135.70, 133.59, 129.82, 129.34, 128.77, 128.47, 128.41, 128.30, 124.97, 118.85, 50.59, 47.57, 40.16, 21.53. HRMS: calcd for $[\text{C}_{27}\text{H}_{27}\text{N}_3\text{O}_2\text{S} + \text{H}]^+$ = 458.18967, found 458.18966.



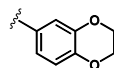
8d: Prepared according to the general procedure. Yield: 19.7 mg, 34.5 μmol , 17.2 %. ^1H NMR (400 MHz, MeOD) δ 9.59 (bs, 1H, H6), 8.75 (d, J = 6.0, 1H, H2), 8.70 (bs, 1H, H1), 8.60 (d, J = 7.4, 1H, H3), 8.52 (d, J = 8.2, 1H, H5), 7.93 (t, J = 7.8, 1H, H4), 7.52 (d, J = 8.1, 2H, H_{Ar}), 7.30 (d, J = 8.0, 2H, H_{Ar}), 7.28 – 7.13 (m, 5H, H_{Ar}), 6.91 (d, J = 15.8, 1H, H11), 6.36 – 6.26 (m, 1H, H10), 3.87 (d, J = 7.2, 2H, H9), 3.20 (m, 4H, H7+H8), 2.23 (s, 3H, CH_3). ^{13}C NMR (101 MHz, MeOD) δ 152.90, 144.13, 142.57, 141.76, 139.94, 136.43, 136.22, 135.93, 135.42, 133.64, 131.40, 130.68, 130.49, 128.81, 128.56, 127.77, 126.91, 118.94, 50.58, 47.58, 40.17, 20.53. HRMS: calcd for $[\text{C}_{27}\text{H}_{27}\text{N}_3\text{O}_2\text{S} + \text{H}]^+$ = 458.18967, found 458.18967.



8e: Prepared according to the general procedure. Yield: 14.9 mg, 25.4 μmol , 12.7 %. ^1H NMR (400 MHz, MeOD) δ 9.63 (bs, 1H, H6), 8.77 (d, J = 5.4, 1H, H2), 8.72 (bs, 1H, H1), 8.61 (d, J = 7.4, 1H, H3), 8.53 (d, J = 8.3, 1H, H5), 7.94 (t, J = 7.8, 1H, H4), 7.60 – 7.54 (m, 4H, H_{Ar}), 7.50 (d, J = 8.3, 2H, H_{Ar}), 6.99 (d, J = 8.6, 2H, H_{Ar}), 6.88 (d, J = 15.8, 1H, H11), 6.33 – 6.23 (m, 1H, H10), 3.85 (d, J = 7.2, 2H, H9), 3.82 (s, 3H, OCH_3), 3.20 (m, 4H, H7+H8). ^{13}C NMR (101 MHz, MeOD) δ 161.05, 152.66, 142.64, 141.19, 139.96, 136.67, 136.04, 135.11, 133.97, 133.80, 129.83, 129.00, 128.88, 128.43, 127.78, 118.48, 115.38, 55.78, 50.64, 47.56, 40.18. HRMS: calcd for $[\text{C}_{27}\text{H}_{27}\text{N}_3\text{O}_3\text{S} + \text{H}]^+$ = 474.18459, found 474.18453.

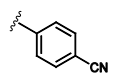


8f: Prepared according to the general procedure. Yield: 10.9 mg, 18.1 μmol , 9.1 %. ^1H NMR (400 MHz, MeOD) δ 9.48 (bs, 1H), 8.67 (bs, 1H, H1), 8.62 (bs, 1H, H2), 8.52 (d, J = 7.4, 1H, H3), 8.45 (d, J = 8.1, 1H, H5), 7.86 (t, J = 7.8, 1H, H4), 7.67 – 7.59 (m, 5H, H_{Ar}), 7.57 – 7.47 (m, 7H, H_{Ar}), 7.13 – 7.10 (m, 2H, H_{Ar}), 6.92 – 6.84 (m, 2H, H_{Ar} +H11), 6.33 – 6.24 (m, 1H, H10), 5.97 (s, 1H, OCH_2O), 3.85 (d, J = 7.2, 2H, H9), 3.17 (m, 4H, H7+H8). ^{13}C NMR (101 MHz, MeOD) δ 153.15, 149.78, 148.89, 142.72, 142.27, 139.86, 136.19, 135.92, 135.85, 135.39, 133.77, 133.74, 133.45, 133.12, 133.02, 130.02, 129.90, 128.78, 128.64, 128.42, 128.03, 121.53, 120.33, 118.67, 115.10, 109.56, 108.12, 102.59, 50.61, 47.58, 40.16. HRMS: calcd for $[\text{C}_{27}\text{H}_{25}\text{N}_3\text{O}_4\text{S} + \text{H}]^+$ = 488.16385, found 488.16370.

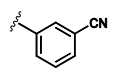


8g: Prepared according to the general procedure. Yield: 11.3 mg, 18.4 μmol , 9.2 %. ^1H NMR (400 MHz, MeOD) δ 9.55 (bs, 1H, H6), 8.68 (bs, 2H, H1+H2), 8.55 (d, J = 7.3, 1H, H3), 8.48 (d, J = 8.2, 1H, H5), 7.89 (t, J = 7.8, 1H, H4), 7.55 (d, J = 8.4, 2H, H_{Ar}), 7.49 (d, J = 8.3, 2H, H_{Ar}), 6.89 – 6.86 (m, 2H, H_{Ar} +H11) 6.32

– 6.23 (m, 1H, H10), 4.25 (d, $J = 2.6$, 4H, $\text{OCH}_2\text{CH}_2\text{O}$), 3.85 (d, $J = 7.2$, 2H, H9), 3.18 (m, 4H, H7+H8). ^{13}C NMR (101 MHz, MeOD) δ 153.43, 145.33, 145.04, 142.94, 142.43, 139.91, 135.89, 135.73, 135.28, 134.91, 133.23, 128.77, 128.40, 127.82, 120.75, 118.62, 118.57, 116.42, 115.12, 65.75, 65.69, 50.61, 47.56, 40.15. HRMS: calcd for $[\text{C}_{28}\text{H}_{27}\text{N}_3\text{O}_4\text{S} + \text{H}]^+ = 502.17950$, found 502.17928.



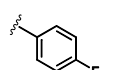
8h: Prepared according to the general procedure. Yield: 32.3 mg, 55.5 μmol , 27.7 %. ^1H NMR (400 MHz, MeOD) δ 9.59 (bs, 1H, H6), 8.75 (d, $J = 5.8$, 1H, H2), 8.70 (bs, 1H, H1), 8.60 (d, $J = 6.8$, 1H, H3), 8.51 (d, $J = 8.2$, 1H, H5), 7.92 (t, $J = 7.8$, 1H, H4), 7.80 (d, $J = 8.6$, 2H, H_{Ar}), 7.77 (d, $J = 8.6$, 2H, H_{Ar}), 7.68 (d, $J = 8.3$, 2H, H_{Ar}), 7.58 (d, $J = 8.3$, 2, H_{Ar} H), 6.90 (d, $J = 15.9$, 1H, H11), 6.41 – 6.31 (m, 1H, H10), 3.87 (d, $J = 7.2$, 2H, H9), 3.21 (m, 4H, H7+H8). ^{13}C NMR (101 MHz, MeOD) δ 152.75, 146.13, 141.46, 140.58, 139.29, 137.23, 136.55, 135.96, 135.88, 133.81, 133.68, 128.88, 128.71, 128.67, 128.59, 120.69, 120.06, 119.74, 112.07, 50.48, 47.64, 40.16. HRMS: calcd for $[\text{C}_{27}\text{H}_{24}\text{N}_4\text{O}_2\text{S} + \text{H}]^+ = 469.16927$, found 469.16916.



8i: Prepared according to the general procedure. Yield: 42.8 mg, 73.5 μmol , 36.8 %. ^1H NMR (400 MHz, MeOD) δ 9.69 (bs, 1H, H6), 8.87 (d, $J = 6.2$, 1H, H2), 8.72 (bs, 1H, H1), 8.66 (dd, $J = 1.0$, 7.4, 1H, H3), 8.58 (d, $J = 8.3$, 1H, H5), 8.02 – 7.89 (m, 3H, H_{Ar} +H4), 7.71 – 7.54 (m, 7H, H_{Ar}), 6.89 (d, $J = 15.9$, 1H, H11), 6.40 – 6.30 (m, 1H, H10), 3.87 (d, $J = 7.1$, 2H, H9), 3.22 (m, 4H, H7+H8). ^{13}C NMR (101 MHz, MeOD) δ 151.83, 142.89, 140.32, 139.43, 139.31, 137.46, 136.92, 136.32, 136.17, 134.30, 132.48, 132.09, 131.34, 131.10, 129.54, 128.71, 128.42, 121.61, 119.89, 119.67, 114.04, 50.50, 47.62, 40.17. HRMS: calcd for $[\text{C}_{27}\text{H}_{24}\text{N}_4\text{O}_2\text{S} + \text{H}]^+ = 469.16927$, found 469.16909.



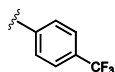
8j: Prepared according to the general procedure. Yield: 24.1 mg, 41.4 μmol , 20.7 %. ^1H NMR (400 MHz, MeOD) δ 9.64 (s, 1H), 8.79 (bs, 1H, H6), 8.76 (bs, 1H, H2), 8.66 (bs, 1H, H1), 8.62 (dd, $J = 0.9$, 7.4, 1H, H3), 8.54 (d, $J = 8.2$, 1H, H5), 7.98 – 7.91 (t, $J = 7.8$, 1H, H4), 7.83 (dd, $J = 0.7$, 7.8, 1H, H_{Ar}), 7.73 (dt, $J = 1.2$, 7.8, 1H, H_{Ar}), 7.63 – 7.56 (m, 7H, H_{Ar}), 7.53 (dt, $J = 1.0$, 7.7, 1H, H_{Ar}), 6.92 (d, $J = 15.9$, 1H, H11), 6.41 – 6.31 (m, 1H, H10), 3.87 (d, $J = 7.1$, 3H, H9), 3.20 (m, 4H, H7+H8). ^{13}C NMR (101 MHz, MeOD) δ 152.51, 146.06, 140.90, 140.07, 139.34, 137.25, 136.81, 136.09, 136.00, 134.90, 134.43, 133.85, 131.14, 130.36, 129.25, 129.10, 128.25, 120.21, 119.54, 112.02, 50.45, 47.56, 40.16. HRMS: calcd for $[\text{C}_{27}\text{H}_{24}\text{N}_4\text{O}_2\text{S} + \text{H}]^+ = 469.16927$, found 469.16915.



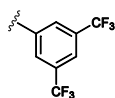
8k: Prepared according to the general procedure. Yield: 49.1 mg, 85.4 μmol , 42.7 %. ^1H NMR (400 MHz, MeOD) δ 9.68 (s, 1H, H6), 8.87 (d, $J = 6.5$, 1H, H2), 8.71 (d, $J = 5.8$, 1H, H1), 8.66 (dd, $J = 1.0$, 7.4, 1H, H3), 8.57 (d, $J = 8.3$, 1H, H5), 8.01 – 7.94 (t, $J = 7.9$, 1H, H4), 7.64 – 7.59 (m, 2H, H_{Ar}), 7.57 (d, $J = 8.4$, 2H, H_{Ar}), 7.51 (d, $J = 8.4$, 2H, H_{Ar}), 7.17 – 7.11 (m, 2H, H_{Ar}), 6.87 (d, $J = 15.9$, 1H, H11), 6.35 – 6.26 (m, 1H, H10), 3.86 (d, $J = 7.2$, 2H, H9), 3.21 (m, 4H, H7+H8). ^{13}C NMR (101 MHz, MeOD) δ 165.23, 162.79, 151.76, 141.74, 139.62, 139.27, 137.88, 137.52, 136.34, 136.19, 135.83, 134.35, 130.39, 129.70, 129.61, 129.57, 128.49, 128.17, 121.64, 119.11, 116.70, 116.49, 50.56, 47.57, 40.17. HRMS: calcd for $[\text{C}_{26}\text{H}_{24}\text{FN}_3\text{O}_2\text{S} + \text{H}]^+ = 462.16460$, found 462.16445.



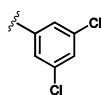
8l: Prepared according to the general procedure. Yield: 54.7 mg, 95.1 μmol , 47.5 %. ^1H NMR (400 MHz, MeOD) δ 9.47 (bs, 1H, H₆), 8.65 (s, 2H, H₁+H₂), 8.54 (d, J = 7.4, 1H, H₃), 8.44 (d, J = 8.0, 1H, H₅), 7.88 – 7.83 (t, J = 7.8, 1H, H₄), 7.53 (s, 4H, H_{Ar}), 7.46 (dt, J = 1.7, 7.8, 1H, H_{Ar}), 7.38 – 7.31 (m, 1H, H_{Ar}), 7.26 – 7.20 (m, 1H, H_{Ar}), 7.20 – 7.13 (m, 1H, H_{Ar}), 6.88 (d, J = 15.8, 1H, H₁₁), 6.37 – 6.26 (m, 1H, H₁₀), 3.86 (d, J = 7.2, 2H, H₉), 3.19 (m, 4H, H₇+H₈). ^{13}C NMR (101 MHz, MeOD) δ 159.85, 153.43, 143.02, 139.62, 137.60, 136.18, 135.87, 135.66, 135.61, 133.18, 131.67, 131.63, 130.61, 130.52, 130.41, 130.38, 129.59, 129.46, 128.36, 128.04, 125.76, 125.72, 119.93, 119.46, 117.16, 116.93, 50.51, 47.56, 40.13. HRMS: calcd for $[\text{C}_{26}\text{H}_{24}\text{FN}_3\text{O}_2\text{S} + \text{H}]^+ = 462.16460$, found 462.16448.



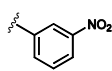
8m: Prepared according to the general procedure. Yield: 35.2 mg, 56.3 μmol , 28.2 %. ^1H NMR (400 MHz, MeOD) δ 9.69 (bs, 1H, H₆), 8.85 (d, J = 5.9, 1H, H₂), 8.73 (bs, 1H, H₁), 8.65 (dd, J = 1.0, 7.4, 1H, H₃), 8.57 (d, J = 8.3, 1H, H₅), 7.97 (t, J = 7.8, 1H, H₄), 7.81 (d, J = 8.2, 2H, H_{Ar}), 7.72 (d, J = 8.3, 2H, H_{Ar}), 7.68 (d, J = 8.3, 2H, H_{Ar}), 7.58 (d, J = 8.3, 2H, H_{Ar}), 6.91 (d, J = 15.9, 1H, H₁₁), 6.41 – 6.31 (m, 1H, H₁₀), 3.87 (d, J = 7.1, 2H, H₉), 3.22 (m, 4H, H₇+H₈). ^{13}C NMR (101 MHz, MeOD) δ 152.03, 145.36, 141.08, 139.84, 139.42, 137.28, 136.86, 136.26, 136.13, 134.19, 130.69, 130.37, 129.42, 128.64, 128.58, 128.41, 127.13, 126.82, 126.78, 124.43, 119.77, 50.52, 47.63, 40.18. HRMS: calcd for $[\text{C}_{27}\text{H}_{24}\text{F}_3\text{N}_3\text{O}_2\text{S} + \text{H}]^+ = 512.16141$, found 512.16118.



8n: Prepared according to the general procedure. Yield: 30.6 mg, 44.1 μmol , 22.1 %. ^1H NMR (400 MHz, MeOD) δ 9.65 (bs, 1H, H₆), 8.80 (d, J = 5.3, 1H, H₂), 8.72 (bs, 1H, H₁), 8.63 (dd, J = 1.0, 7.4, 1H, H₃), 8.55 (d, J = 8.3, 1H, H₅), 8.18 (s, 2H, H_{Ar}), 7.99 – 7.93 (m, 2H, H_{Ar}+H₄), 7.74 (d, J = 8.4, 2H, H_{Ar}), 7.63 (m, 5H, H_{Ar}), 7.57 – 7.51 (m, 2H, H_{Ar}), 6.93 (d, J = 15.9, 1H, H₁₁), 6.43 – 6.34 (m, 1H, H₁₀), 3.88 (d, J = 7.1, 2H, H₉), 3.21 (m, 4H, H₇+H₈). ^{13}C NMR (101 MHz, MeOD) δ 152.48, 144.21, 140.82, 139.42, 139.17, 137.55, 136.85, 136.10, 135.99, 133.89, 133.77, 133.74, 133.55, 133.22, 133.11, 133.01, 130.02, 129.90, 129.12, 128.91, 128.72, 128.22, 126.19, 123.49, 121.99, 121.03, 120.35, 50.47, 47.68, 40.17. HRMS: calcd for $[\text{C}_{28}\text{H}_{23}\text{F}_3\text{N}_3\text{O}_2\text{S} + \text{H}]^+ = 580.14879$, found 580.14891.

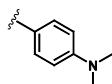


8o: Prepared according to the general procedure. Yield: 34.6 mg, 55.4 μmol , 27.7 %. ^1H NMR (400 MHz, MeOD) δ 9.62 (bs, 1H, H₆), 8.79 (d, J = 6.3, 1H, H₂), 8.70 (bs, 1H, H₁), 8.62 (dd, J = 1.1, 7.4, 1H, H₃), 8.54 (d, J = 8.3, 1H, H₅), 7.97 – 7.92 (t, J = 7.9, 1H, H₄), 7.60 (d, J = 8.5, 2H, H_{Ar}), 7.58 – 7.53 (m, 4H, H_{Ar}), 7.38 (t, J = 1.8, 1H, H_{Ar}), 6.89 (d, J = 15.9, 1H, H₁₁), 6.39 – 6.30 (m, 1H, H₁₀), 3.87 (d, J = 7.2, 2H, H₉), 3.21 (m, 4H, H₇+H₈). ^{13}C NMR (101 MHz, MeOD) δ 152.43, 144.93, 140.74, 139.76, 139.28, 137.13, 136.87, 136.61, 136.08, 135.98, 133.91, 129.11, 128.70, 128.44, 128.23, 126.42, 121.00, 119.98, 50.48, 47.63, 40.16. HRMS: calcd for $[\text{C}_{26}\text{H}_{23}\text{Cl}_2\text{N}_3\text{O}_2\text{S} + \text{H}]^+ = 512.09608$, found 512.09595.

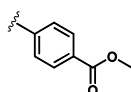


8p: Prepared according to the general procedure. Yield: 34.1 mg, 56.6 μmol , 28.3 %. ^1H NMR (400 MHz, MeOD) δ 9.68 (bs, 1H, H₆), 8.84 (d, J = 5.7, 1H, H₂), 8.72 (bs, 1H, H₁), 8.64 (d, J = 7.4, 1H, H₃), 8.56 (d, J = 8.3, 1H, H₅), 8.43 (t, J = 1.9, 1H, H_{Ar}), 8.18 (dd, J = 1.4, 8.2, 1H, H_{Ar}), 8.02 (d, J = 7.8, 1H, H_{Ar}), 8.00 –

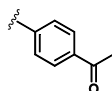
7.94 (t, $J = 7.9$, 1H, H₄), 7.72 – 7.65 (m, 3H, H_{Ar}), 7.59 (d, $J = 8.3$, 2H, H_{Ar}), 6.91 (d, $J = 15.9$, 1H, H₁₁), 6.41 – 6.32 (m, 1H, H₁₀), 3.88 (d, $J = 7.2$, 2H, H₉), 3.22 (m, 4H, H₇+H₈). ¹³C NMR (101 MHz, MeOD) δ 152.11, 150.19, 143.26, 140.18, 140.03, 139.30, 137.18, 137.07, 136.21, 136.09, 134.12, 133.95, 131.23, 129.35, 128.77, 128.49, 123.20, 122.90, 122.32, 121.36, 119.98, 115.06, 110.86, 50.50, 47.64, 40.18. HRMS: calcd for [C₂₆H₂₄N₄O₄S + H]⁺ = 489.15910, found 489.15892.



8q: Prepared according to the general procedure. Yield: 31.6 mg, 52.6 μ mol, 26.3 %. ¹H NMR (400 MHz, MeOD) δ 9.65 (bs, 1H, H₆), 8.83 (d, $J = 6.3$, 1H, H₂), 8.71 (bs, 1H, H₁), 8.64 (dd, $J = 1.0$, 7.4, 1H, H₃), 8.56 (d, $J = 8.3$, 1H, H₅), 8.00 – 7.94 (t, $J = 7.9$, 1H, H₄), 7.79 (d, $J = 8.6$, 2H, H_{Ar}), 7.64 (d, $J = 8.0$, 2H, H_{Ar}), 7.56 (d, $J = 8.3$, 4H, H_{Ar}), 6.89 (d, $J = 15.8$, 1H, H₁₁), 6.39 – 6.29 (m, 1H, H₁₀), 3.86 (d, $J = 7.1$, 2H, H₉), 3.26 (s, 6H, 2xNCH₃), 3.21 (m, 4H, H₇+H₈). ¹³C NMR (101 MHz, MeOD) δ 152.19, 145.12, 141.04, 140.22, 139.48, 137.13, 136.39, 136.19, 136.08, 134.09, 129.58, 129.30, 128.63, 128.21, 121.26, 120.49, 119.48, 50.54, 47.60, 45.81, 40.17. HRMS: calcd for [C₂₈H₃₀N₄O₂S + H]⁺ = 487.21622, found 487.21624.



8r: Prepared according to the general procedure. Yield: 40.7 mg, 66.2 μ mol, 33.1 %. ¹H NMR (400 MHz, MeOD) δ 9.60 (s, 1H, H₆), 8.78 (d, $J = 6.4$, 1H, H₂), 8.68 (d, $J = 6.2$, 1H, H₁), 8.63 – 8.59 (dd, 0.84, 7.36, 1H, H₃), 8.52 (d, $J = 8.3$, 1H, H₅), 8.05 (d, $J = 8.4$, 2H, H_{Ar}), 7.93 (t, $J = 7.8$, 1H, H₄), 7.72 (d, $J = 8.4$, 2H, H_{Ar}), 7.66 (d, $J = 8.3$, 2H, H_{Ar}), 7.55 (d, $J = 8.3$, 2H, H_{Ar}), 6.89 (d, $J = 15.9$, 1H, H₁₁), 6.39 – 6.29 (m, 1H, H₁₀), 3.90 (s, 3H, OCH₃), 3.87 (d, $J = 7.2$, 2H, H₉), 3.21 (m, 4H, H₇+H₈). ¹³C NMR (101 MHz, MeOD) δ 168.34, 152.48, 146.12, 141.33, 140.91, 139.45, 136.81, 136.76, 136.04, 135.96, 133.86, 131.12, 130.47, 130.28, 129.05, 128.60, 128.54, 127.88, 120.90, 119.69, 52.64, 50.52, 47.62, 40.16. HRMS: calcd for [C₂₈H₂₇N₃O₄S + H]⁺ = 502.17950, found 502.17921.

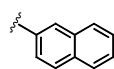


8s: Prepared according to the general procedure. Yield: 16.2 mg, 27.0 μ mol, 13.5 %. ¹H NMR (400 MHz, MeOD) δ 9.59 (bs, 1H, H₆), 8.75 (d, $J = 6.0$, 1H, H₂), 8.70 (bs, 1H, H₁), 8.60 (d, $J = 7.4$, 1H, H₃), 8.52 (d, $J = 8.1$, 1H, H₅), 8.06 (d, $J = 8.4$, 2H, H_{Ar}), 7.96 – 7.90 (t, $J = 7.8$, 1H, H₄), 7.77 (d, $J = 8.4$, 2H, H_{Ar}), 7.70 (d, $J = 8.3$, 2H, H_{Ar}), 7.58 (d, $J = 8.3$, 2H, H_{Ar}), 6.91 (d, $J = 15.9$, 1H, H₁₁), 6.40 – 6.30 (m, 1H, H₁₀), 3.88 (d, $J = 7.1$, 2H, H₉), 3.21 (m, 4H, H₇+H₈), 2.62 (s, 3H, C(O)CH₃). ¹³C NMR (101 MHz, MeOD) δ 200.07, 152.85, 146.26, 141.62, 141.37, 139.51, 137.31, 136.84, 136.49, 135.95, 133.67, 130.58, 130.16, 128.85, 128.63, 128.58, 128.48, 128.29, 128.03, 127.53, 119.70, 50.54, 47.64, 40.17, 26.71. : calcd for [C₂₈H₂₇N₃O₃S + H]⁺ = 486.18459, found 486.18461.



8t: Prepared according to the general procedure. Yield: 53.8 mg, 88.6 μ mol, 44.3 %. ¹H NMR (400 MHz, MeOD) δ 9.63 (bs, 1H, H₆), 8.79 (bs, 2H, H₁+H₂), 8.61 (dd, $J = 0.9$, 7.4, 1H, H₃), 8.49 (d, $J = 8.1$, 1H, H₅), 7.95 – 7.76 (m, 4H, H_{Ar}+H₄), 7.57 (d, $J = 8.1$, 2H, H_{Ar}), 7.51 – 7.33 (m, 7H, H_{Ar}), 6.93 (d, $J = 15.9$, 1H, H₁₁), 6.40 – 6.31 (m, 1H, H₁₀), 3.87 (d, $J = 7.1$, 2H, H₉), 3.22 (m, 4H, H₇+H₈). ¹³C NMR (101 MHz, MeOD) δ 152.47, 142.67, 140.99, 140.74, 139.77, 136.70, 135.99, 135.89, 135.33, 133.74, 132.65, 131.45, 129.41,

129.01, 128.92, 127.93, 127.84, 127.14, 126.87, 126.51, 126.39, 119.25, 50.55, 47.57, 40.16. HRMS: calcd for $[\text{C}_{30}\text{H}_{27}\text{N}_3\text{O}_2\text{S} + \text{H}]^+ = 494.18967$, found 494.18946.



8u: Prepared according to the general procedure. Yield: 37.8 mg, 62.3 μmol , 31.1 %. ^1H NMR (400 MHz, MeOD) δ 9.56 (s, 1H, H₆), 8.77 (d, $J = 6.4$, 1H, H₂), 8.67 (bs, 1H, H₁), 8.59 (dd, $J = 1.1$, 7.4, 1H, H₃), 8.48 (d, $J = 8.3$, 1H, H₅), 8.06 (s, 1H, H_{Ar}), 7.93 – 7.81 (m, 5H, H_{Ar}+H₄), 7.77 – 7.70 (m, 3H, H_{Ar}), 7.55 (d, $J = 8.3$, 2H, H_{Ar}), 7.48 – 7.44 (m, 2H, H_{Ar}), 6.88 (d, $J = 15.8$, 1H, H₁₁), 6.36 – 6.27 (m, 1H, H₁₀), 3.85 (d, $J = 7.1$, 2H, H₉), 3.20 (m, 4H, H₇+H₈). ^{13}C NMR (101 MHz, MeOD) δ 152.47, 142.60, 140.92, 139.72, 138.77, 136.74, 136.01, 135.89, 135.14, 134.23, 133.81, 130.45, 129.58, 129.27, 129.00, 128.60, 128.54, 128.26, 127.41, 127.16, 126.51, 126.00, 120.86, 119.05, 50.57, 47.57, 40.15. HRMS: calcd for $[\text{C}_{30}\text{H}_{27}\text{N}_3\text{O}_2\text{S} + \text{H}]^+ = 494.18967$, found 494.18948.

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Chapter 5 | Novel GS analogues

Introduction

Gramicidin S (GS, **1**) is a cationic antimicrobial peptide¹ (CAP) that was first isolated from *Bacillus Brevis* by Gause and Brazhnikova². GS is active against a wide range of bacteria, both Gram-positive and Gram-negative strains, and as such represents an attractive lead compound for the development of new antibiotic strategies. Unfortunately, GS itself is highly hemolytic, and its use in the clinic is therefore limited to topical applications.² Both bacterial and hemolytical activities are rooted in its molecular mode of action.^{1a} GS acts on the cell membrane and disrupts the integrity of the lipid bilayer, with cell lysis as a result. Here lies the difficulty in GS-inspired research towards new antibiotic strategies: although highly different in nature and functioning, the bacterial cell is not that different from an erythrocyte (GS induced lysis of which causes hemolysis) where it comes to the cell membrane. That GS is at all considered as an attractive starting point is based on the same molecular mode of action: unlike most other antibiotic compound classes GS does not target a specific molecule (protein, DNA or RNA) within the bacterial cell and thus the development of resistance towards GS and related CAPs is commonly thought to be less likely to develop.³ In this respect, an interesting finding underscoring the mode of action of GS is that

enantiomeric GS is equally active as the natural compound.⁴ Clearly, such would not be the case when a specific molecule, for instance an integrated membrane protein, would be the main target of GS.

Research after GS analogues with improved medicinal properties is mainly aimed at the development of analogues that have bactericidal properties inherent to GS, while not being active against erythrocytes. In practice, this entails the development of compounds able to distinguish between a mammalian cell membrane and that of a bacterial cell. This is a daunting task, first because, as said before, there is not much difference between the two types of lipid bilayers (and not much information on this issue is available). And second, there is little known about the exact mode of action by which GS inserts into a lipid bilayer.⁵ Contemporary research after new and improved GS analogues therefore takes a three-pronged approach, namely 1) the design and synthesis of new GS derivatives, 2) the in-depth analysis of the secondary structure these compounds may adopt in (aqueous) solution and 3) the assessment of these compounds on their activities on both bacterial strains and erythrocytes. Recent years have further witnessed approaches to study the conformational behavior of GS in the context of membrane fractions, but no conclusive results have emerged from these studies yet.⁶ Current state-of-the-art dictates that, whereas the conformational behavior of GS⁷ (and that of quite a few synthetic analogues)⁸ in aqueous solution is known in detail, it is not known whether, and to what extent, this behavior holds true for the conformational behavior (and therefore mode of action) of GS in lipid membranes. Röntgen diffraction studies on GS assemblies further give conflicting results^{7d} and in any case it is debatable whether such artificial assemblies reflect those possibly found in lipid bilayers as pore entities. The approach taken in this chapter is therefore as outlined above, namely, 1) modify GS at specific positions, 2) evaluate the structure in solution and 3) measure their biological activities.

At the basis of the here presented study is the solution structure of GS itself, together with that of a number of modified structures that have recently appeared in the literature.^{7,8} GS is a cyclic decapeptide with sequence (Pro-Val-Orn-Leu-^DPhe-)₂ and has C₂ symmetry. In aqueous solution, and as measured by proton NMR,^{7b} it adopts an anti-parallel β -sheet with the two Val-Orn-Leu stretches aligning as two β -strands and the two ^DPhe-Pro dipeptides as two type II' β -turn. The β -sheet is held in place (see Figure 1) by four interstrand hydrogen

bonds and the molecule adopts a slight twist.^{7c} This overall conformational assembly gives GS its amphiphilic nature that is thought to be at the basis of its lytic properties.⁹ Here the two basic ornithine residues point to one side of the molecule and the four hydrophobic Val / Leu residues are situated at the opposite side. GS analogues of varying nature have been reported, including extended homologues,⁹ derivatives featuring different α -amino acids¹⁰ and derivatives incorporating amino acid analogues.¹¹ may be thus brought to both β -strand and β -turn regions. With respect to the latter, of specific interest is the recently reported structure of **2a**,¹² in which one of the two β -turn dipeptides is replaced by a so-called sugar amino acid (SAA) dipeptide isoster **3a**.¹³ SAAs have attracted some interest in recent years due to their ease of access, the conformational control they may exert on a target peptide, their (putative) protease stability and the number of functional groups that are inherent to the parent carbohydrate and that opens ways for further functionalization.¹⁴ Rather surprisingly, the secondary structure of **2a** and **2b**, that contains one copy of SAA **3a** and **3b** respectively, deviate from GS in that the leucine carbonyl adjacent to the SAA amine is flipped (in comparison to GS) out of the ring, and is not involved in hydrogen bonding.¹⁴ Rather, there appears to be a hydrogen bond between the SAA-NH and one of the oxygen functionalities attached to the SAA furan core. As a result, the solution structures of **2a** and **2b** deviate from that of GS in that, although the β -sheet character is to a large extent intact, one of the β -turn regions is drastically altered and the overall molecule has a much more pronounced twist around the β -strand. Interestingly, both bacterial and hemolytical activities of GS and **2b** (R=Bn) are comparable and a tentative conclusion from these studies may be that some conformational freedom in the amphiphilic structure is allowed, and possibly may be capitalized upon in the development of species specific GS analogues. Incidentally, the hydrophobic nature of the turn region is much more important: earlier studies proved that the analogeous compound **2a**, without a benzyl group at a position corresponding to ^DPhe in the GS structure, has no activity at all, even though its solution structure is highly similar to that of **2b**.^{12,14}

Subject of this chapter is to further study to what extent the SAA design in **3** is amenable for modification. Three analogues are presented here, of which the first (compound **7**, see Figure 1) lacks the -OH group involved in hydrogen bonding and the other two (**8** and **9**, see Figure 1) are rendered more flexible through the incorporation of dipeptide isosters. The synthesis of these molecules, and the SAA building blocks on which they are based, are

presented. Further, their conformational behavior in solution and their bactericidal/hemolytical activities are discussed, taking into consideration the data known on GS and GS analogues **2a** and **2b**.

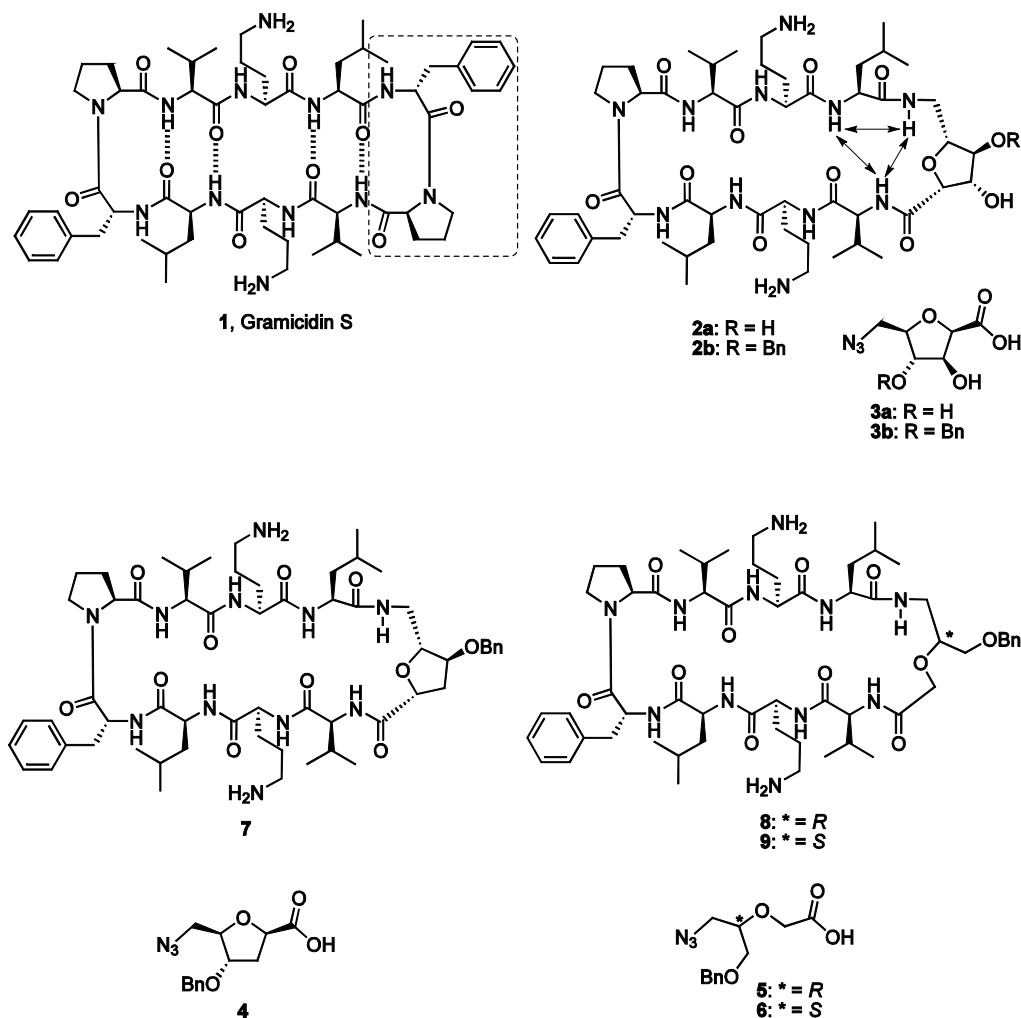
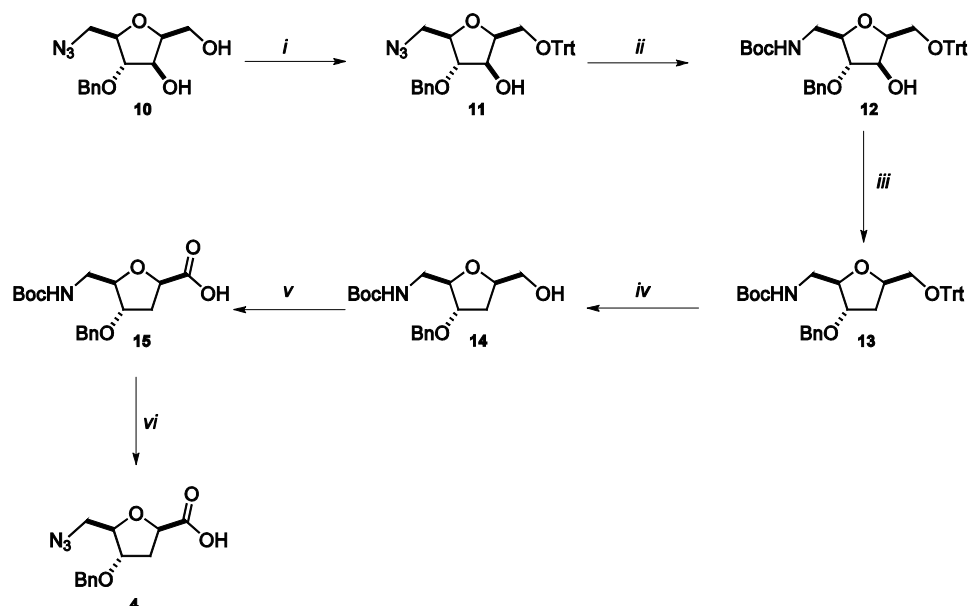


Figure 1; Gramicidin S and analogues thereof in which the ^DPhe-Pro motif (highlighted in GS) has been replaced by different dipeptide isosters. Dashed lines in GS denote Hydrogen bonds. Arrows in **2a** and **2b** indicate NOE interactions.

Results

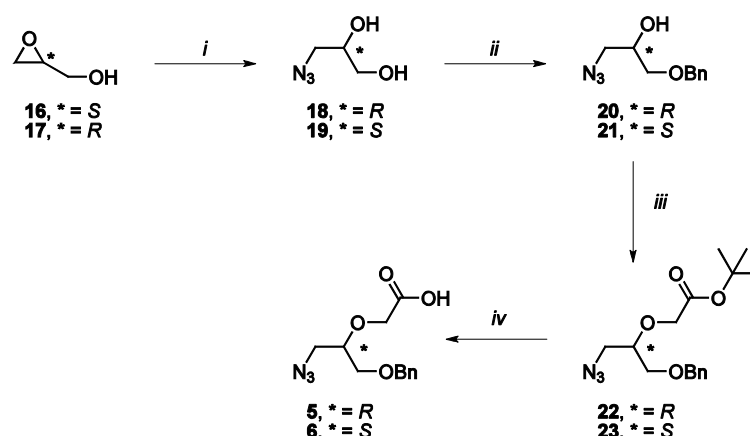
The synthesis of SAA **4** commenced from the known intermediate **10**¹⁴ of which the primary alcohol was selectively protected using trityl chloride in pyridine to give compound **11** in 68% yield (Scheme 1). Conversion of the azide-group in **11** into the more compatible Boc-protective group gave compound **12** in 93% yield. Next, Barton deoxygenation using thiocarbonyldiimidazole, AIBN and Bu₃SnH gave deoxygenated compound **13** in 74% yield. Deprotection of the titylated alcohol under slightly acidic condition (**13** → **14**, 70%) and

oxidation of the primary alcohol to the carboxylic acid under classical TEMPO conditions (**14** → **15**, 80%) followed by Boc removal and diazotransfer using freshly prepared triflic azide afforded SAA **4** in 77% yield.



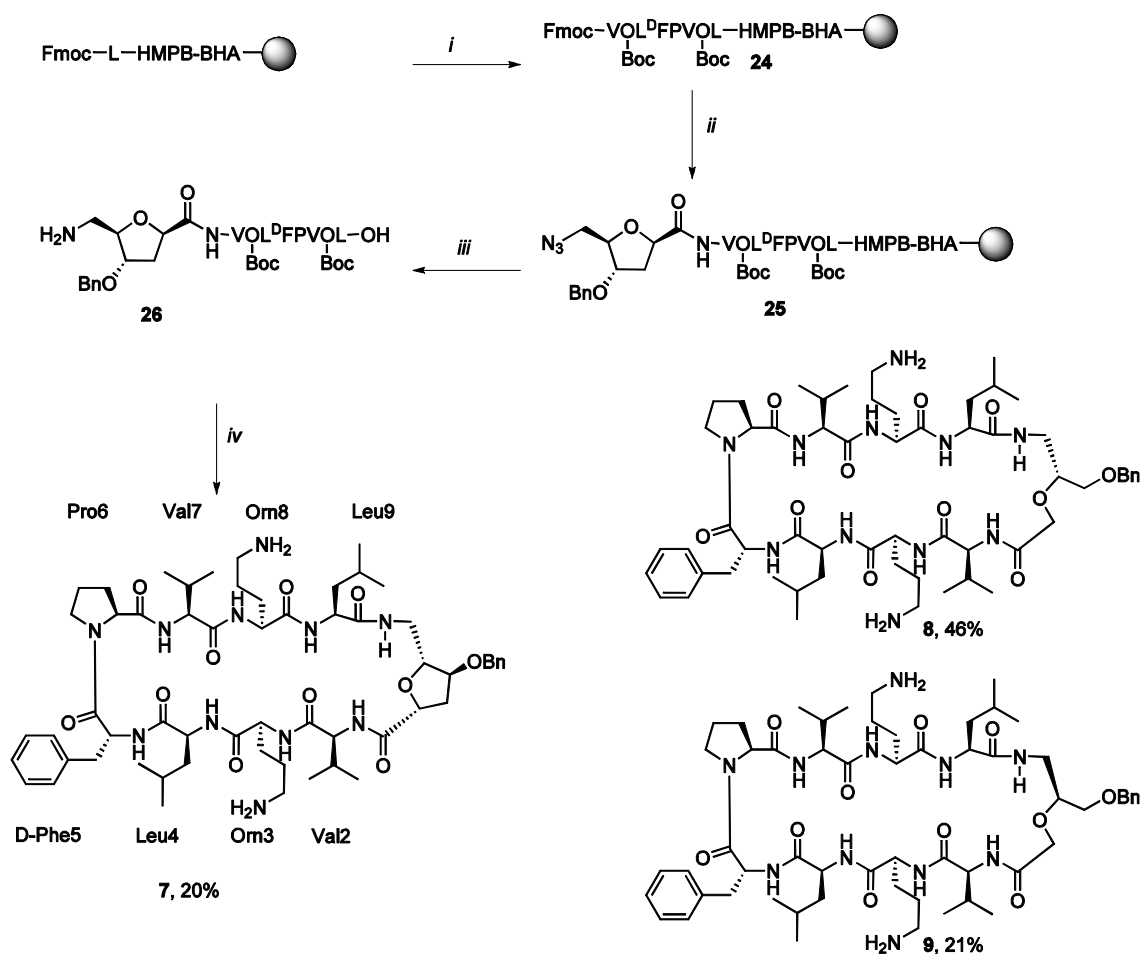
Scheme 1; *Reagents and conditions*: (i) TrtCl (1.5 eq.), pyridine, 16h, 0°C, 68%. (ii) a: PMe_3 (1M soln. toluene, 2 eq., 0°C, THF, 1h. then H_2O 2 eq., 6h., b: Boc_2O (1.5 eq.), DiPEA (2 eq.) 16h, 93% over two steps. (iii) a: 1,1-thiocarbodiimidazole (4 eq.), 90°C, toluene, 5h., b: AIBN (0.2 eq.), Bu_3SnH (4 eq.), 80°C, toluene, 4h, 74% over two steps. (iv) $p\text{TsoH}$ (0.5 eq.), 40°C, 10% MeOH / DCM 6h, 70%. (v) TEMPO (cat.), KBr (0.1 eq.), NaOCl (2 eq.), ACN, 80%. (vi) a: 50% TFA/DCM, b: tFN_3 (5 eq.), CuSO_4 (0.1 eq.), K_2CO_3 (3 eq.), MeOH / H_2O , 77% over two steps

Azidoethoxy acetic acid (AAA) **5** was synthesized as follows. Commercially available *S*-glycidol **16** was selectively azidolyzed with NaN_3 in wet *t*-BuOH under Lewis acid catalysis according to the literature procedure,¹⁵ affording diol **18** in 50% yield. Tin-ketal formation followed by selective alkylation towards the primary benzyl ether yielded **20** in 62% yield. Alkylation using *t*-butylbromoacetate (**20**→**22**, 64% yield) and acidic deprotection resulted in formation of **5** in 74% yield. The enantiomeric purity of **22** was assessed by chiral HPLC. At this stage, less than 5% of the corresponding enantiomer was found. The stereoisomeric **6** was obtained following the same sequence of reactions starting from *R*-glycidol in comparable yield.



Scheme 2; *Reagents and conditions:* (i) NaN_3 (1.2 eq.) LiBF_4 (0.2 eq.), $t\text{BuOH}/\text{H}_2\text{O}$ (5/1), 49% **18**; 44% **19**; (ii) a: Bu_2SnO (1.5 eq.) 100°C , toluene, 3h., b: BnBr (2 eq.) 100°C , toluene, 16h., 62% **20**; 28% **21**; (iii) NaH (60% suspension in mineral oil, 1.2 eq.), $t\text{butylbromoacetate}$ (2 eq.), 0°C to r.t. DMF, 16h., 64% **22**; 40% **23**; (iv) $\text{TFA}/\text{DCM}/\text{TIS}$ 50/50/1. 72% **5**; 86% **6**.

The synthesis of GS analogues **7** – **9** is exemplified by the synthesis of **7** and is depicted in Scheme 3. Commercially available polystyrene based resin, preloaded with Fmoc-Leu, attached *via* the hyper acid labile benzhydrylamine / 4-hydroxymethyl-3-methoxyphenoxybutanoic acid linker was elongated with ornithine, valine, proline, D-phenylalanine, leucine, ornithine and valine using repetitive cycles of standard Fmoc-based SPPS as depicted in Scheme 3. Coupling of SAA **4** was performed with a 1.5-fold excess of SAA with respect to the resin-loading and the coupling time was extended to 16 hours affording **25**. On-resin Staudinger reduction for 16 h. using excess PMe_3 in wet THF ¹⁶ followed by acidic cleavage using 1% TFA/DCM yielded linear protected nonamers **26**. Cyclization under dilute conditions (10 mM in DMF) using PyBOP/HOBt and DIPEA and acidic deprotection (50% TFA/DCM) afforded GS analogue **7** in 20% after HPLC purification. Analogues **8** and **9** were prepared in a similar fashion.



Scheme 3; Reagents and conditions: (i) 8 cycles of a: 20% pip/NMP, 3x5 min.; b: Fmoc-AA-OH (5 eq.), HCTU (5 eq.), DiPEA (10 eq.) 90 min. (ii) a: 20% pip/NMP, 3x5 min. b: **4**, **5** or **6** (1.5 eq.), HCTU (1.5 eq.), DiPEA (3 eq.), 16 h. (iii) a: PMe₃ (1M in 9:1 THF/H₂O, 25 eq.), 16 h. b: 1% TFA/DCM. (iv) a: PyBOP (5 eq.), HOBT (5 eq.), DiPEA (10 eq.), 16h. b: 50% TFA/DCM, 1h.

Structural analysis of the newly synthesized analogues was performed by extensive 1D and 2D NMR experiments. From the ¹H-NMR, all the NH-coupling constants could be determined and compared to those obtained from native GS (**1**) and **2b** (Figure 2A). The presence of a β-turn conformation around D-Phe5 was evidenced in all analogues by its small coupling constant¹⁷ $^3J_{\text{NH-H}\alpha} < 4 \text{ Hz}$ and a negative value for the chemical shift perturbation of the α-proton of the D-Phe and Pro residues¹⁸ (Figure 2B). For **7**, the remaining coupling constants are similar to both native GS and **2b**, indicating a comparable overall β-sheet structure¹⁹ ($8.5 \text{ Hz} < ^3J_{\text{NH-H}\alpha} < 9 \text{ Hz}$), except Val2 and Orn8 which are less pronounced. The coupling constants and chemical shift perturbation of **8** and **9** indicate a similar structure of these two analogues.

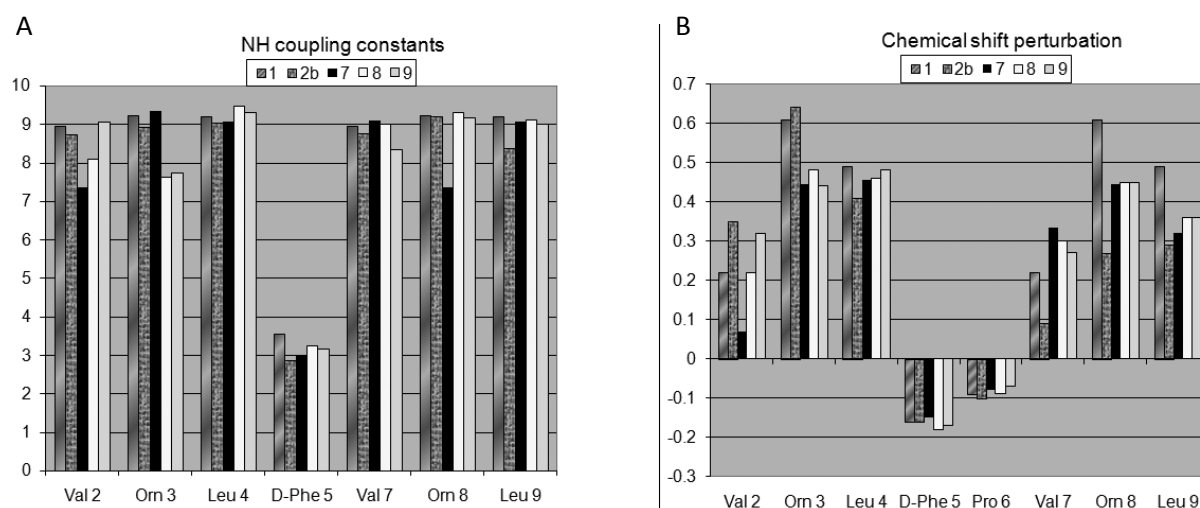


Figure 2; A: $^3J_{\text{NH-H}\alpha}$ of GS, **2b** and **7–9** (values on the y-axis are in Hz). B: Chemical shift perturbation of GS, **2b** and **7–9**.

A close inspection of the amide region in the NOE spectrum of **7** (Figure 3) reveals, besides numerous sequential NOEs, several interstrand NOEs indicating the presence of a β -sheet character in this analogue. Interestingly, NH-NH NOE cross correlations were found between the SAA and Leu9 (A, in Figure 3), between the SAA and the Val2 amide protons (B in Figure 3) and between Leu9 and Val2, similar to those found for **2a** and **2b** which is indicative for the same altered orientation of the SAA amide proton (Figure 3). These results clearly indicate that a hydrogen bond between the SAA amide and the hydroxyl is not the determining factor for the observed re-orientation of the Leu - SAA amide bond in **2a** and **2b**. A stabilizing effect of this hydrogen bond can of course not be ruled out. It does, however, implicate the involvement of conformational restrictions imposed by the furanoid ring as major factor in the amide re-orientation. This conclusion is substantiated by results obtained from flexible analogues **8** and **9** where the NOE spectra lack the NOE cross correlation signals between the AAA and Val2 amide protons.

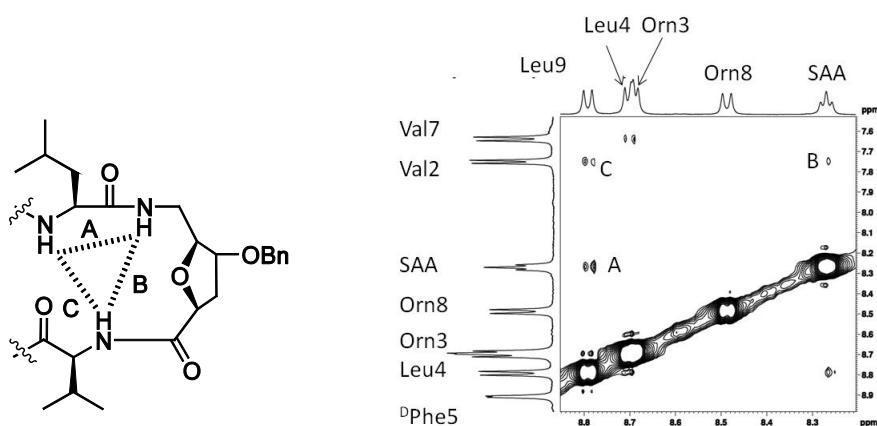


Figure 3; Turn region of **7** and part of the amide region of the NOE spectrum of **7**.

Biological evaluation

Next, the newly synthesized analogues were assessed for their antibacterial activity and compared to GS and **2b**. The results are depicted in Table 1. Both the Gram negative bacterial strains used in this assay, *E. coli* and *P. aeruginosa*, were largely unaffected by the presence of any of the peptides. Compound **8** was most effective against *E. coli* with a minimal inhibitory concentration (MIC) of 16 µg/mL. The GS analogues **7**, **8** and **9** are potent against gram positive strains with MIC values between 8 and 32 µg/mL. Compound **8** is about as potent as GS, **7** and **9** are about 2-fold less potent.

Table 1; MIC values for **1**, **2b** and **7 - 9**

	Gram pos. Staph. aureus ATCC 29213	Gram pos. Staph. epidermis ATCC 12228	Gram pos. Enterococ. faecalis ATCC 29212	Gram neg. E. Coli ATCC 25922	Gram neg. P. aeruginosa ATCC 27853	Gram pos. Bacillus cereus ATCC 11778
1	8	8	8	>64	>64	8
2b	16	8	32	32	>64	8
7	16	16	32	64	>64	16
8	8	8	16	16	64	8
9	16	8	32	32	>64	8

The hemolytic activity of the GS analogues was determined and the results are plotted in Figure 4.²⁰ Analogues **7** and **9** are about as toxic towards human erythrocytes as GS and **2b**. The former (**7**) cause 100% lysis at 125 µM peptide concentration and the latter (**9**) at 62.5 µM. Interestingly, compound **8** shows a reduced hemolytic activity with only 90% lysis at a peptide concentration of 500 µM.

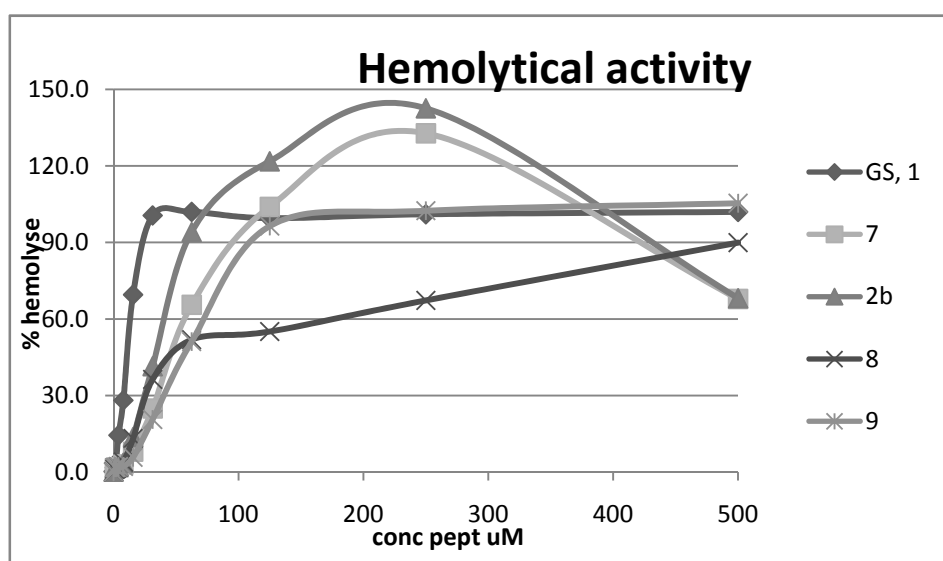


Figure 4; Hemolytical activity of GS and analogues thereof

Discussion

Three novel dipeptide isosters were successfully synthesized and used to replace the D-Phe/Pro turn motif of gramicidin S. A cyclic SAA was designed without a hydroxyl group on C3 that could possibly function as a hydrogen bond accepting moiety that could stabilize the re-orientated backbone conformation found in GS analogue **2a** and **2b**. Two stereoisomeric acyclic dipeptide isosters were also synthesized and incorporated into GS. All three GS analogues were analyzed by means of 1D- and 2D-NMR experiments to investigate their structural conformation in organic solvent. In the case of **7**, containing the cyclic SAA **4**, the backbone showed a similar re-orientation as was found in **2a** and **2b**. The backbone conformations of the acyclic dipeptide isoster containing GS analogues **8** and **9** were not distorted and resembled the conformation found in native GS.

The biological evaluation revealed that **7** was similarly active as **2b** and slightly less active compared to GS in both the bacterial and hemolytical assays. Replacing the β -turn motif in GS with a flexible dipeptide isoster resulted in analogues with a similar or improved biological profile depending on the stereochemistry of the aromatic moiety in the dipeptide isoster. Compound **9** is approximately 2-fold less toxic for human erythrocytes compared to GS. However, a similar decrease in potency was observed in the antibacterial assay. The stereoisomeric analogue **8** possessed bactericidal potency comparable to GS combined with a marked decrease in hemolytical activity. These findings indicate that the distorted backbone conformation of GS, imposed by the cyclic nature of furanoid SAAs **2b** and **4**, is a determining factor in the decreased antibacterial activity. Although the precise correlation between the increased conformational freedom in the modified turn region in **8** and the decreased hemolytical activity remains unidentified, these results demonstrate the potential of peptide isosters as elements to replace secondary structural elements in biologically active peptides in an attempt to optimize behavior in biological surroundings.

Experimental section

General: PE with a boiling range of 40 - 60°C was used. THF and Et₂O were distilled over LiAlH₄ prior to use. DCM was distilled over CaH₂ prior to use. All other solvents used under anhydrous conditions were stored over molecular sieves (4Å) except for methanol which was stored over 3Å molecular sieves. Solvents used for work-up and column chromatography were of technical grade and distilled before use. Unless stated otherwise, solvents were removed by rotary evaporation under reduced pressure at 40°C. Reactions were monitored by TLC-analysis using Merck 25 DC plastikfolien 60 F₂₅₄ with detection by spraying with 20% H₂SO₄ in EtOH, (NH₄)₆Mo₇O₂₄·4H₂O (25 g/L) and (NH₄)₄Ce(SO₄)₄·2H₂O (10 g/L) in 10% sulfuric acid or by spraying with a solution of ninhydrin (3 g/L) in EtOH / AcOH (20/1 v/v), followed by charring at approx. 150°C. Column chromatography was performed on Fluka silicagel (0.04 – 0.063 mm). For LC/MS analysis, an JASCO HPLC-system (detection simultaneously at 214 and 254 nm) equipped with an analytical C₁₈ column (4.6 mmD × 250 mmL, 5μ particle size) in combination with buffers A: H₂O, B: MeCN and C: 0.5% aq. TFA and coupled to a mass instrument with a custom-made Electrospray Interface (ESI) was used. For reversed-phase HPLC purification of the final compounds, an automated HPLC system supplied with a semi-preperative C₁₈ column (10.0 mmD × 250 mmL, 5μ particle size) was used. The applied buffers were A: H₂O, B: MeCN and C: 1.0% aq. TFA. High resolution mass spectra were recorded by direct injection (2 μL of a 2 μM solution in water/acetonitrile; 50/50; v/v and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150-2000) and dioctylphthalate (m/z = 391.28428) as a "lock mass".²¹ The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). ¹H- en ¹³C-NMR spectra were measured on a Joel JNM-FX-200 (200/50 Mhz), a Brüker AV-400 (400/100 MHz), a Brüker AV-500 (500/125 MHz) or a Brüker DMX-600 (600/125 MHz). Chemical shifts are given in ppm (δ) relative to TMS (0 ppm) or MeOD (3.30 ppm) and coupling constants are given in Hz. Optical rotation are measured at r.t. at a concentration of 10 mg/mL.

General procedure for peptide synthesis:

a) stepwise elongation: Fmoc-Leu-HMPB-MBHA resin Resin **6** (196 mg, 0.51 mmol/g, 0.1 mmol) was submitted to seven cycles of Fmoc solid-phase synthesis with the appropriate commercially available amino acid building blocks Fmoc-Orn(Boc)-OH, Fmoc-Val-OH, Fmoc-Pro-OH, Fmoc-^DPhe-OH, as follows: a) deprotection with piperidine / NMP (1/4, v/v, 5 mL, 15 min); b) wash with NMP (5 mL, 3x, 3 min); c) coupling of the appropriate Fmoc amino acid (5 eq., 0.5 mmol) in the presence of HCTU (5 eq., 0.5 mmol, 206 mg) and DiPEA (10 equiv., 1 mmol, 162 μL) which was preactivated for 2 min in NMP (5 mL) and shaken for 90 min; d) wash with NMP (5 mL, 3x, 3 min). Couplings were monitored for completion by the Kaiser test.²² Finally, the *N*-terminal amine was liberated by Fmoc-deprotection with piperidine / NMP (1/4, v/v, 5 mL, 15 min) followed by washing with NMP (5 mL, 3x, 3 min). Coupling of SAA **6** was performed as follows: To the resin bound peptide, a preactivated solution of SAA **6** (1.5 eq. 44 mg, 0.150 mmol), HCTU (1.5 eq., 62 mg, 0.150 mmol) and DiPEA

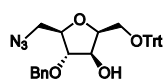
(3.0 eq., 74 μ L, 0.45 mmol) in NMP (3 mL) was added and the resulting suspension was shaken for 16h. The resin was finally washed with NMP (5 mL, 3x, 3 min) to give title compound.

b) on-resin Staudinger reduction. The appropriate resin-bound azide is treated with a pre-mixed cocktail of H_2O (0.5 mL) and PMe_3 (3.5 mL, 1M in THF) and shaken for 16h. The resin is washed with methanol (4 mL, 3x, 3 min) and DMF (4 mL, 3x, 3 min.)

c) cleavage from the resin: Resin-bound nonapeptide is repeatedly treated with 1% TFA/DCM (4 mL). The filtrate was coevaporated with toluene (3x) and used as such in the cyclization step.

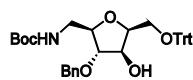
d) cyclization: The linear nonapeptide was taken up in DMF (5 mL) and added dropwise over the course of an hour to a solution of benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) (5 equiv., 270 mg, 0.5 mmol), HOBT (5 equiv., 67 mg, 0.5 mmol) and DIPEA (15 equiv., 254 μ L, 1.5 mmol) in DMF (70 mL) and allowed to stir for 16h. The solvent was removed in vacuo and the resulting mixture was used without further purification in the deprotection step.

e) deprotection: The crude cyclised peptide was treated with 50% TFA/DCM (10 mL) for 1 h. before it was concentrated and purified by HPLC purification.



(2S,3R,4S,5R)-5-(azidomethyl)-4-(benzyloxy)-2-(trityloxymethyl)tetrahydrofuran-3-ol (**11**)

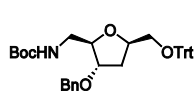
Diol (**10**) (1.11 g, 2.13 mmol) was dissolved in pyridine (11 mL) and cooled to 0 °C. Trityl chloride (0.89 g, 3.2 mmol, 1.5 equiv) was added at 0 °C. The reaction mixture was stirred for 18h and when TLC analysis (3/20 v/v EtOAc in PE) indicated complete conversion of the diol into a higher running product, the reaction mixture was evaporated to dryness. The residue was taken up in EtOAc, washed with sat. aq. NaHCO_3 and Brine, dried over Na_2SO_4 , filtered and evaporated. Purification was performed by column chromatography (mixture of eluents: EtOAc/ PE 0/1 $\rightarrow$ 3/20 v/v) and afforded compound (**11**) (0.75 g, 1.44 mmol, 68% yield) as a yellow syrup ^1H NMR (200 MHz, CDCl_3) δ 7.82 – 7.11 (m, 20H), 4.69 (d, J = 11.8, 1H), 4.57 (d, J = 11.8, 1H), 4.17 (d, J = 4.9, 1H), 4.04 (d, J = 3.7, 1H), 3.84 – 3.76 (m, 1H), 3.60 – 3.34 (m, 4H), 3.18 (d, J = 5.7, 1H), 2.36 (s, 1H). ^{13}C NMR (50 MHz, CDCl_3) δ 151.49, 147.94, 142.05, 133.04, 132.55, 132.46, 132.38, 132.23, 131.75, 131.68, 91.88, 91.21, 86.59, 84.62, 81.06, 76.37, 66.84, 57.52.



tert-butyl ((2R,3S,4R,5S)-3-(benzyloxy)-4-hydroxy-5-(trityloxymethyl)tetrahydrofuran-2-yl)methylcarbamate (**12**)

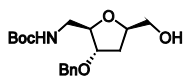
Tritylated (**11**) (5.54 g, 10.64 mmol) was co-evaporated with toluene (2x), re-dissolved in THF (55 mL), cooled to 0 °C under argon atmosphere and PMe_3 (21.3 mL (1 M solution in toluene), 2 equiv) was added. The reaction mixture was stirred for 1h and then H_2O (0.38 mL, 21.3 mmol, 2 equiv) was added, after which the stirring was continued for another 6h. After that, the reaction mixture was concentrated, co-evaporated with toluene (2x), re-dissolved in toluene (50 mL) and cooled at 0 °C and DIPEA (2.75 g, 3.5 mL, 21.3 mmol, 2 equiv) and Boc_2O (3.5 g, 15.96 mmol, 1.5 equiv) were added. The reaction was stirring for 12h at r.t. and after TLC analysis (3/10 v/v EtOAc in PE) showed complete consumption of (**11**), EtOAc was added and the organic layer was washed with sat. aq. NaHCO_3 , NH_4Cl and brine, dried over Na_2SO_4 , filtered and concentrated. Protected amine (**12**) was isolated by column chromatography (eluents: 1/20 $\rightarrow$ 3/20 v/v EtOAc in PE) (5.89 g, 9.9 mmol, 93% yield) as a

white foam. ^1H NMR (200 MHz, CDCl_3) δ 7.68 – 7.01 (m, 20H), 4.98 (s, 1H), 4.64 (t, J = 9.1, 2H), 4.32 (d, J = 3.8, 1H), 4.21 – 4.05 (m, 2H), 3.97 (s, 1H), 3.77 (d, J = 2.1, 1H), 3.61 – 3.25 (m, 4H), 1.42 (s, 9H). ^{13}C NMR (50 MHz, CDCl_3) δ 155.96, 143.38, 143.35, 143.31, 143.29, 143.28, 137.49, 128.35, 128.30, 127.83, 127.65, 127.51, 127.05, 87.17, 86.60, 81.81, 79.60, 79.10, 77.64, 77.00, 76.51, 71.60, 62.32, 42.84, 28.23.



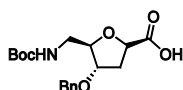
tert-butyl ((2R,3S,5R)-3-(benzyloxy)-5-(trityloxymethyl)tetrahydrofuran-2-yl)methylcarbamate (**13**)

Protected amine (**12**) (0.18 g, 0.3 mmol) was co-evaporated with toluene (1x), 1,1-thiocarbonyldiimidazole (0.23 g, 1.2 mmol, 4 equiv) was added, the reaction mixture was heated to 90 °C and refluxed for 5h after which TLC analysis (3/10 v/v EtOAc in PE) revealed conversion to a lower running product. EtOAc was added and the organic layer was washed with demi water (3x), dried over Na_2SO_4 , filtered and evaporated. The residue was co-evaporated with toluene (2x), re-dissolved in toluene and AIBN (9.8 mg, 0.06 mmol, 0.2 equiv) was added. Argon was bubbled through the flask for 15 min at 80 °C and then Bu_3SnH (0.27 g, 0.244 mL, 0.92 mmol, 4 equiv) was added. TLC analysis indicated completion of the reaction after 4h and the mixture was concentrated. The residue was purified by column chromatography (eluent: 1/20 $\rightarrow$ 2/10 v/v EtOAc in PE) yielding compound (**13**) (0.13 g, 0.22 mmol, 74%) as a colorless oil. ^1H NMR (200 MHz, CDCl_3) δ 7.58 – 7.11 (m, 20H), 4.92 – 4.75 (m, 1H), 4.52 (s, 2H), 4.34 (s, 1H), 4.23 – 3.87 (m, 4H), 3.37 (s, 1H), 3.17 (d, J = 4.5, 4H), 1.42 (s, 9H). ^{13}C -NMR (CDCl_3): δ 143.75 (C_q Trt), δ 137.90 (C_q Bn), δ 128.53 – 126.80 (CH_{arom} Trt, Bn), δ 82.89 (C5), δ 80.62 (C2), δ 77.74 (C4), δ 71.13 (C10), δ 65.76 (C1), δ 43.17 (C6), δ 34.61 (C3), δ 28.18 (CH_3 Boc)



tert-butyl ((2R,3S,5R)-3-(benzyloxy)-5-(hydroxymethyl)tetrahydrofuran-2-yl)methylcarbamate (**14**)

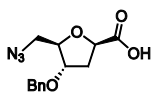
De-oxygenated (**13**) (9.9 g, 17 mmol) was diluted in a mixture of DCM / MeOH (9:1 v/v, 106 mL), heated up to 40 °C, pTsOH (10.2 mmol, 1.94 g) was added and the system was refluxed for 6h. When TLC analysis (1/5 v/v EtOAc in PE) indicated completion, sat. aq. NaHCO_3 and brine were added, the aqueous layer was washed with ether (5x) and the organic layer was dried over MgSO_4 , filtered and evaporated. Purification by silica gel column (eluent: 2/5 $\rightarrow$ 3/5 v/v EtOAc in PE) yielded (**14**) (4 g, 11.9 mmol, 70%) as a colorless oil. ^1H NMR (200 MHz, CDCl_3) δ 7.39 – 7.28 (m, 4H), 4.84 (s, 1H), 4.52 (s, 2H), 4.29 (s, 1H), 4.21 – 4.08 (m, 1H), 4.08 – 3.86 (m, 3H), 2.03 – 1.97 (m, 2H), 1.44 (s, 10H). ^{13}C -NMR (50 MHz, CDCl_3): δ 156.43, 137.66, 128.17 – 127.35, 83.53, 81.13, 79.37, 70.94, 63.03, 43.26, 32.37, 28.09. MS: m/z = 338.2 [$\text{M}+\text{H}$] $^+$, 282.1 [$\text{M}-t\text{Bu}+\text{H}$] $^+$, 238.1 [$\text{M}-\text{Boc}+\text{H}$] $^+$



(2S,4S,5R)-4-(benzyloxy)-5-((tert-butoxycarbonylamino)methyl)tetrahydrofuran-2-carboxylic acid (**15**)

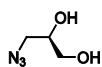
Alcohol (**14**) (2.4 g, 7 mmol) was diluted in a solution of TEMPO in CH_3CN (18.2 mg in 14 mL) and KBr in sat. NaHCO_3 (88 mg, 0.7 mmol in 18.2 mL) and cooled to 0 °C. 87 mL of a solution of NaOCl (31 mL), NaHCO_3 (19.6 mL) and NaCl (36.4 mL) was slowly added over a 1h period, after which there was no more change of color with extra addition of the solution. TLC analysis (4/5 v/v EtOAc in PE) indicated completion of the reaction and the reaction mixture was quenched with MeOH, after which it was washed with DCM and the aqueous layer was

acidified with 1M HCl solution to pH 6, washed with DCM (4x), dried over MgSO_4 , filtered and evaporated. Compound (**15**) was retrieved as a yellow solid (1.96 g, 5.6 mmol, 80%). $^1\text{H-NMR}$ (CDCl_3): δ 7.27 – 7.09 7.33 (m, 5H, Bn), δ 1.41 (s, 9H, 3x Me), $^{13}\text{C-NMR}$ (CDCl_3): δ 175.22, 156.84, δ 137.25, 128.70 – 127.30, 84.61, 79.60, 76.05, 70.96, 42.61, 35.81, 27.96. MS: m/z = 352.1 $[\text{M}+\text{H}]^+$, 252.1 (M-Boc+H) $^+$



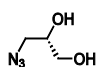
(2R,4S,5R)-5-(azidomethyl)-4-(benzyloxy)tetrahydrofuran-2-carboxylic acid (**4**)

Boc-protected amine **15** (175 mg, 0.5 mmol) was treated with 50% TFA/DCM (2 mL) for 30 minutes before it was concentrated. Residual traces of TFA were removed by repeated coevaporation with toluene (5x 2 mL) to yield the crude TFA-salt which was dissolved in H_2O (1.6 mL). To this solution K_2CO_3 (207 mg, 1.5 mmol, 3 eq.) was added, followed by a solution of CuSO_4 (8 mg, 0.05 mmol, 0.1 eq.) in H_2O (0.5 mL) and MeOH (3.2 mL). To this homogenous solution, freshly prepared TfN_3 (5 mmol) in DCM (7 mL) was added and the reaction was allowed to stir for 16h before the reaction was diluted with DCM (10 mL) and H_2O (10 mL) and the aqueous phase washed with DCM (2x5 mL). The aqueous phase was acidified to pH = 2 by addition of sat. aq. citric acid and extracted with DCM (3x5 mL) to yield the title compound (107 mg, 0.39 mmol, 77%) as colorless oil. $^1\text{H NMR}$ (200 MHz, MeOD) δ 10.09 (s, 1H), 7.44 – 7.17 (m, 4H), 4.71 (t, J = 7.9, 1H), 4.54 (d, J = 5.4, 2H), 4.26 – 4.16 (m, 1H), 4.10 – 4.00 (m, 1H), 3.46 (dd, J = 1.4, 4.7, 2H), 2.46 (ddd, J = 3.4, 7.4, 13.4, 1H), 2.23 (ddd, J = 5.1, 7.2, 8.4, 1H). NMR (50 MHz, MeOD) δ 176.10, 137.16, 128.54, 128.05, 127.70, 83.70, 79.60, 76.41, 71.67, 52.38, 35.91. MS: m/z = 555.2 $[2\text{M}+\text{H}]^+$, 277.1 $[\text{M}+\text{H}]^+$, 250.1 $[\text{M}-\text{N}_2+\text{H}]^+$.

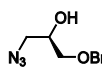


(R)-3-azidopropane-1,2-diol (**18**)

A solution of S(-)-glycidol **16** (1.26 mL, 20 mmol) in t-BuOH (100 mL) was charged with a solution of LiBF_4 (375 mg, 4 mmol) in H_2O (4 mL) and NaN_3 (1.6 g, 25 mmol) and stirred for 5 h at 70°C before the reaction mixture was concentrated. The residue was directly applied to a silica column and eluted with 5% MeOH/EtOAc to afford the title compound (1.15 g, 9.8 mmol, 49%) as colorless oil. $^1\text{H NMR}$ (200 MHz, MeOD) δ 3.85 – 3.68 (m, 1H), 3.53 (s, 1H), 3.34 (s, 2H), 3.34 – 3.29 (m, 2H). $^{13}\text{C NMR}$ (50 MHz, MeOD) δ 71.98, 64.20, 54.20. α_{D}^{20} (MeOH) = +12.2°



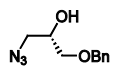
(S)-3-azidopropane-1,2-diol (**19**) was prepared similarly to **18** and obtained as colorless oil (1.06 g, 9.1 mmol, 44%). $^1\text{H NMR}$ (200 MHz, MeOD) δ 3.91 – 3.70 (m, 1H), 3.52 (s, 1H), 3.37 (s, 2H), 3.34 – 3.28 (m, 2H). $^{13}\text{C NMR}$ (50 MHz, MeOD) δ 72.05, 64.29, 54.09. α_{D}^{20} (MeOH) = -17.4°



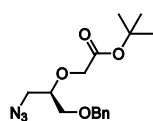
(R)-1-azido-3-(benzyloxy)propan-2-ol (**20**)

Diol **17** (1.15 g, 9.8 mmol) was coevaporated with toluene (3x 25 mL) and dissolved in toluene (25 mL) before addition of Bu_2SnO (3.6 g, 15 mmol, 1.5 eq.). The reaction mixture was stirred at 100°C for 3 h, before it was concentrated. The residue was coevaporated with toluene (3x25 mL) and redissolved in toluene (25 mL). To this solution, Benzylbromide (2.4 mL, 20 mmol 2 eq.) was added and reaction stirred at 100°C until TLC analysis (25% EtOAc/PE) indicated the formation of a higher running product. The reaction mixture was concentrated, the residue partitioned between EtOAc/ H_2O and the aqueous phase extracted with EtOAc (3x25 mL). The combined organic phase was washed with sat. aq. bicarb. (25 mL) and brine (25 mL), dried over

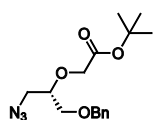
Na_2SO_4 , filtered and concentrated. The residue was purified by silica column chromatography (0-8% EtOAc/PE) to yield the title compound (1.25 g, 6.0 mmol, 62%) as colorless oil. NMR (200 MHz, CDCl_3) δ 7.59 – 7.13 (m, 7H), 4.87 (bs, 1H), 4.53 (s, 2H), 3.99 – 3.78 (m, 1H), 3.55 – 3.44 (m, 3H), 3.36 – 3.24 (m, 3H). NMR (50 MHz, CDCl_3) δ 139.27, 129.46, 128.86, 74.30, 72.64, 70.67, 54.82. α_D^{20} (MeOH) = +17.0°



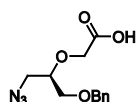
(S)-1-azido-3-(benzyloxy)propan-2-ol (**21**) Benzyl ether **21** was prepared according to the procedure described for **20** to yield the product (1.87g, 9 mmol, 28%) as colorless oil. ^1H NMR (400 MHz, MeOD) δ 7.38 – 7.32 (m, 4H), 5.13 (bs, 2H), 4.53 (s, 2H), 3.83 – 3.70 (m, 1H), 3.61 (dd, J = 5.2, 10.2, 2H), 3.53 – 3.35 (m, 2H). ^{13}C NMR (101 MHz, CDCl_3) δ 137.38, 128.27, 127.70, 127.61, 127.29, 126.75, 73.26, 71.18, 69.39, 64.76, 53.25. α_D^{20} (MeOH) = -12.6°



(R)-tert-butyl 2-(1-azido-3-(benzyloxy)propan-2-yloxy)acetate (**22**) Alcohol **20** (1.15 g, 5.55 mol) was dissolved in DMF (25 mL) at 0°C under an atmosphere of argon before addition of NaH (266 mg, 6.67 mmol, 1.2 eq. 60% susp. mineral oil). After the gas evolution ceased, *tert*-butylbromoacetate (1.6 mL, 11.1 mmol, 2 eq.) was added and the reaction mixture was allowed to stir at r.t. until TLC analysis (10% EtOAc/PE) indicated the formation of a higher running spot. The reaction was quenched by the addition of MeOH (5 mL) and partitioned between Et₂O (50 mL) and H₂O (100 mL). The aqueous phase was washed with Et₂O (2x 25 mL) and the combined organic phase with sat. aq. bicarb. (25 mL) and brine (25 mL), dried (Na_2SO_4), filtered and concentrated. The residue was applied to a silica column and eluted with 0-3% EtOAc/PE to afford the title compound (1.14 g, 3.56 mmol, 64%) as colorless oil. NMR (200 MHz, CDCl_3) δ 7.29 (d, J = 13.8, 6H), 4.54 (s, 2H), 4.15 (s, 2H), 3.80 – 3.66 (m, 1H), 3.66 – 3.57 (m, 2H), 3.52 – 3.44 (m, 2H), 1.47 (s, 9H). NMR (50 MHz, CDCl_3) δ 169.50, 137.78, 128.41, 127.76, 127.68, 81.71, 78.25, 73.53, 69.87, 68.24, 52.15, 28.04. α_D^{20} (MeOH) = +7.2°; MS: m/z = 344.0 $[\text{M}+\text{Na}]^+$, 339.0 $[\text{M}+\text{NH}_4]^+$, 322.0 $[\text{M}+\text{H}]^+$, 288.1 $[\text{M}-\text{tBu}+\text{Na}]^+$, 296.1 $[\text{M}-\text{N}_2+\text{H}]^+$, 266.0 $[\text{M}-\text{tBu}+\text{H}]^+$, 238 $[\text{M}-\text{tBu}-\text{N}_2+\text{H}]^+$

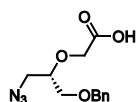


(S)-tert-butyl 2-(1-azido-3-(benzyloxy)propan-2-yloxy)acetate (**23**) *Tert*-butylester **23** was prepared in a similar fashion as described for **22** resulting in the isolation of the target compound (153 mg, 0.48 mmol, 40%) as colorless oil. NMR (200 MHz, CDCl_3) δ 7.32 (s, 5H), 4.53 (s, 2H), 4.14 (s, 2H), 3.78 – 3.65 (m, 1H), 3.65 – 3.55 (m, 2H), 3.50 – 3.41 (m, 2H), 1.46 (s, 9H). NMR (50 MHz, CDCl_3) δ 169.30, 137.65, 128.22, 127.55, 127.47, 81.43, 78.07, 73.30, 69.69, 68.03, 51.94, 27.86. α_D^{20} (MeOH) = -9.0°; MS: m/z = 344.1 $[\text{M}+\text{Na}]^+$, 321.9 $[\text{M}+\text{H}]^+$, 296.1 $[\text{M}-\text{N}_2+\text{H}]^+$, 265.9 $[\text{M}-\text{tBu}+\text{H}]^+$



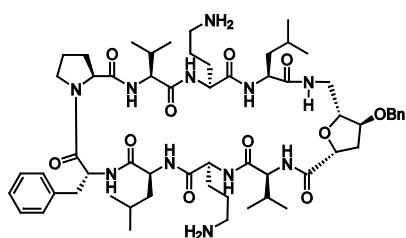
(R)-2-(1-azido-3-(benzyloxy)propan-2-yloxy)acetic acid (**5**) *Tert*-butylester **22** (1.04 g, 3.2 mmol) was dissolved a mixture of DCM/TFA/TIS (10 mL, 1/1/0.05) and stirred for 1h before TLC analysis (EtOAc/AcOH 99/1) indicated complete conversion of the starting material into a lower running spot. The reaction mixture was concentrated and repeatedly evaporated with toluene (3x10 mL) and partitioned between DCM (10 mL) and sat. aq. bicarb. (10 mL). The aqueous phase was washed with DCM (2x 5mL), acidified to pH=2 with 1M HCl and washed with DCM (3x 10 mL). The combined organic phase was dried (Na_2SO_4), filtered and concentrated to afford the title compound (611 mg,

2.3 mmol, 72%) as colorless oil. NMR (200 MHz, MeOD) δ 7.32 (d, J = 2.6, 5H), 4.50 (s, 2H), 4.23 (s, 2H), 3.79 – 3.65 (m, 1H), 3.59 (d, J = 2.9, 1H), 3.57 (d, J = 3.1, 1H), 3.41 (t, J = 5.0, 2H). NMR (50 MHz, MeOD) δ 173.83, 139.29, 129.38, 128.83, 79.71, 74.36, 70.95, 68.24, 52.76. α_{20} (MeOH) = +13.6°; MS: m/z = 283.3 $[M+Na]^+$, 266.1 $[M+H]^+$, 238.1 $[M-N_2+H]^+$.

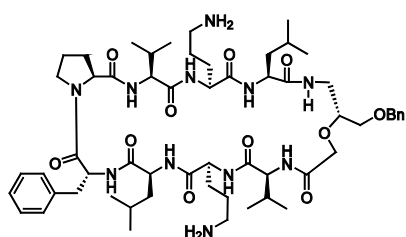


(S)-2-(1-azido-3-(benzyloxy)propan-2-yloxy)acetic acid (**6**)

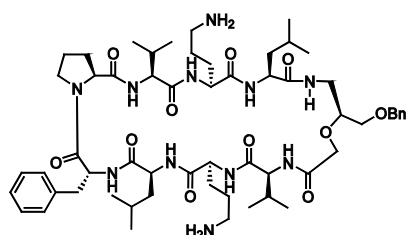
Following the same procedure as described for **5**, *S*-Tert-butylester **23** (150 mg, 0.47 mmol) yielded the title compound (100 mg, 0.40 mmol, 86%) as colorless oil. NMR (200 MHz, MeOD) δ 7.24 (q, J = 2.5, 5H), 4.43 (s, 2H), 4.16 (s, 2H), 3.75 – 3.59 (m, 1H), 3.53 (d, J = 2.9, 1H), 3.50 (d, J = 3.1, 1H), 3.34 (t, J = 4.9, 2H). NMR (50 MHz, MeOD) δ 173.81, 139.27, 129.36, 128.82, 79.68, 74.34, 70.93, 68.22, 52.74. α_{20}^d (MeOH) = -16.8°; MS: m/z = 266.1 $[M+H]^+$, 238.1 $[M-N_2+H]^+$.



Compound **7** was prepared according to the general procedure on a 200 μ mol scale. Yield: 54.5 mg, 40 μ mol, 20% ^1H NMR (500 MHz, MeOD) δ 8.91 (d, J = 3.1, 1H), 8.79 (d, J = 9.1, 1H), 8.74 – 8.65 (m, 2H), 8.49 (d, J = 9.4, 1H), 8.27 (t, J = 6.2, 1H), 7.75 (d, J = 7.4, 1H), 7.64 (d, J = 9.2, 1H), 7.37 – 7.21 (m, 9H), 4.54 (d, J = 4.7, 0H), 4.53 – 4.47 (m, 0H), 4.43 (dd, J = 5.4, 11.2, 0H), 4.36 (d, J = 7.5, 0H), 4.31 – 4.24 (m, J = 8.9, 0H), 4.08 (d, J = 5.2, 0H), 4.01 (t, J = 7.6, 0H), 3.80 (dd, J = 6.3, 15.9, 0H), 3.75 – 3.67 (m, 1H), 3.44 (s, 0H), 3.21 (dd, J = 7.5, 15.5, 1H), 3.16 (s, 0H), 3.11 – 2.98 (m, 3H), 2.98 – 2.84 (m, 4H), 2.54 – 2.42 (m, 1H), 2.36 (dd, J = 5.2, 13.1, 1H), 2.29 – 2.13 (m, 3H), 2.02 (d, J = 16.2, 2H), 1.82 – 1.39 (m, 20H), 1.38 – 1.23 (m, 10H), 1.20 (s, 2H), 1.03 (d, J = 6.8, 3H), 0.97 (d, J = 6.9, 3H), 0.93 (d, J = 6.8, 4H), 0.91 – 0.84 (m, 17H). MS: m/z = 1130.8 $[M+H]^+$, 566.2 $[M+2H]^{2+}$. HRMS calcd for $[C_{59}H_{91}N_{11}O_{11}+H]^+$ 1130.68713, found 1130.69908.



Compound **8** was prepared according to the general procedure on a 200 μ mol scale. Yield: 123 mg, 91 μ mol, 46% ^1H NMR (600 MHz, MeOD) δ 8.95 (d, J = 3.2, 1H), 8.74 – 8.68 (m, 2H), 8.65 (d, J = 9.2, 1H), 8.54 (d, J = 9.3, 1H), 8.03 (t, J = 6.1, 1H), 7.91 (s, 2H), 7.86 (s, 1H), 7.64 (d, J = 9.0, 1H), 7.55 (d, J = 8.1, 1H), 7.34 – 7.21 (m, 11H), 4.85 – 4.77 (m, 1H), 4.67 – 4.61 (m, 1H), 4.54 (dd, J = 3.9, 10.7, 3H), 4.49 (dd, J = 7.3, 11.5, 1H), 4.36 (d, J = 7.8, 1H), 4.25 (t, J = 8.4, 1H), 4.22 – 4.13 (m, 2H), 3.96 (s, 1H), 3.91 (d, J = 14.7, 1H), 3.71 (d, J = 8.2, 1H), 3.63 (s, 1H), 3.55 (d, J = 5.2, 3H), 3.44 (d, J = 19.2, 0H), 3.28 – 3.26 (m, 1H), 3.07 (dd, J = 4.8, 12.6, 1H), 3.04 – 2.97 (m, 1H), 2.92 (dd, J = 12.7, 24.6, 5H), 2.45 (t, J = 9.0, 1H), 2.25 (dd, J = 6.9, 13.9, 1H), 2.13 (dd, J = 6.9, 13.7, 1H), 2.06 – 1.97 (m, 2H), 1.96 – 1.91 (m, 1H), 1.81 – 1.75 (m, 2H), 1.72 – 1.46 (m, 17H), 1.41 (t, J = 7.1, 1H), 0.98 (d, J = 6.7, 4H), 0.96 (d, J = 6.8, 4H), 0.93 (d, J = 6.7, 4H), 0.91 (d, J = 6.3, 4H), 0.90 – 0.83 (m, 17H). HRMS calcd for $[C_{58}H_{91}N_{11}O_{11}+H]^+$ 1118.69723, found 1118.69896

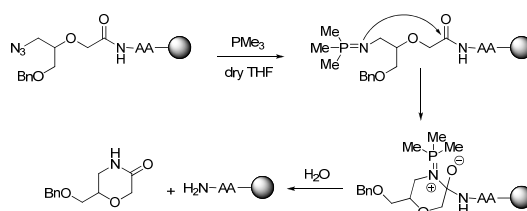


Compound **9** was prepared according to the general procedure on a 200 μmol scale. Yield: 56.1 mg, 41.7 μmol , 21% ^1H NMR (600 MHz, MeOD) δ 8.94 (d, J = 3.2, 1H), 8.74 (d, J = 3.4, 1H), 8.72 (d, J = 5.0, 1H), 8.65 (d, J = 9.0, 1H), 8.58 (d, J = 9.2, 1H), 7.94 (t, J = 8.7, 2H), 7.90 (s, 1H), 7.77 (s, 1H), 7.63 (d, J = 9.1, 1H), 7.37 – 7.20 (m, 13H), 4.90 (d, J = 3.9, 4H), 4.81 (d, J = 8.3, 1H), 4.67 – 4.62 (m, 1H), 4.54 (s, 2H), 4.53 – 4.47 (m, 3H), 4.37 (d, J = 7.8, 1H), 4.30 – 4.25 (m, 1H), 4.24 – 4.16 (m, 3H), 4.05 (d, J = 13.2, 1H), 3.94 – 3.88 (m, 1H), 3.76 (dd, J = 3.8, 7.0, 1H), 3.73 – 3.67 (m, 2H), 3.59 – 3.50 (m, 3H), 3.07 (dd, J = 4.8, 12.5, 1H), 3.02 (dd, J = 4.1, 14.2, 3H), 2.97 – 2.85 (m, 4H), 2.80 (s, 1H), 2.71 (s, 1H), 2.45 (t, J = 8.8, 1H), 2.24 (dd, J = 6.9, 13.8, 1H), 2.19 – 2.10 (m, 1H), 2.01 (dd, J = 10.0, 13.4, 3H), 1.80 – 1.72 (m, 4H), 1.70 – 1.59 (m, 6H), 1.58 – 1.49 (m, 10H), 1.47 – 1.39 (m, 3H), 0.99 (d, J = 6.7, 4H), 0.97 (d, J = 6.8, 5H), 0.93 (d, J = 6.7, 4H), 0.92 – 0.84 (m, 24H). HRMS calcd for $[\text{C}_{58}\text{H}_{91}\text{N}_{11}\text{O}_{11}+\text{H}]^+$ 1118.69723, found 1118.69870

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Chapter 6 | Structural and Biological Evaluation of Novel Loloatin C Analogues

Introduction

The Loloatins are cationic antimicrobial peptides (CAPs) isolated from laboratory cultures of bacteria collected from the reefs of Loloata Island, Papua New Guinea.¹ Four different loloatins were discovered. These four cyclic peptides are named Loloatin A, B, C and D and their structures are depicted in Figure 1. Loloatins A-D are active against Gram positive bacteria with MIC values between 1 and 8µg/mL against a panel of 8 organisms tested.² Besides being the most potent of the four family members, Loloatin C was also the only one active against the Gram negative bacteria strain *Escherichia Coli*, making it an interesting lead compound for further development towards a broad spectrum antibiotic. There is no literature precedence concerning the hemolytical activity of these CAPs.

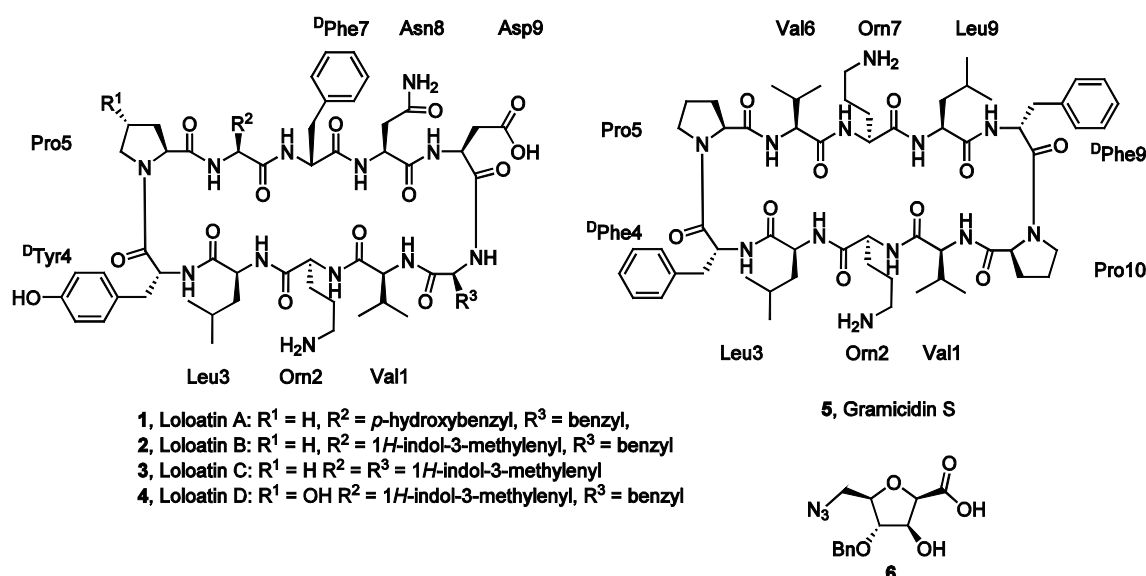


Figure 1; Chemical structures of Loloatin A-D, GS and SAA 6

Loloatin C shows a remarkable resemblance to Gramicidin S. Both are cyclic decapeptides, and they share 4 out of the 10 amino acids in their primary sequence. GS is composed of two identical pentapeptides, each consisting of a β -strand (Val-Orn-Leu) connected to a type II' β -turn motif ($^D\text{Phe-Pro}$). Loloatin C has one closely related pentapeptide motif (with a ^DTyr instead of a ^DPhe) supplemented with a Trp- $^D\text{Phe-Asn}$ moiety and a type I β -turn formed by Asp-Trp. Whereas GS is likely to carry two positive charges in biological surroundings, Loloatin C has a zwitterionic character. The solution structure of GS has been extensively studied in a variety of solvents.³ In each of these solvents GS adopts a rigid β -sheet conformation that is stabilized by four interstrand hydrogen bonds. This β -sheet conformation is characterized by an amphiphilic orientation of the individual side chains, with the hydrophobic Val and Leu residues on one side of the molecule and the hydrophilic Orn side chains on the other. The solution structure of Loloatin C is much less defined.⁴ The secondary structure is found to be solvent dependent. In DMSO, a hydrogen bond accepting solvent, the secondary structure of Loloatin C was analyzed by means of chemical shift perturbation and NOESY analysis. A β -strand composed of Leu-Orn-Val and an opposing α -helical⁵ Trp- $^D\text{Phe-Asn}$ strand are interconnected *via* two turn motifs composed of Pro- ^DTyr and Asp-Trp. The lack of chemical shift reference data in water / trifluoroethanol (TFE, a hydrogen bond donating solvent known to stabilize secondary structural elements)⁶ precluded solid conclusions about the conformation of Loloatin C. In a 30% TFE / H_2O mixture, NOESY analysis revealed a less defined structure with only one secondary structural

element, namely, an inverse γ -turn around the $^{\text{D}}\text{Tyr-Pro-Trp}$ sequence. Increasing the TFE content in the solvent mixture to 70% induced a remarkable structural change. A dumbbell-like conformation was identified centered around the Orn and $^{\text{D}}\text{Phe}$ residues. Interestingly, this conformation orientates the hydrophilic sidechains (Orn, Asn and Asp) to one side of the molecule and the remaining hydrophobic side chains to the other, resulting in an amphiphilic overall structure.

The flexibility of Loloatin C and the zwitterionic character present attractive features amenable for optimization towards Loloatin C analogues with improved antibacterial properties and reduced hemolytic activity.

In the previous chapter, a study is described in which GS was modified by the incorporation of a series of SAAs replacing the β -turn motif consisting of a Pro and a $^{\text{D}}\text{Phe}$ residue. It was shown that SAA **6** could be used as a turn inducing dipeptide isoster with a slightly distinct architecture compared to the natural β -turn. In the study described in this chapter, the primary structure of Loloatin C is modified by replacing single amino acids and / or incorporating SAA **6**. The newly synthesized analogues were evaluated for their antibacterial and hemolytical activities and their secondary structure were assessed by means of NMR analysis.

The target molecules are depicted in Figure 2. As can be seen, SAA **6** replaces each of the three putative turn motifs in Loloatin C. Firstly, the type II' β -turn comprised of the Pro- $^{\text{D}}\text{Tyr}$ motif, similar to the turns found in GS, is replaced (**7**, Figure 2). Secondly, the Asn- $^{\text{D}}\text{Phe}$ part of the α -helical turn is replaced (**8**, Figure 2) and replacing the type I turn inducing Asp-Trp motif with SAA **6** (**9**, Figure 2) completes the series. In analogue **10** (Figure 2) SAA **6** replaces the Asp-Trp motif and the $^{\text{D}}\text{Phe}$ within the α -helical turn is replaced by $^{\text{L}}\text{Phe}$. The influence of using $^{\text{L}}\text{Phe}$ instead of $^{\text{D}}\text{Phe}$ is further studied in **11** and in **12** which has an additional replacement of the $^{\text{L}}\text{Asp}$ with a $^{\text{D}}\text{Asp}$ intended as modified turn inducing motif (Figure 2).

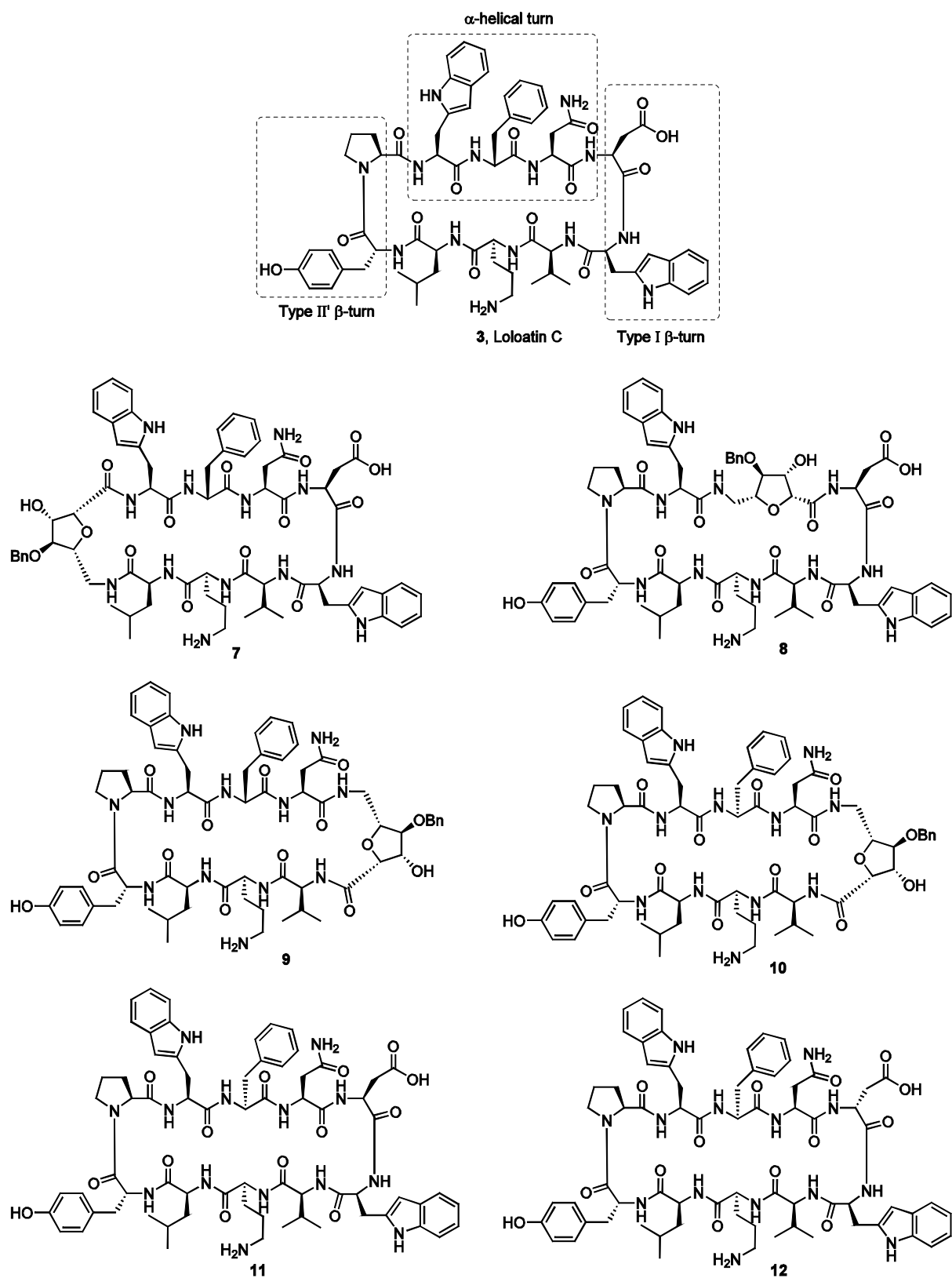
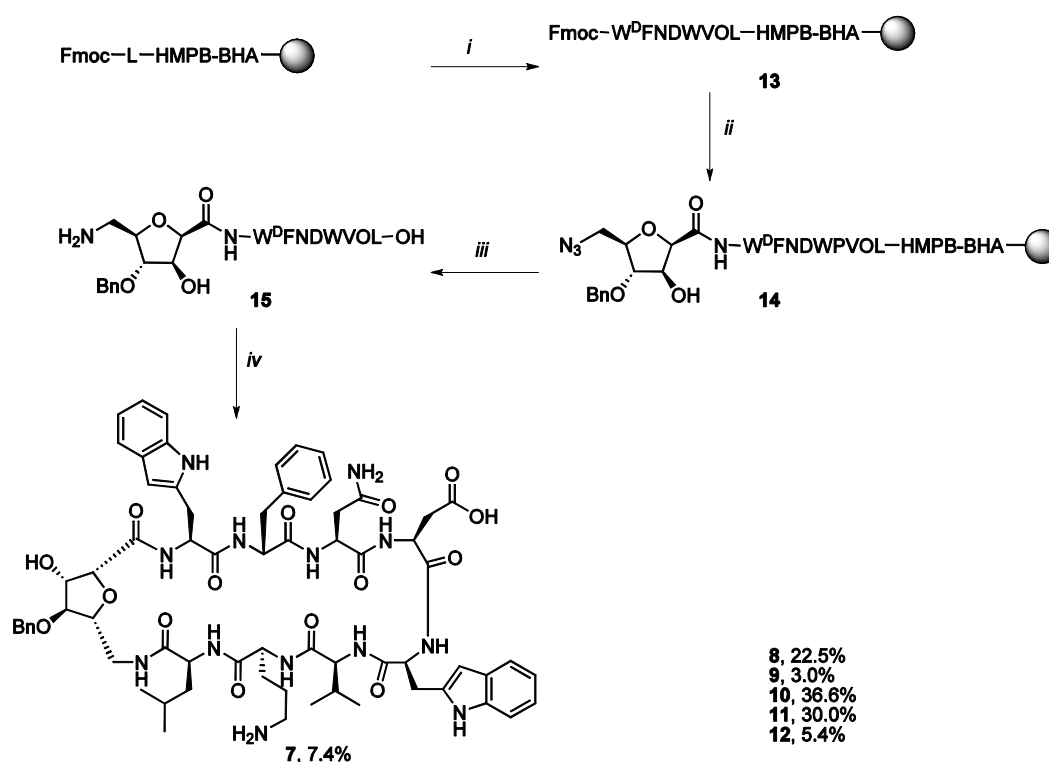


Figure 2; Loloatin C and newly designed derivatives 7 - 12

Results and discussion

The synthesis of the Loloatin C analogues is exemplified in Scheme 1 for the construction of compound **7**. Standard Fmoc based solid phase peptide synthesis (SPPS) starting from commercially available HMPB-BHA resin preloaded with Fmoc-Leucine is subjected to 7 coupling cycles under the agency of HCTU en DiPEA affording linear octapeptide **13**. Staudinger reduction of the terminal azide functionality in **14** using PMe_3 in wet THF followed by acidic cleavage from the resin afforded **15** which was subsequently cyclized upon PyBOP/HOBt/DiPEA treatment in DMF under dilute conditions and deprotected using 50%TFA/DCM. HPLC purification yielded the target compound in 7.4 % yield. Analogues **8** to **12** were prepared following the same strategy, and although the final yields varied considerably, suitable quantities of each compound for the ensuing biological and hemolytical studies were obtained.



Scheme 1; Synthesis of Lolatin C derivatives using SPPS. *Reagents and conditions:* 8 cycles of a: 20% pip/NMP, 3x5 min.; b: Fmoc-AA-OH (5 eq.), HCTU (5 eq.), DiPEA (10 eq.) 90 min. (ii) **6** (1.5 eq.), HCTU (1.5 eq.), DiPEA (3 eq.), 16 h. (iii) a: PMe_3 (1M in 9:1 THF/ H_2O , 25 eq.), 16 h. b: 1% TFA/DCM. (iv) a: PyBOP (5 eq.), HOBt (5 eq.), DiPEA (10 eq.), 16h. b: 50% TFA/DCM, 1h.

NMR studies of Loloatin derivatives 7 - 12

Next, attention was focused on the structural analysis of the newly designed Loloatin C analogues in solution by means of NMR spectroscopy. The structure of each of the analogues was assessed by the analysis of the vicinal $^3J_{\text{NH-H}\alpha}$ coupling constant⁷, the chemical shift perturbation⁸ and the NOESY spectrum. The vicinal $^3J_{\text{NH-H}\alpha}$ coupling constant correlates with the dihedral angle within an amino acid residue. It can be used in a qualitative manner to identify secondary structural elements within a peptide. Consecutive large values (8.5 – 9.5 Hz) correspond to dihedral angles commonly found in β -sheets, small values for $^3J_{\text{NH-H}\alpha}$ (3.5 – 4.5 Hz) correspond to those found in an α -helix. Isolated occurrences of small values for $^3J_{\text{NH-H}\alpha}$ (< 4 Hz) indicate the presences of a turn within the peptide chain. Another qualitative method to distinguish between secondary structural elements is the chemical shift perturbation. This method relies on the comparison of the measured chemical shift of the α -proton of a certain residue and the standard value of that same residue in a random coil configuration ($\Delta\delta\text{H}_\alpha = \text{observed } \delta\text{H}_\alpha - \text{random coil } \delta\text{H}_\alpha$). This is based on the observation that α -protons of residues within an α -helix experience an upfield shift and residues within a β -sheet experience a downfield shift compared to the chemical shift of that residue in a random coil configuration.

The observed values for the vicinal coupling constants $^3J_{\text{NH-H}\alpha}$ for the Loloatin C analogues are depicted in Figure 3. The chemical shift perturbations are depicted in Figure 4. The secondary structure of all derivatives contain a turn motif around $^{\text{D}}\text{Tyr}$ ($^3J_{\text{NH-H}\alpha} < 4 \text{ Hz}$), flanked by two strand regions ($8.5 < ^3J_{\text{NH-H}\alpha} < 9.5 \text{ Hz}$). The Leu-Orn-Val sequence forms a β -strand in all analogues. However, the relatively low value for $^3J_{\text{NH-H}\alpha}$ of Val in **7** indicates distortion of the residue. Interpretation of the chemical shift perturbation underlines these findings. The Trp_6 - $^{\text{(D or L)}}\text{Phe-Asn}$ motif is more variable among the different compounds and no general conclusions can be drawn. In compound **9**, the $^{\text{D}}\text{Tyr}$, Trp_6 and $^{\text{D}}\text{Phe}$ residues appear to form a helical region, as evidenced by their negative chemical shift perturbation values. The position of residues 9 and 10 in analogues are connected either by SAA **6** (in **10**) or by $^{\text{(D or L)}}\text{Asp-Trp}$ motif (in **11** and **12** resp.). The structure of compounds **10** and **11** are remarkably similar except for the regions round the $^{\text{L}}\text{Phe}$ residues. The ^1H -NMR spectra of **8** and **12** show severe peak broadening, and the limited number of sequential NOE signals in the 2D-NMR prevented complete assignment of the spectra.

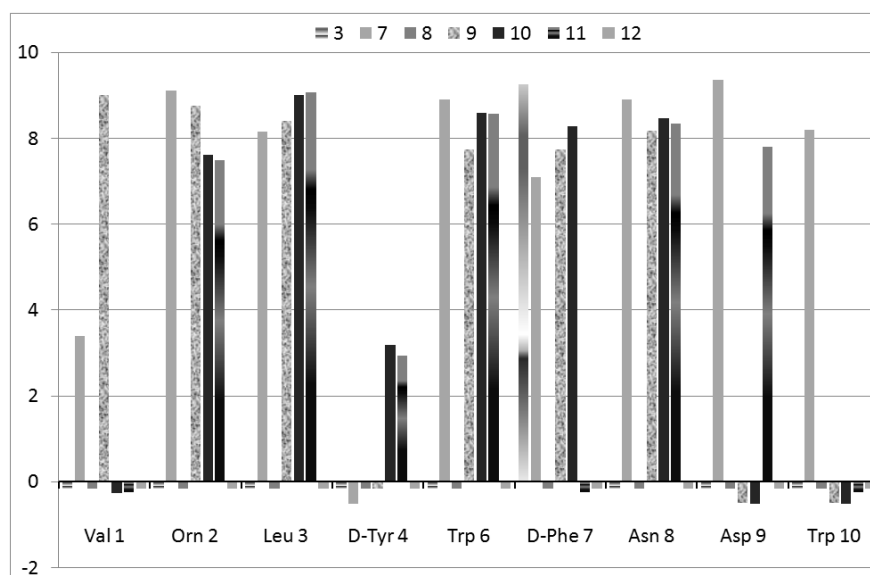


Figure 3; Values of the vicinal $^3J_{\text{NH-H}\alpha}$ (Hz) for Loloatin C and its derivatives.

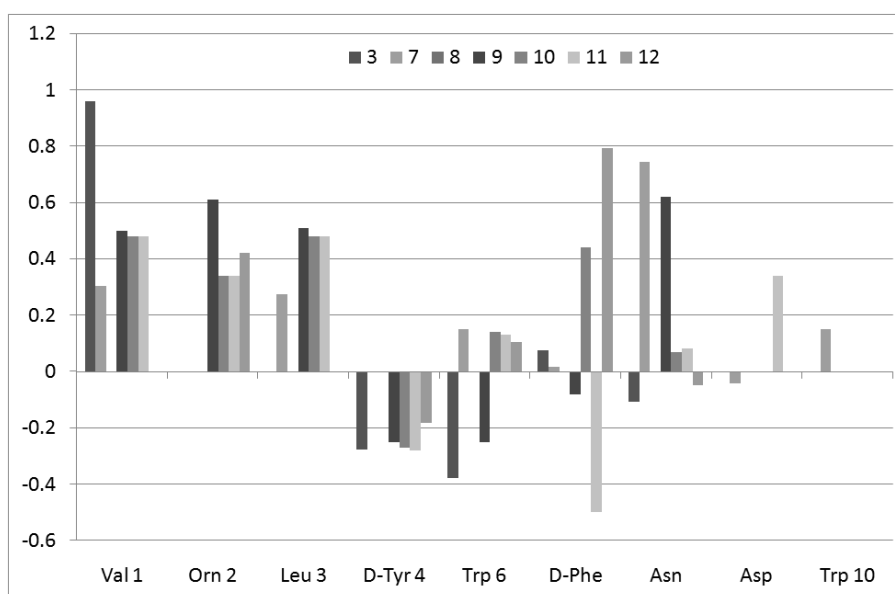


Figure 4; Chemical shift perturbation for Loloatin C and its derivatives.

Biological evaluation

Finally, the antimicrobial and hemolytical potencies of the novel Loloatin C derivatives were determined (Table 1 and Figure 5). Whereas the native Loloatin C (**3**) shows some antimicrobial potency against gram positive strains, it is inactive against the gram negative strains used in this assay. Unfortunately, Loloatin C is also hemolytically active. As for the analogues **7**, **8**, **9** and **12** both the antimicrobial and hemolytical⁹ activities are highly reduced compared to Loloatin C. Only analogues **10** and **11** are marginally active in both assays.

Table 1; MIC values for compounds **3** and **7** - **13**

	Gram pos. Staph. aureus ATCC 29213	Gram pos. Staph. epidermis ATCC 12228	Gram pos. Enterococ. faecalis ATCC 29212	Gram neg. E. Coli ATCC 25922	Gram neg. P. aeruginosa ATCC 27853	Gram pos. Bacillus cereus ATCC 11778
3	8	8	8	>64	>64	8
7	>64	>64	>64	>64	>64	>64
8	>64	>64	>64	>64	>64	>64
9	>64	>64	>64	>64	>64	>64
10	>64	32	>64	>64	>64	64
11	64	32	>64	>64	>64	32
12	>64	>64	>64	>64	>64	>64

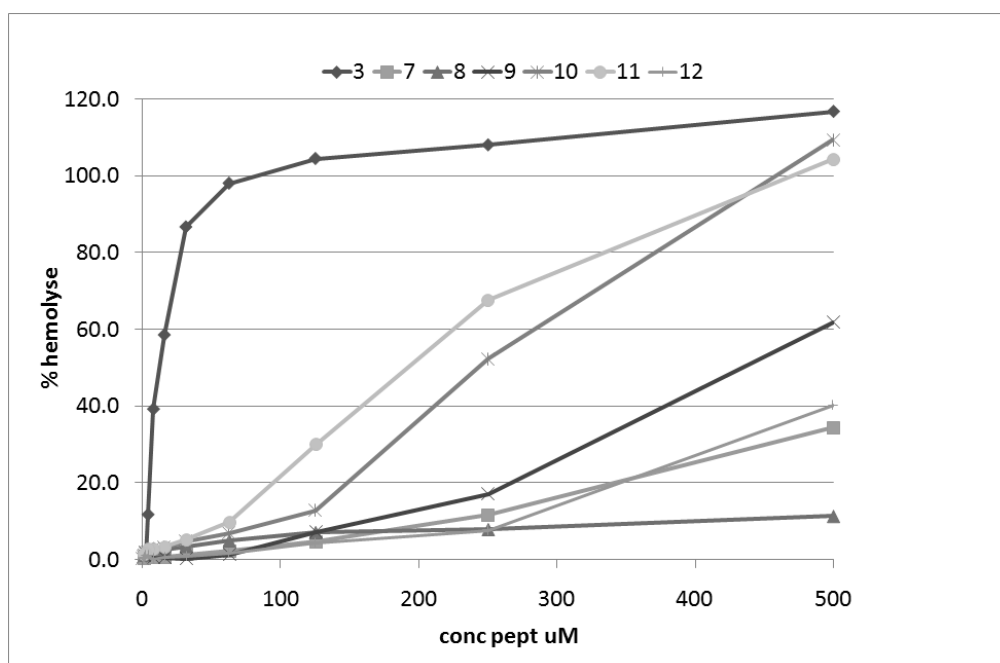


Figure 5; Hemolytical activity of the Loloatin C derivatives

Conclusion

Six novel analogues of Loloatin C were synthesized and analyzed by means of NMR spectroscopy. Four of these analogues contain sugar amino acid **6**, replacing 2 natural amino acids. Replacing either the Pro – D-Tyr or the Asp-Trp motif resulted in analogues (**7** – **10**) with defined secondary structures that could be analyzed based on 1D- and 2D-NMR spectra. Loloatin C and analogues **11** and **12** proved to be much more flexible in CD₃OH. The biological activities of all analogues were rather low with only marginal activities of analogues **10** and **11** in both the antimicrobial and hemolytical assay. The activity of native Loloatin C has been explained by the dumbbell-like conformation it adopts. This conformation induces an amphiphilic character of the peptide.² The reduced activity of analogues **7** – **12** might be explained by inability of these peptides to adopt a similar conformation.

Experimental section

General procedure for peptide synthesis:

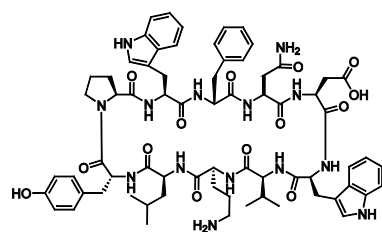
a) stepwise elongation: Fmoc-Leu-HMPB-BHA resin Resin (196 mg, 0.51 mmol/g, 0.1 mmol) was submitted to seven cycles of Fmoc solid-phase synthesis with the appropriate commercially available amino acid building blocks Fmoc-Orn(Boc)-OH, Fmoc-Val-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Pro-OH, Fmoc-D³Phe-OH, Fmoc-Phe-OH, Fmoc-Asp(tBu)-OH, Fmoc-Asn(Trt)-OH and Fmoc-Trp(Boc)-OH as follows: a) deprotection with piperidine / NMP (1/4, v/v, 5 mL, 15 min); b) wash with NMP (5 mL, 3x, 3 min); c) coupling of the appropriate Fmoc amino acid (5 eq., 0.5 mmol) in the presence of HCTU (5 eq., 0.5 mmol, 206 mg) and DiPEA (10 equiv., 1 mmol, 162 µL) which was preactivated for 2 min in NMP (5 mL) and shaken for 90 min; d) wash with NMP (5 mL, 3x, 3 min). Couplings were monitored for completion by the Kaiser test.¹⁰ Finally, the *N*-terminal amine was liberated by Fmoc-deprotection with piperidine / NMP (1/4, v/v, 5 mL, 15 min) followed by washing with NMP (5 mL, 3x, 3 min). Coupling of SAA **6** was performed as follows: To the resin bound peptide, a preactivated solution of SAA **6** (1.5 eq. 44 mg, 0.150 mmol), HCTU (1.5 eq., 62 mg, 0.150 mmol) and DiPEA (3.0 eq., 74 µL, 0.45 mmol) in NMP (3 mL) was added and the resulting suspension was shaken for 16h. The resin was finally washed with NMP (5 mL, 3x, 3 min) to give the title compound.

b) on-resin Staudinger reduction. The appropriate resin-bound azide was treated with a pre-mixed cocktail of H₂O (0.5 mL) and PMe₃ (3.5 mL, 1M in THF) and shaken for 16h. The resin was washed with methanol (4 mL, 3x, 3 min) and DMF (4 mL, 3x, 3 min.)

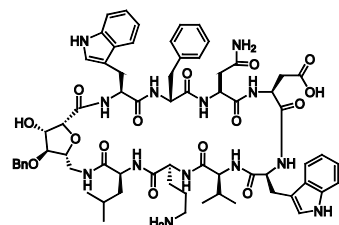
c) cleavage from the resin: Resin-bound nonapeptide was repeatedly treated with 1% TFA/DCM (4 mL). The filtrate was coevaporated with toluene (3x) and the residue was used as such in the cyclization step.

d) cyclization: The linear nonapeptide was taken up in DMF (5 mL) and added dropwise over the course of an hour to a solution of benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) (5 equiv., 270 mg, 0.5 mmol), HOBt (5 equiv., 67 mg, 0.5 mmol) and DiPEA (15 equiv., 254 μ L, 1.5 mmol) in DMF (70 mL) and allowed to stir for 16h. The solvent was removed *in vacuo* and the resulting mixture was used without further purification in the deprotection step.

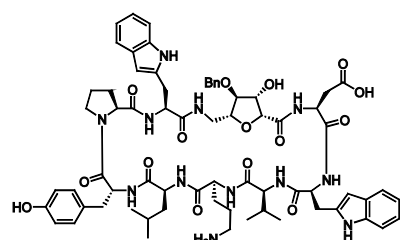
e) deprotection: The crude cyclised peptide was treated with 50% TFA/DCM (10 mL) for 1 h., before it was concentrated and purified by HPLC purification.



cyclo-[Val-Orn-Leu-^DTyr-Pro-Trp-^DPhe-Asn-Asp-Trp] **3**: Prepared according to the general procedure. Yield: 19.7 mg, 13.5 μ mol, 13.5%. ¹H NMR (500 MHz, CD₃OH) δ 10.25 (s, 1H), 10.22 (s, 1H), 9.43 (bs, 1H), 9.34 (bs, 1H), 9.23 (bs, 1H), 8.93 (bs, 1H), 8.80 (d, *J* = 9.3, 1H), 8.68 (bs, 1H), 8.28 (d, *J* = 2.6, 1H), 8.14 (s, 1H), 8.00 (d, *J* = 8.6, 1H), 7.65 (d, *J* = 7.7, 1H), 7.55 (dd, *J* = 13.3, 29.2, 4H), 7.43 – 7.32 (m, 3H), 7.28 – 7.13 (m, 5H), 7.09 – 6.89 (m, 6H), 6.84 (s, 1H), 6.72 (bs, 1H), 6.63 (d, *J* = 8.1, 2H), 5.94 – 5.84 (m, 1H), 5.52 (d, *J* = 5.9, 1H), 4.78 – 4.68 (m, 1H), 4.68 – 4.60 (m, 2H), 4.32 (bs, 2H), 4.06 (d, *J* = 7.1, 1H), 3.65 – 3.63 (m, 1H), 3.45 – 3.35 (m, 2H), 3.22 – 3.00 (m, 4H), 3.00 – 2.79 (m, 4H), 2.64 (t, *J* = 13.5, 1H), 2.40 – 2.14 (m, 5H), 2.14 – 2.05 (m, 1H), 2.03 – 1.93 (m, 2H), 1.93 – 1.84 (m, 2H), 1.83 – 1.75 (m, 4H), 1.74 – 1.65 (m, 2H), 1.61 – 1.48 (m, 2H), 1.39 – 1.23 (m, 3H), 1.20 (s, 3H), 1.16 (m, 6H), 1.12 – 1.04 (m, 6H), 0.94 – 0.82 (m, 1H), 0.19 (s, 1H).

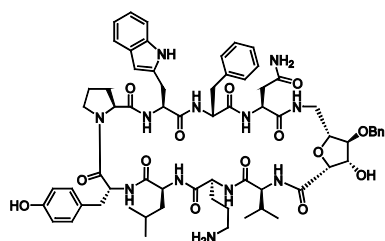


cyclo-[Val-Orn-Leu-SAA-Trp-^DPhe-Asn-Asp-Trp] **7**: prepared according to the general procedure. Yield: 9.73 mg, 6.76 μ mol, 6.8%. ¹H NMR (500 MHz, CD₃OH) δ 10.39 (s, 1H), 10.33 (s, 1H), 8.88 (d, *J* = 7.1, 1H), 8.53 (d, *J* = 8.3, 1H), 8.39 (d, *J* = 8.9, 1H), 8.34 (d, *J* = 3.4, 1H), 8.25 (d, *J* = 8.2, 1H), 8.19 (d, *J* = 9.3, 1H), 8.08 – 8.03 (m, 1H), 7.98 – 7.93 (m, 1H), 7.75 (d, *J* = 8.9, 1H), 7.73 (s, 1H), 7.66 (d, *J* = 7.8, 1H), 7.52 (d, *J* = 7.9, 1H), 7.38 (bs, 1H), 7.34 – 6.94 (m, 20H), 5.48 (d, *J* = 7.7, 1H), 4.82 (dd, *J* = 8.1, 13.8, 1H), 4.71 (dt, *J* = 4.8, 10.2, 1H), 4.67 – 4.62 (m, 1H), 4.56 (d, *J* = 11.7, 1H), 4.52 (d, *J* = 11.6, 1H), 4.46 – 4.39 (m, 1H), 4.38 – 4.30 (m, 3H), 4.21 (d, *J* = 10.0, 2H), 3.84 (s, 1H), 3.76 – 3.68 (m, 1H), 3.34 (s, 1H), 3.24 – 3.15 (m, 3H), 3.11 – 3.04 (m, 3H), 2.99 – 2.92 (m, 1H), 2.91 – 2.79 (m, 3H), 2.73 (bs, 1H), 2.69 (dd, *J* = 3.9, 17.4, 2H), 2.30 – 2.22 (m, 1H), 2.06 – 1.94 (m, 2H), 1.85 – 1.76 (m, 1H), 1.72 – 1.57 (m, 4H), 1.57 – 1.46 (m, 2H), 1.36 – 1.34 (m, 1H), 1.32 – 1.25 (m, 2H), 1.25 – 1.16 (m, 2H), 1.01 (d, *J* = 6.7, 3H), 0.97 (d, *J* = 6.6, 3H), 0.85 (d, *J* = 5.9, 3H), 0.79 (d, *J* = 5.9, 3H).



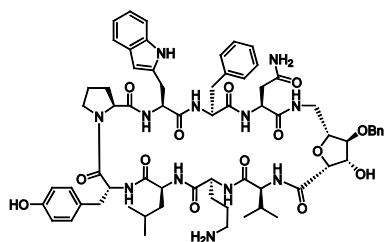
cyclo-[Val-Orn-Leu-^DTyr-Pro-Trp-SAA-Asp-Trp] **8**. Prepared according to the general procedure. Yield: 29.8 mg, 22.5 μ mol, 22.5%. ¹H NMR (600 MHz, MeOD) δ 10.67 (s, 1H), 10.65 (s, 1H), 10.32 (s, 1H), 8.99 (s, 1H), 8.38 (d, *J* = 9.5, 1H), 7.85 (bs, 1H), 7.82 (bs, 1H), 7.74 (d, *J* = 7.7, 2H), 7.57 (d, *J* = 8.1, 1H), 7.55 (bs, 1H), 7.49 (d, *J* = 7.8, 1H), 7.45 (s, 1H), 7.38 (d, *J* =

7.6, 3H), 7.32 (t, $J = 7.5$, 2H), 7.27 (t, $J = 7.9$, 2H), 7.22 – 7.14 (m, 3H), 7.08 (s, 1H), 7.03 (dd, $J = 7.2$, 12.4, 2H), 6.96 (t, $J = 7.4$, 1H), 6.92 (d, $J = 8.3$, 2H), 6.83 – 6.73 (m, 1H), 6.65 (d, $J = 8.4$, 2H), 4.65 – 4.59 (m, 2H), 4.59 – 4.50 (m, 3H), 4.33 (d, $J = 14.4$, 1H), 4.29 (d, $J = 13.2$, 2H), 4.19 (t, $J = 5.0$, 1H), 4.15 (bs, 1H), 4.11 – 4.06 (m, 2H), 4.06 – 4.00 (m, 1H), 3.90 (d, $J = 8.1$, 1H), 3.88 (bs, 1H), 3.72 (s, 1H), 3.52 – 3.45 (m, 2H), 3.45 – 3.35 (m, 1H), 3.34 (s, 1H), 3.28 – 3.16 (m, 4H), 2.90 (t, $J = 12.6$, 1H), 2.77 (dt, $J = 5.4$, 17.6, 1H), 2.77 (bs, 1H), 2.61 (bs, 1H), 2.19 – 2.06 (m, 2H), 2.01 – 1.87 (m, 2H), 1.78 – 1.69 (m, 1H), 1.63 (dd, $J = 11.0$, 22.2, 2H), 1.60 – 1.53 (m, 2H), 1.47 – 1.38 (m, 2H), 1.38 – 1.25 (m, 4H), 1.25 – 1.17 (m, 7H), 1.15 – 1.06 (m, 2H), 1.06 – 0.98 (m, 5H), 0.98 – 0.81 (m, 6H), 0.74 – 0.62 (m, 1H), 0.61 – 0.47 (m, 1H), 0.44 – 0.29 (m, 1H). HRMS calcd for $[C_{69}H_{86}N_{12}O_{15}+H]^+$ 1323.64084, found 1323.64236.



cyclo-[Val-Orn-Leu-^DTyr-Pro-Trp-^DPhe-Asn-SAA] **9**. Prepared according to the general procedure. Yield: 4.0 mg, 3.0 μ mol, 3.0%. ¹H NMR (600 MHz, CD₃OH) δ 10.30 (s, 2H), 8.95 (s, 1H), 8.79 (d, $J = 8.4$, 1H), 8.59 (d, $J = 8.1$, 1H), 8.55 (d, $J = 7.7$, 1H), 8.49 (d, $J = 8.7$, 1H), 8.18 (bs, 1H), 8.14 (d, $J = 7.7$, 1H), 8.02 (d, $J = 9.0$, 1H), 7.78 (d, $J = 9.2$, 2H), 7.73 (d, $J = 7.9$, 1H), 7.55 (d, $J = 7.9$, 1H), 7.32 (d, $J = 8.1$, 1H), 7.27 (d, $J = 8.2$, 1H), 7.21 – 7.14 (m, 4H),

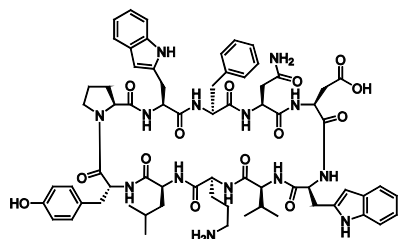
7.14 – 7.09 (m, 4H), 7.09 – 6.96 (m, 10H), 6.94 (t, $J = 7.4$, 2H), 6.66 (d, $J = 8.4$, 2H), 5.41 – 5.31 (m, 1H), 4.82 – 4.75 (m, 1H), 4.69 (dd, $J = 7.9$, 15.5, 1H), 4.60 – 4.54 (m, 1H), 4.46 (t, $J = 8.0$, 1H), 4.39 – 4.33 (m, 1H), 4.16 (dd, $J = 2.4$, 7.5, 1H), 4.12 – 4.07 (m, 1H), 3.37 (d, $J = 7.6$, 3H), 3.26 (d, $J = 9.3$, 1H), 3.18 (dd, $J = 4.4$, 14.4, 1H), 3.07 (dd, $J = 6.8$, 13.8, 1H), 3.05 – 2.98 (m, 2H), 2.96 (dd, $J = 4.7$, 12.7, 2H), 2.91 – 2.74 (m, 5H), 2.72 – 2.55 (m, 3H), 2.27 (dd, $J = 9.5$, 17.2, 1H), 2.20 (dd, $J = 9.3$, 17.1, 1H), 2.13 (dd, $J = 6.8$, 13.7, 1H), 1.96 – 1.88 (m, 1H), 1.79 – 1.72 (m, 1H), 1.72 – 1.61 (m, 6H), 1.61 – 1.54 (m, 2H), 1.44 – 1.33 (m, 3H), 1.31 – 1.24 (m, 1H), 1.02 – 0.91 (m, 18H), 0.43 – 0.32 (m, 1H). HRMS calcd for $[C_{69}H_{86}N_{14}O_{14}+H]^+$ 1335.65207, found 1335.65402.



cyclo-[Val-Orn-Leu-^DTyr-Pro-Trp-Phe-Asn-SAA] **10**. Prepared according to the general procedure. Yield: 46.6 mg, 36.6 μ mol, 36.6%. ¹H NMR (600 MHz, MeOD) δ 10.41 (bs, 1H), 8.99 (d, $J = 3.1$, 1H), 8.82 (d, $J = 8.4$, 1H), 8.78 (d, $J = 9.1$, 2H), 8.72 (d, $J = 8.4$, 2H), 8.64 (d, $J = 7.6$, 2H), 7.94 (d, $J = 8.4$, 1H), 7.85 – 7.79 (m, 5H), 7.70 (d, $J = 8.6$, 2H), 7.69 – 7.65 (m, 2H), 7.63

– 7.60 (m, 1H), 7.57 – 7.53 (m, 1H), 7.51 – 7.48 (m, $J = 12.5$, 1H), 7.40 – 7.37 (m, 5H), 7.37 – 7.30 (m, 7H), 7.29 – 7.22 (m, 7H), 7.18 – 7.14 (m, 1H), 7.12 (t, $J = 7.3$, 2H), 7.07 – 6.99 (m, 5H), 5.18 (q, $J = 7.6$, 1H), 5.02 (s, 1H), 4.94 – 4.85 (m, 1H), 4.82 – 4.76 (m, 1H), 4.76 – 4.71 (m, 1H), 4.71 – 4.67 (m, 2H), 4.62 (d, $J = 11.7$, 1H), 4.57 – 4.47 (m, 5H), 4.43 – 4.36 (m, 2H), 4.28 – 4.25 (m, 1H), 4.24 (d, $J = 3.0$, 1H), 4.17 (dd, $J = 1.7$, 8.2, 1H), 4.12 (t, $J = 10.6$, 1H), 3.92 (d, $J = 2.8$, 2H), 3.81 (d, $J = 16.7$, 1H), 3.76 – 3.70 (m, 1H), 3.65 (s, 1H), 3.31 (dd, $J = 9.1$, 14.7, 18H), 3.23 – 3.18 (m, 25H), 3.03 – 2.96 (m, 3H), 2.89 – 2.81 (m, 3H), 2.80 – 2.73 (m, 1H), 2.70 (dd, $J = 5.7$, 15.5, 1H), 2.55 – 2.47 (m, 1H), 2.31 – 2.20 (m, 3H), 2.09 – 2.02 (m, 11H), 2.01 – 1.93 (m, 2H), 1.93 – 1.87 (m, 21H), 1.84 – 1.76 (m, 2H), 1.76 – 1.67 (m, 2H), 1.67 – 1.52 (m, 7H), 1.52 – 1.38 (m, 4H), 1.38 – 1.30 (m, 2H), 1.28 (s, 1H), 1.11

– 1.03 (m, 20H), 1.03 – 0.93 (m, 5H), 0.42 – 0.30 (m, 1H). HRMS calcd for $[C_{67}H_{86}N_{12}O_{14}+H]^+$ 1283.64592, found 1283.64735.



cyclo-[Val-Orn-Leu-^DTyr-Pro-Trp-Phe-Asn-Asp-Trp] **11**. Prepared

according to the general procedure. Yield: 39 mg, 30 μmol, 30%. ¹H

NMR (600 MHz, MeOD) δ 10.32 (s, 1H), 8.91 (d, *J* = 2.9, 1H), 8.75 (d, *J* =

7.9, 1H), 8.71 (d, *J* = 9.0, 1H), 8.64 (d, *J* = 8.4, 1H), 8.57 (d, *J* = 7.5, 1H),

7.86 (d, *J* = 8.4, 1H), 7.78 – 7.71 (m, 3H), 7.62 (d, *J* = 8.6, 1H), 7.60 (bs,

1H), 7.55 – 7.50 (m, 1H), 7.47 (t, *J* = 7.6, 1H), 7.42 (s, 1H), 7.33 – 7.22 (m, 9H), 7.21 – 7.14 (m, 5H), 7.04 (t, *J* =

7.5, 1H), 6.98 – 6.93 (m, 3H), 6.67 (s, 1H), 6.64 (d, *J* = 8.4, 2H), 5.10 (d, *J* = 5.7, 1H), 4.74 – 4.69 (m, 1H), 4.66 (dd,

J = 7.8, 16.2, 1H), 4.61 (d, *J* = 11.7, 1H), 4.54 (d, *J* = 11.7, 1H), 4.47 (d, *J* = 3.9, 1H), 4.45 – 4.40 (m, 2H), 4.34 –

4.29 (m, 1H), 4.16 (d, *J* = 2.9, 1H), 4.09 (d, *J* = 8.3, 1H), 3.85 (d, *J* = 2.6, 1H), 3.73 (d, *J* = 14.3, 1H), 3.62 (d, *J* =

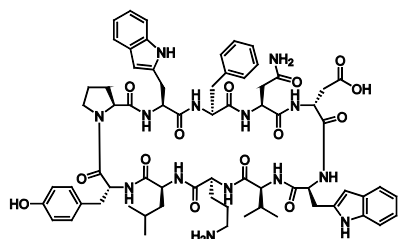
11.1, 1H), 3.58 – 3.54 (m, 1H), 3.29 – 3.17 (m, 8H), 3.17 – 3.11 (m, 2H), 2.95 – 2.88 (m, 2H), 2.81 – 2.73 (m, 2H),

2.73 – 2.66 (m, 1H), 2.62 (dd, *J* = 5.6, 15.4, 1H), 2.47 – 2.38 (m, 1H), 2.23 – 2.13 (m, 2H), 2.01 – 1.93 (m, 4H),

1.92 – 1.80 (m, 1H), 1.77 – 1.68 (m, 1H), 1.68 – 1.59 (m, 1H), 1.59 – 1.45 (m, 4H), 1.45 – 1.31 (m, 2H), 1.30 –

1.24 (m, 1H), 1.02 – 0.95 (m, 14H), 0.94 – 0.85 (m, 2H), 0.33 – 0.24 (m, 1H). HRMS calcd for $[C_{67}H_{86}N_{12}O_{14}+H]^+$

1283.64592, found 1283.64729



cyclo-Val-Orn-Leu-^DTyr-Pro-Trp-Phe-Asn-^DAsp-Trp] **12**. Prepared

according to the general procedure. Yield: 7.2 mg 5.4 μmol, 5.4 %. ¹H

NMR (600 MHz, CD₃OH) δ 10.32 (s, 2H), 10.30 (s, 2H), 9.05 (bs, 2H), 8.72

(bs, 2H), 8.61 (d, *J* = 8.6, 2H), 8.53 (d, *J* = 6.6, 2H), 8.45 (bs, 3H), 7.95 (d, *J* =

10.2, 2H), 7.78 (bs, 2H), 7.71 (d, *J* = 7.9, 3H), 7.66 (d, *J* = 8.8, 2H), 7.47

(bs, 2H), 7.28 (d, *J* = 8.0, 7H), 7.25 – 7.11 (m, 18H), 7.10 – 6.96 (m, 20H), 6.93 (t, *J* = 7.5, 3H), 6.69 (d, *J* = 8.2, 5H),

5.45 (s, 1H), 4.84 – 4.57 (m, 2H), 4.49 – 4.24 (m, 3H), 4.19 (s, 2H), 3.45 – 3.32 (m, 6H), 3.27 – 3.22 (m, 4H), 3.10

(s, 9H), 3.03 – 2.83 (m, 6H), 2.74 (dd, *J* = 4.2, 17.3, 4H), 2.67 (dd, *J* = 5.2, 16.7, 5H), 2.30 – 2.04 (m, 6H), 1.88 –

1.52 (m, 21H), 1.42 (s, 7H), 1.35 – 1.21 (m, 4H), 1.13 – 0.74 (m, 43H), 0.50 (s, 2H). HRMS calcd for

$[C_{69}H_{86}N_{14}O_{14}+H]^+$ 1335.65207, found 1335.65430.

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Chapter 7 | Activity-Based Serine Hydrolase Profiling Reveals Broad Reactivity of O-Alkyl- Methylphosphonofluoridates

INTRODUCTION

Activity-based profiling of enzymes has emerged as a powerful and broadly applicable technique to report on enzyme activities in complex biological samples, and to reveal the inhibitory potential and selectivity of newly designed inhibitors aimed at (a subset of) the enzymes.¹ The general layout of an activity-based probe is as follows. A recognition element directs the probe to the selected enzyme, or enzyme family, and specificity can be geared such that either a single enzyme or a broad family of enzymes is targeted. These are then covalently and irreversibly bound through reaction of nucleophilic functional groups residing within the active site with an electrophilic trap that matches the reactivity of the nucleophile.

In cases where an amino acid side chain is involved in catalysis, active site labelling is relatively easy and a variety of electrophilic traps aimed at serine hydrolases,² cysteine hydrolases³ and threonine hydrolases⁴ have been described. Some enzyme families (metalloproteases,⁵ phosphatases⁶) however employ water as the nucleophile and here covalent modification is aimed for by installation of a photoreactive group within the activity-based probe. The third general element that distinguishes an activity-based probe is an identification and/or enrichment element, normally biotin or a fluorophore. Here activity-based probes come in two flavours. In the first the biotin or fluorophore is attached directly to the probe,² whereas in the second, for reasons of cell-permeability or enzyme recognition, a 2-step labeling approach is applied where a functionalised fluorophore or biotin is made to react with the covalently and irreversibly modified enzymes by means of a bioorthogonal ligation reaction.⁷

The general outline of activity-based probes as described above dictates that these are most suited for the study of enzymes that employ a nucleophilic amino acid side chain within their active sites. This holds true for the serine dependent enzymes, a family that encompasses such diverse activities as esterases, serine proteases and ester synthetases.⁸ Indeed, serine hydrolases are ideal targets for activity-based profiling and one of the seminal studies defining the field of activity-based enzyme profiling is the development and application, by Cravatt and co-workers, of the biotinylated organophosphorus compound, FP-biotin (**1**, Figure 1).^{2,9} Our interest in serine hydrolase enzyme family lies in their reactivity towards small molecule O-alkyl methylphosphonofluoridates such as the ill-reputed nerve agent sarin **2**.¹⁰

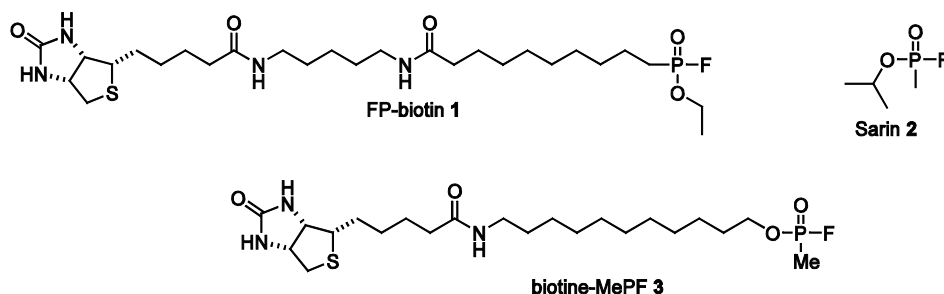


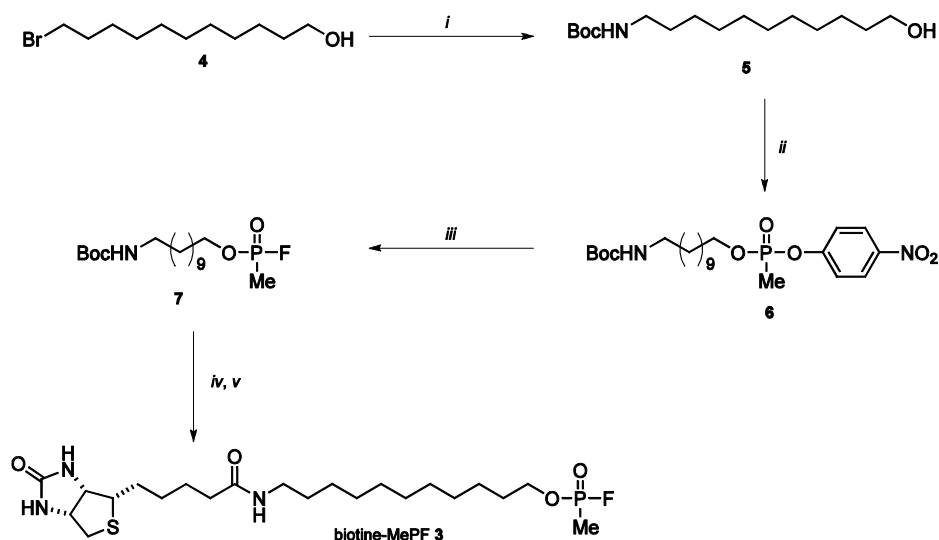
Figure 1; Structure of Cravatt's carbon-modified fluorophosphonate **1**, sarin **2** and oxygen-modified methylfluorophosphonate **3**

The toxicity of **2** and related compounds to the mammalian nervous system is ascribed to their high reactivity towards acetylcholinesterase (AChE).¹¹ However, recent reports reveal a broader reactivity of this type of compounds towards other serine-dependent enzymes.¹² We here describe our results in the activity-based profiling-mediated establishment of a range of sarin-reactive serine-dependent enzymes in liver and lung tissue. FP-biotin **1** would be suitable for this purpose but we feel that the design of **3** is better suited as a mimick of sarin **2**. Both **2** and **3** contain a methyl substituent directly attached to the phosphorous atom which has been proven beneficial in binding to AChE.¹³

We reveal the broad reactivity of **3** to a range of serine-dependent enzymes and show that labelling of eight specific activities can be abolished by the addition of **2** in a competition experiment. Finally, we demonstrate that the enzymatic activity of butyrylcholinesterase (BuChE),¹⁴ which is currently under investigation in several laboratories as a bioscavenger against organophosphorus-based nerve poisoning,¹⁵ can be restored in an experiment in which the enzyme is first activity-tagged with **3**, then liberated by treatment with an oxime¹⁶ or fluoride containing reactivation-cocktail.¹⁷ Together with biotin-streptavidin pull-down, this technology could improve current isolation/purification methods of BuChE.¹⁸

RESULTS AND DISCUSSION

The synthesis of fluorophosphonate **3** is depicted in Scheme 1 and commences with a standard three-step conversion of 11-bromoundecan-1-ol **4** into *N*-*tert*-boc-11-aminoundecan-1-ol **5**. Treatment of the latter compound with di-*p*-nitrophenyl methylphosphonate¹⁹ and DBU gave methylphosphonate **6** in good overall yield. At this stage the *p*-nitrophenol in **6** was substituted by fluorine using tetrabutylammonium triphenyldifluorosilicate (TBAT) as the dry fluorine source, giving fluorophosphonate **7** in 70% yield. Ensuing acidic *N*-deprotection and treatment with biotin-OSu²⁰ provided the target compound **3** in a near quantitative yield over the last two steps. In this fashion we routinely prepared activity-based probe **3** on a 20 mg scale.



Scheme 1; Reagents and conditions. (i) a: 2 eq. NaN_3 , DMF 80°C , 16h.; b: PMe_3 , THF/ H_2O ; c: Boc_2O , THF, 0°C , 73% over three steps. (ii) 1.5 eq. di-*p*-nitrophenyl methylphosphonate, 1.0 eq. DBU, DCM, 1h. 65%. (iii) 3.7 eq. TBAT, THF, 90%. (iv) 50% TFA/DCM, 30 min. (v) 0.9 eq. biotin-OSu, 3.0 eq. DiPEA, DMF, 99%.

For the labelling and competition studies we selected three relevant biological samples, namely, rhesus monkey liver homogenate, human A549 lung cell lysates and intact A549 cells (Figure 2A-C respectively). These samples were incubated with probe **3** at $10\ \mu\text{M}$, either in the absence or in the presence ($120\ \mu\text{M}$) of sarin **2**. After streptavidin pull-down of the thus biotinylated proteins, these were resolved by SDS-PAGE, the protein bands were excised from the gel and trypsin digested after which the resulting oligopeptides were identified by MALDI-TOF mass spectral analysis. The images of the gel slabs of the three tissue labelling experiments are depicted in Figure 2 and represent in each case labelling without (left) and in the presence of (right) sarin **2**.

Both the monkey and human tissues gave a distinct labelling pattern (lanes 1, 3 and 5) for probe **3** and, whereas lysate labelling (lane 3) gives more bands than that of the corresponding living cells (lane 5), the bands visualized in lane 5 are also present in lane 3. The combined labelled proteins constitute (see Table 1) a wide array of different enzymes, but the common denominator is, as expected, that they all contain a serine residue in their active site. In fact, the labelling profile and the range of identified proteins compares well to the literature reports^{2,9} on the activity-based profiling experiments using FP-biotin **1** and its derivatives and we conclude that methylfluorophosphonate **3** is well suited for its intended purpose, that is the establishment of the reactivity profile of the nerve agent sarin **2**. The three samples reveal some differences in the serine-dependent enzymes of which the

activity-based labelling with **3** is abolished (see lanes 2, 4 and 6) upon addition of sarin **2**. In the monkey liver homogenate, five enzymes are inhibited by sarin in a competitive manner, four of which (indicated by arrows in Figure 2) could be identified by excision of the bands followed by MALDI-TOF mass spectral analysis. The four identified enzymes are acylpeptide hydrolase, liver carboxyl esterase, esterase D (or S-formyl glutathione hydrolase) and PAF acetylhydrolase (see lane 2). From the experiments involving the A549, lysate six sarin-reactive proteins are competed in this fashion. Five of these enzymes could be identified and are carboxylesterase, PAF acylhydrolase, fatty acid synthase, dipeptidyl peptidase 9 and prolyl oligopeptidase (see lane 4). The experiments using A549 tissue reveal four enzymes as sarin targets (lane 6) that could be identified. In the intact A549 lung cell, the fatty acid synthase and the dipeptidylpeptidase 9 are not found, but the enzyme long-chain coenzyme A thioesterase is. In total we have identified eight enzymes that are sensitive towards sarin, all belonging to the serine hydrolase superfamily. Likely, more organophosphorus-reactive proteins will be identified in this fashion when one starts from a different tissue. Of all identified proteins, literature data exists that denote them to be reactive towards organophosphorous compounds in general, which corroborates the validity of our activity-based serine hydrolase profiling approach.²¹ Thus, although we are aware of the high doses of sarin **2** used in our experiments we feel it is safe to conclude from our experiments that the reactivity of this nerve agent is much broader.

Proteins covalently bound to sarin-biotin	Liver homogenate	Lysed A549 cells	Intact A549 cells
Fatty acid synthase (1)	+	+,#	+
Carbamoyl phosphate synthetase I	+	-	-
Pyruvate carboxylase	+	+	+
Acylpeptide hydrolase (2)	+,#	-	-
Dipeptidylpeptidase 9 (3)	-	+,#	+
Prolyl oligopeptidase(4)*	+	+,#	+,#
Carboxylesterase (5)	+,#	+,#	+,#
Serine hydroxyl-methyltransferase	+	-	-
Long-chain acyl coenzyme A thioesterase (6)	+	-	+,#
KIAA13263 = aryl acetamide deacylase-like 1	-	+	-
Aldehyde dehydrogenase	-	+	-
PAF acetylhydrolase 1b (7)**	+,#	+,#	+,#
Actin	+	+	-
Alcohol dehydrogenase 1B/ fumarylacetoacetate hydrolase	+	-	-
Prostaglandin F synthase	-	+	+
Aldo-keto reductase family 1, member C1 isoform 2	+	-	-
EsteraseD/S-formyl glutathione hydrolase (8)	+,#	-	-
Lysophospholipase I and II	-	-	+
Glutathione-S-Transferase	+	+	-

Table 1; Proteins from rhesus monkey liver, human A549 cells or lysed human A549 cells that were biotinylated upon exposure to 10 μ M of probe **3** for 1 h. Proteins were identified by mass spectrometry from bands excised from one-dimensional SDS-PAGE gels. Proteins that are specific targets for sarin are bold, and the numbers (in brackets) refer to Figure 2.

“ + ” indicates that the biotinylated form of the protein could be detected in the excised gel

“ - ” indicates that a band for the biotinylated form of the protein could not be detected in the excised gel (either due to the absence of this protein in the specific tissue or due to non-binding of the probe)

“ # ” indicates clear sensitivity of the protein towards competitive binding of sarin for the respective tissue

*: In case of lysed A549 cell, two isoforms of prolyl oligopeptidase were detected

** : In case of lysed and intact A549 cels, the β - and γ -subunit of PAF acetylhydrolase 1b were detected

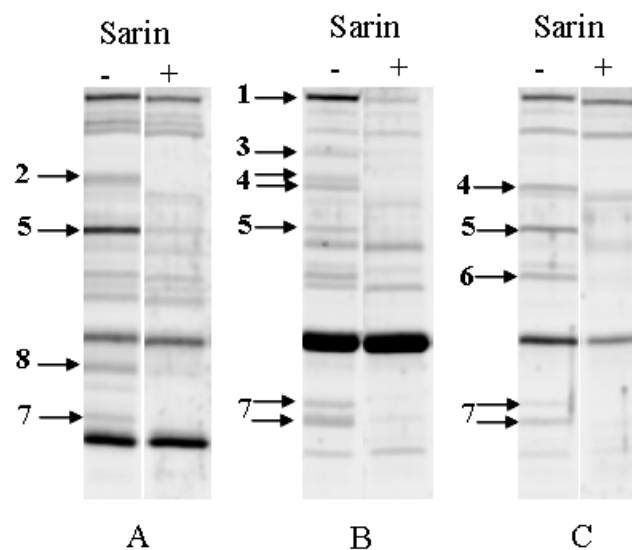


Figure 2; One-dimensional SDS-PAGE lanes showing proteins that are adducted with biotin-MePF (**3**). A. Rhesus monkey liver homogenate. B. Lysed A549 human lung cells. C. Intact A549 human lung cells. Each sample was incubated with 10 μ M of probe **3** for 1h., either in absence or in presence of 120 μ M sarin. The protein bands were excised, digested with trypsin and the resulting digest was subjected to mass spectrometric analysis. Arrows indicate the protein bands that were largely reduced or disappeared when sarin was present during the incubation of the samples with probe **3** and that could be identified by mass spectral analysis. The numbers refer to specific proteins listed in Table 1.

One protein that definitely binds to sarin **2** is the enzyme butylcholinesterase.¹⁴ This enzyme is present in human plasma at concentrations of up to 80 nM.²² Although its natural function is not known, human butyrylcholinesterase (HuBuChE) is subject of several studies based on its capacity to sequester organophosphorus based toxins from the blood, a feat that is based on reaction of the enzyme with the nerve poisons just as their primary target, acetylcholinesterase, does.²³ Current procedures to obtain large amounts of purified HuBuChE rely on a laborious combination of HPLC and affinity purification.¹⁸ We argue that activity-based probes such as **3** may find use as an alternative strategy, or at least an addition, to these purification protocols. In a preliminary experiment aimed at demonstrating the validity of this, we treated 0.5 mL human plasma with probe **3** for 30 minutes, after which we determined the remaining activity of HuBuChE by means of Ellman's colorimetric test (Figure 3).²⁴ As can be seen (compare lanes 1 and 5) all enzyme activity is blocked at concentrations of **3** as little as 0.8 mM. The sample used in lane 5 is then depleted of excess probe by 1kD cut-off filtration and HuBuChE was regenerated by the addition of either KF or pralidoxime (2-pyridiniumalldoxime-N-methyl Chloride, PAM2-Cl),^{16a} each at a 0.5 mM concentration followed by 3 washing/cut-off filtration cycles. Performing Ellman's

colorimetric test on these samples, redissolved in 0.5 mL buffer, revealed that enzyme activity is restored almost to the initial values.

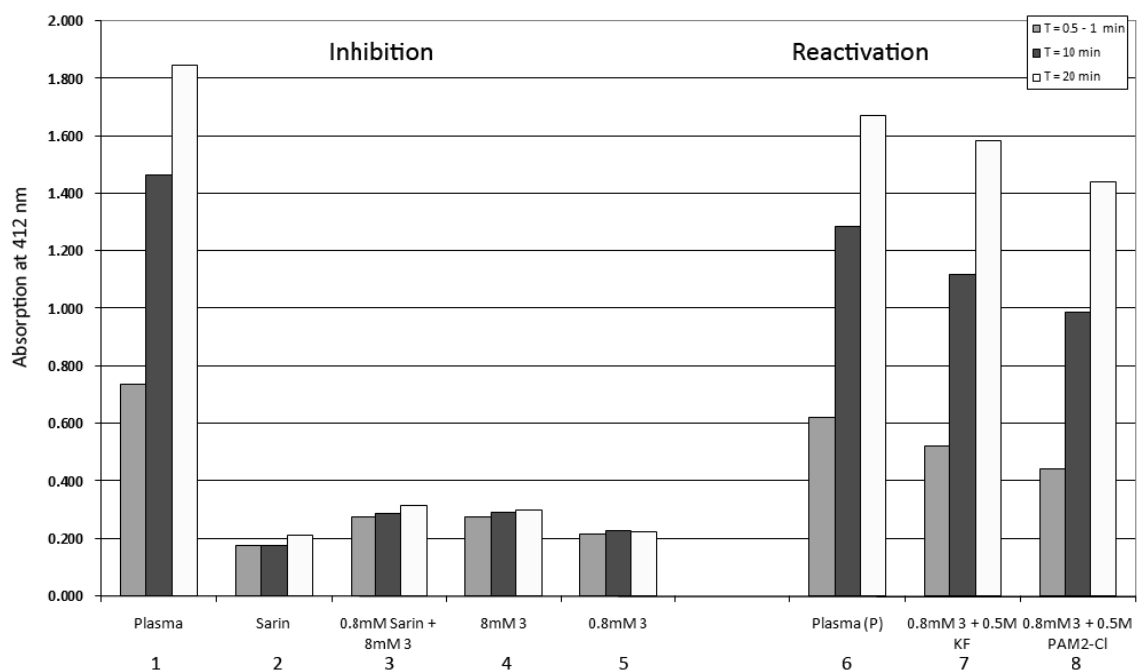


Figure 3; HuBuChE activity as determined by Ellman's colorimetrics test

Conclusion

In summary, we have demonstrated that activity-based profiling is a valid and rapid technique to reveal serine hydrolase reactivity towards organophosphorus-based nerve poisons. From a synthetic point of view we reason that the route of synthesis towards activity-based probe (**3**) is robust. It allows the preparation of relatively large quantities of the probe and is amenable for modification to give analogous probes varying in the nature of, for instance, the spacer or the reporter entity. This in contrast to FP-biotine **1** which, in our hands, proved to be cumbersome to synthesize, specifically the transformation into and purification of the phosphonate mono-ethylester intermediate.

Our initial profiling experiments suggest that our probe displays the favourable characteristics featured by FP-biotin **1**. In a competition assay, we have identified eight serine hydrolase activities namely, Fatty acid synthase, Acylpeptide hydrolase, Dipeptidylpeptidase 9, Prolyl oligopeptidase, Carboxylesterase, Longchain acyl coenzyme A thioesterase, PAF acetylhydrolase 1b and EsteraseD/S-formyl glutathione hydrolase as

targets that are modified by the toxic agent sarin. Finally, the deactivation-purification-reactivation scheme we employed on HuBuChE reveals that activity-based probes may find their way in the development of alternative purification protocols related to affinity purification.

Experimental section

General

Caution: All experiments with the highly toxic sarin and the potentially toxic methylphosphonofluoridate **3** were carried out in fume cupboards of the Hi-Tox facility at TNO Defense, Security and Safety, Rijswijk, The Netherlands.

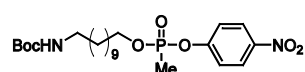
PE with a boiling range of 40 - 60°C was used. THF and Et₂O were distilled over LiAlH₄ prior to use. DCM was distilled over CaH₂ prior to use. All other solvents used under anhydrous conditions were stored over molecular sieves (4Å) except for methanol which was stored over 3Å molecular sieves. Solvents used for work-up and column chromatography were of technical grade and distilled before use. Unless stated otherwise, solvents were removed by rotary evaporation under reduced pressure at 40°C. Reactions were monitored by TLC-analysis using Merck 25 DC plastikfolien 60 F₂₅₄ with detection by spraying with 20% H₂SO₄ in EtOH, (NH₄)₆Mo₇O₂₄·4H₂O (25 g/L) and (NH₄)₄Ce(SO₄)₄·2H₂O (10 g/L) in 10% sulfuric acid or by spraying with a solution of ninhydrin (3 g/L) in EtOH / AcOH (20/1 v/v), followed by charring at approx. 150°C. Column chromatography was performed on Fluka silicagel (0.04 – 0.063 mm). For LC/MS analysis, an JASCO HPLC-system (detection simultaneously at 214 and 254 nm) equipped with an analytical C₁₈ column (4.6 mmD × 250 mmL, 5μ particle size) in combination with buffers A: H₂O, B: MeCN and C: 0.5% aq. TFA and coupled to a mass instrument with a custom-made Electrospray Interface (ESI) was used. For reversed-phase HPLC purification of the final compounds, an automated HPLC system supplied with a semi-preperative C₁₈ column (10.0 mmD × 250 mmL, 5μ particle size) was used. The applied buffers were A: H₂O, B: MeCN and C: 1.0% aq. TFA. High resolution mass spectra were recorded by direct injection (2 μL of a 2 μM solution in water/acetonitrile; 50/50; v/v and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150-2000) and dioctylphthalate (m/z = 391.28428) as a “lock mass”.²⁵ The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). ¹H- en ¹³C-NMR spectra were measured on a Joel JNM-FX-200 (200/50 Mhz), a Brüker AV-400 (400/100 MHz), a Brüker AV-500 (500/125 MHz) or a Brüker DMX-600 (600/125 MHz). Chemical shifts are given in ppm (δ) relative to TMS (0 ppm) or MeOD (3.30 ppm) and coupling constants are given in Hz.

Protease inhibitor cocktail tablets (Complete Mini) were purchased from Roche Diagnostics, (Mannheim, Germany). Human lung tumor A549 cells were purchased from American Type Culture Collection. 10% heat-inactivated Fetal Calf Serum was obtained from PAA laboratories GmbH (Pasching, Austria). Dulbecco's modified Eagle's medium (DMEM) was purchased from Invitrogen (Merelbeke, The Netherlands). CellLytic™-M

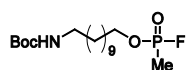
lysis extraction reagent was obtained from Sigma-Aldrich Chemie (Zwijndrecht, The Netherlands). PD-10 columns were purchased from GE Healthcare (Uppsala, Sweden). Avidin-agarose beads were obtained from Sigma-Aldrich Chemie (Zwijndrecht, The Netherlands). DNase I/RNaseA mix containing 1 mg/ml DNase, 0.25 mg/ml RNase was obtained from Roche Diagnostics (Mannheim, Germany). Proteom IQ blue was purchased from Proteome Systems Inc (Woburn, United States). Sypro Ruby (SR) was obtained from Sigma-Aldrich Chemie (Zwijndrecht, The Netherlands). Modified trypsin was obtained from Promega Benelux BV (Leiden, The Netherlands). ZipTip μ -C18 columns were obtained from Millipore. RC DC protein assay was obtained from BioRad (Veenendaal, The Netherlands).

 11-Bromoundecan-1-ol (12 g, 48 mmol) was treated with NaN_3 (6.5 g, 100 mmol) in DMF (250 mL) at 80°C overnight. Water (500 mL) was added and the mixture was extracted with ether (3x 250 mL), the combined organic phase was washed with brine, dried over MgSO_4 , concentrated to afford the crude azide (11 g) which was used in the subsequent reaction as such.

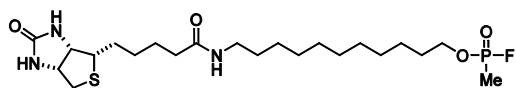
The intermediate azide (2.5g, 11.7 mmol) was dissolved in THF/ H_2O (20/1; 100 mL) before addition of PMe_3 (20 mL, 1M soln. in THF). After complete consumption of the starting material as judged by TLC analysis, the reaction mixture was concentrated and coevaporated with dry toluene to remove residual water and reagent, redissolved in THF (100 mL) at 0°C and allowed to react with Boc_2O (4.3 g, 20 mmol) for 2h. The reaction mixture was diluted with EtOAc (200 mL) and water (100 mL), the organic phase extracted, washed with sat. aq. NaHCO_3 (50 mL), water (50 mL) and brine (50 mL), dried over MgSO_4 , filtered and concentrated. The residue was purified by column chromatography (EtOAc/PE) to afford the title compound (2.5 g, 8.5 mmol, 73 % over 3 steps) as white solid. $^1\text{H-NMR}$ (CDCl_3) δ : 3.67 – 3.61 (t, 2H, $J = 6.58 \text{ Hz}$), 3.15 – 3.05 (dd, 2H, $J_1 = 13.13 \text{ Hz}$, $J_2 = 6.58 \text{ Hz}$), 1.56 – 1.17 (m, 27H); $^{13}\text{C-APT NMR}$ (CDCl_3) δ : 62.60, 40.52, 32.60, 29.90, 29.33, 28.2, 26.63, 25.63; LCMS: 15 min. 10-90%ACN/ H_2O , rt = 10.45 min, $m/z = 288.1 [\text{M}+\text{H}]^+$, 232.1 $[\text{M}+\text{H}-t\text{-Bu}]^+$, 187.7 $[\text{M}+\text{H}-\text{Boc}]^+$.



To a mixture of alcohol **5** (750 mg, 2.6 mmol) and bis-*p*-nitrophenyl methylphosphonate (1.05 g, 3.2 mmol) in anhydrous DCM (20 mL) was slowly added a solution of DBU (393 μL , 2.6 mmol) in DCM (5 mL) with concomitant appearance of a yellow color. The reaction mixture was allowed to stir for 1 h after which 0.5M HCl (20 mL) was added and the organic phase was separated, washed with water (2x 10 mL), brine (1x 10 mL), dried (MgSO_4), filtered and concentrated. The residue was applied to a silica column and eluted with EtOAc/PE to yield the desired product (821 mg, 1.69 mmol, 65%) as slightly yellow solid. $^1\text{H-NMR}$ (CDCl_3) δ : 8.25 – 8.22 (dd, 2H, $J_1 = 0.6 \text{ Hz}$, $J_2 = 9.33 \text{ Hz}$), 7.40 – 7.38 (dd, 2H, $J_1 = 1.0 \text{ Hz}$, $J_2 = 9.33 \text{ Hz}$), 4.50 (bs, 1H), 4.17 – 4.08 (m, 3H), 3.11 – 3.09 (m, 2H), 1.72 – 1.65 (m, 6H), 1.48 – 1.44 (m, 9H), 1.33 – 1.25 (m, 14H). $^{13}\text{C-APT NMR}$ (CDCl_3) δ : 156.08, 155.70 – 155.63 (d, $J = 7.5 \text{ Hz}$), 144.66, 125.74, 121.05 – 121.00 (d, $J = 5 \text{ Hz}$), 79.06, 67.12 – 67.05 (d, $J = 6.88 \text{ Hz}$), 40.70, 30.46, 30.40, 30.13, 29.75, 29.54, 29.48, 29.31, 29.11, 28.50, 26.84, 25.45, 12.42 – 10.93 (d, $J = 145.71 \text{ Hz}$). $^{31}\text{P-NMR}$ (CDCl_3) δ : 28.29. MS: $m/z = 487.2 [\text{M}+\text{H}]^+$, 431.1 $[\text{M}-t\text{Bu}+\text{H}]^+$, 387.0 $[\text{M}-\text{Boc}+\text{H}]^+$



A solution of p-nitrophenyl phosphonate **6** (70 mg, 0.14 mmol) in THF (5 mL) was charged with TBAT (283 mg, 0.52 mmol). After the starting material had completely disappeared according to TLC analysis (50% EtOAc/PE), the reaction mixture was diluted with DCM (10 mL) and 0.5 M aq. HCl (5 mL). The organic phase was extracted, the aqueous phase washed with DCM (3x 5 mL) and the combined organic phase washed with brine (1x 5 mL), dried (MgSO₄) and concentrated. The residual oil was further purified by silica chromatography (EtOAc/PE) to afford the title compound (48 mg, 0.13 mmol, 90%) as colorless solid. ¹H-NMR (CDCl₃) δ: 4.50 (bs, 1H), 4.21 – 4.16 (m, 2H), 3.12 – 3.08 (dd, 2H, *J*₁ = 6.15 Hz, *J*₂ = 12.70 Hz), 1.74 – 1.68 (m, 2H), 1.67 – 1.61 (dd, 3H, *J*₁ = 5.76 Hz, *J*₂ = 18.66 Hz), 1.60 – 1.19 (m, 25H). ¹³C-APT NMR (CDCl₃) δ: 156.08, 79.10, 67.28 – 67.21 (d, *J* = 7.0 Hz), 40.73, 30.44, 30.38, 30.17, 29.57, 29.50, 29.34, 29.12, 28.53, 26.87, 25.41, 10.88 – 9.12 (dd, *J*₁ = 27.5 Hz, *J*₂ = 149.5 Hz). ³¹P-NMR (CDCl₃) δ: 33.51 – 27.04 (d, *J* = 1048.10 Hz). MS: *m/z* = 390.2 [M+Na]⁺, 368.2 [M+H]⁺, 312.1 [M-tBu+H]⁺. MS/MS: *m/z* = 368.3 [M+H]⁺, 312.2 [M-tBu+H]⁺, 268.2 [M-Boc+H]⁺, 170.3 [M-Boc-OP(O)CH₃F+H]⁺.



Bocylated amine **7** (18 mg, 50 μmol) was treated with 50% TFA/DCM (2 mL) for 30 min after which it was concentrated en coevaporated with toluene trice to remove excess TFA. The crude TFA-salt was subsequently dissolved in DMF (2 mL) before addition of DiPEA (24.5 μL, 150 μmol) and biotin-OSu (15.3 mg, 45 μmol) were and the reaction was allowed to stir for 16 hours after which TLC analysis (15% MeOH/EtOAc) indicated complete conversion of the starting material in a slightly lower running spot. The reaction mixture was concentrated and filtered over a small plug of silica and recrystallised from warm EtOAc to afford the title compound (22 mg, 44 μmol, quant) as colorless solid. ¹H-NMR (CDCl₃) δ: 6.10 (bs, 1H), 5.95 bs, 1H), 4.51 (bs, 1H), 4.32 (bs, 1H), 4.23 – 4.11 (m, 2H), 3.21 (m, 1H), 3.13 – 3.10 (m, 2H), 2.96 – 2.88 (m, 1H), 2.78 – 2.66 (m, 1H), 2.25 – 2.14 (m, 2H), 1.73 – 1.68 (m, 2H), 1.68 – 1.62 (dd, *J*₁ = 5.76 Hz, *J*₂ = 18.45 Hz, 3H), 1.27 – 1.10 (m, 23H). ¹³C-APT NMR (CDCl₃) δ: 173.04, 162.00, 67.33 – 67.23 (d, *J* = 6.88 Hz), 55.47, 53.61, 41.97, 40.56, 39.65, 36.10, 30.40, 30.34, 29.68, 29.51, 29.44, 29.32, 29.05, 28.27, 28.19, 26.98, 25.64, 25.36, 10.84 – 9.09 (dd, *J*₁ = 26.89 Hz, *J*₂ = 149.46 Hz). ³¹P-NMR (CDCl₃) δ: 33.64 – 27.17 (d, *J* = 1047.7 Hz). ¹⁹F-NMR (CDCl₃) δ: -58.23 – -61.03 (dd, *J*₁ = 5.13 Hz, *J*₂ = 1047.89 Hz). MS: *m/z* = 494.3 [M+H]⁺; MS/MS: *m/z* = 494.3 [M+H]⁺, 396.3 [M-OP(O)MeF+H]⁺, 268.2 [M-biotine+H]⁺, 227.1 [M-H₂N(CH₂)₁₁OP(O)MeF+H]⁺, 170.2 [M-biotine-NH(CH₂)₁₁+H]⁺.

Preparation of liver homogenates

Liver of rhesus monkey was homogenized with a Potter homogenizer at 800-1000 rpm in 10 volumes of ice cold 50 mM TrisCl buffer pH 8, supplemented with 0.32 M Sucrose and 1/10 ml buffer protease inhibitor cocktail tablet. The suspension was centrifuged at 1000g for 10 minutes at 4°C and the supernatant was centrifuged at 17,000g for 55 min at 4°C. Then the supernatant was centrifuged at 100,000g for 60 minutes at 4°C. The thus obtained supernatant was stored in aliquots at -70°C. For protein content determination, samples of 150 μl were treated with 1.5 ml 11% trichloroacetic acid in ice-cold acetone supplemented with 25 mM DTT to precipitate the proteins. Pellets were washed twice with ice-cold acetone and subsequently dissolved in 9.5 M urea, 4% CHAPS and 25 mM DTT. Protein concentrations were determined with the RC DC protein assay.

Cell culture of A549 lung cells

Human lung tumor A549 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) complemented with 10% heat-inactivated Fetal Calf Serum, 100 units/ml penicillin and 100 µg/ml streptomycin. A549 cells were maintained in an atmosphere of 5% CO₂ and 37°C in 75 cm² tissue culture flasks and subcultured twice a week with a concentration of 6×10^5 cells per flask.

Preparation of A549 lung cell lysates

A549 lung cell lysates were prepared according to the manufacturer's protocol. In brief, cells were washed with PBS and 1 ml CellLyticTM-M lysis extraction reagent was added. A portion (1/6, v/v, cocktail solution/cell lysate) of protease inhibitor cocktail tablet dissolved in 1.5 ml MQ was added to the lysate. Cells were incubated for 15 min with continuous shaking. The lysed cells were centrifuged at 14,000 g for 15 min and the protein-containing supernatant was put into clean 15 ml tubes. Protein concentrations were determined with the RC DC protein assay and samples were stored in aliquots at -20°C.

Exposure of Liver homogenates and A549 lung cell lysates to biotin-MePF (3)*Pre-incubation of the protein samples*

To prevent aspecific binding of biotin-containing proteins, 2,5 ml protein samples (1,4 mg/ml in 50 mM Tris, pH8, containing 5 mM EDTA) were incubated with 1/10, v/v, avidin-agarose beads overnight at 4°C with continuous mixing. The suspension was centrifuged at 3,200g for 10 min and the supernatant with the solubilized protein fraction was put into new reaction tubes for exposure to 3.

Exposure to biotin-MePF (3)

A stock solution of 3 was prepared in CHCl₃ (12 mM) and dilutions were made in DMSO to afford concentrations of 0.5, 1 and 4 mM. Subsequently, the protein samples (1.4 mg/ml in 50 mM Tris, pH 8, containing 5 mM EDTA) were incubated with 1/100 v/v stock solutions of 3 (final concentration 5, 10 and 40 µM 3 in 1% DMSO) for 1 hour at 25°C.

Competitive exposure between sarin and biotin-MePF (3)

A stock solution of sarin (71 mM) was prepared in IPA and diluted in MQ to make a concentration of 12 mM. The protein samples (1,4 mg/ml in 50 mM Tris, pH 8, containing 5 mM EDTA) were incubated with 1/100 v/v of 12 mM sarin (final concentration 120 µM sarin in 1% MQ) for 1 hour at 25°C with continuous mixing. Then the proteins were incubated with 1/100 v/v stock solutions of 3 (final concentration 5 and 10 µM 3 in 1% DMSO) for 1 hour at 25°C. Next, the biotin-labeled proteins were purified as described below.

Exposure of A549 lung cells to biotin-MePF (3)

Stock dilutions of 3 were made in DMSO as described above. When cells had reached confluence they were washed with PBS and exposed to freshly prepared solutions PBS with 0, 5, 10 and 40 µM 3 in a 75 cm² culture dish (with 1% DMSO). The cells were incubated for 1 hour at 25°.

Competitive exposure between biotin-MePF (3) and sarin

Stock dilutions of **3** and sarin were prepared as described above. When cells had reached confluence, they were washed with PBS and exposed to 120 μ M sarin (with 1% MQ) and cells were incubated for 1 hour at 25°. Immediately after the incubation cells were exposed to freshly prepared solutions PBS with 0, 5, 10 and 40 μ M Biotin-MePF in a 75 cm² culture dish (with 1% DMSO). The cells were incubated for 1 hour at 25° and after the exposure cells were solubilized as described below.

Protein extraction of exposed A549 lung cells

Pellets of cells (ca 4 mg protein) were solubilized in 0.525 ml buffer containing 9.5 M urea, 4% Chaps and 25 mM DTT and were immediately put on ice. A portion of 90 μ l protease inhibitor cocktail tablet dissolved in 1.5 ml MQ was added to the lysate and 1/10 (v/v) DNase I/RNaseA mix containing 1 mg/ml DNase, 0.25 mg/ml RNase in 0.5 M Tris buffer pH 7.5, 0.05 M MgCl₂ was added. The lysates were incubated for 1 h on ice. Protein concentrations were determined with the RC DC protein assay. The protein samples were stored in aliquots at -20°C.

Purification of Biotin-MePF labeled proteins

For selective isolation of the biotinylated proteins, the method as used by Kidd et al (2001) and Lockridge (2003) was used. To remove excess **3** from the protein samples, each sample (2.5 ml) was passed over a PD-10 size exclusion chromatography column. The eluate with protein material was treated with 0.5% SDS, heated at 85°C for 3 minutes and diluted 2.5-fold with 50 mM Tris/EDTA. Then the mixtures were incubated with 250 μ l avidin-agarose beads overnight at room temperature with continuous mixing. The beads were washed twice with 50 mM Tris/EDTA containing 0.2% SDS and twice with 50 mM Tris/EDTA. Beads were dissolved in 200 μ l 50 mM Tris/EDTA and 50 μ l of 6x SDS PAGE loading buffer (0.2 Tris, pH 6.8, 10% SDS, 30% glycerol, 0.6 M DTT and 0.012 bromophenol blue was added. Mixtures were heated at 85°C for 5 minutes.

Visualization of purified biotinylated proteins

Biotinylated proteins were separated on homogeneous 10% polyacrylamide gels with 4% stacking gel in a BioRad Mini-Protean II electrophoresis unit. Bead mixtures of 20 μ l were loaded per lane and run at 100V/gel, at 20°C until bromophenol blue had migrated to the bottom of the gel. Gels were stained with Proteom IQ blue as a post-staining of the quantitative stained gel with Sypro Ruby (SR; Wijte et al., 2006). In brief, gels were fixated for 30 min in MeOH/acetic acid (10%/7% in Milli-Q). Staining with SR was performed overnight in the dark followed by a 30 min fixation in MeOH/acetic acid (10%/7% in Milli-Q). SR was used diluted 1:2 as recommended by Krieg et al., 2003. Before post-staining of the gels with Proteom IQ Blue the gels were fixated again for 60 min in MeOH/acetic acid (25%/10% in Milli-Q) and stained overnight with Proteom IQ Blue. Comparative analysis of protein patterns has been performed by eye. Changes in protein spots were considered as genuine differences in protein expression when they showed similar qualitative changes in expression in all examined gels originating from one sample.

Protein identification

Protein spots were excised and in-gel digested with trypsin, using the protocol described by Havlis et al., 2003. In brief, gel pieces were washed with water for 5-10 min and dehydrated in neat acetonitrile for 20 min. Acetonitrile was aspirated, and gel pieces were dried in a vacuum centrifuge and rehydrated for 60 min in a 1.5 μ M solution of modified trypsin in 50 mM NH_4HCO_3 buffer at room temperature. The digestion was carried out in the same trypsin solution for 30 min at 58 °C. The reaction was stopped by adding 1 μ L formic acid. Extraction of the protein fragments was achieved by adding 50 μ L 0.1% (v/v) trifluoroacetic acid (TFA) in 50% acetonitrile. The extracts were pooled and dried in a vacuum centrifuge. Before desalting the digested proteins were redissolved in 20 μ L of 5% (v/v) formic acid and 10 μ L of this solution was applied to a ZipTip μ -C18 column. The ZipTip column was washed with 10 μ L of 0.1% (v/v) aqueous TFA, before the peptides were eluted. Elution from the ZipTip column onto the MALDI-target was achieved using 1 μ L of a saturated solution of α -cyano-4-hydroxycinnamic acid in 50% acetonitrile and 0.1% (v/v) TFA. Peptides were analyzed using a Biflex III instrument (Bruker Daltonics), using delayed extraction and reflectron mode with positive ion detection. The mass scale was calibrated with the trypsin autodigestion product of known $[\text{M}+\text{H}]^+$ mass, 2211.1 Da, and the keratin 9 fragment $[\text{M}+\text{H}]^+$, 2705.2 Da. Proteins were identified using MASCOT software (Perkins et al., 1999) and peptide map searches were performed against the NCBI non-redundant database.

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Chapter 8 | Summary and Future Prospects

Isoquinolinesulfonamide based kinase inhibitors

An increasing number of bacterial strains develop resistance against multiple antibiotics, including some of the so-called last resort drugs.¹ These Multi-drug resistant bacterial strains pose a serious health threat. In order to remain able to treat future outbreaks several, steps must be taken. First, besides the optimization of existing antibiotics, new classes of antibiotics must be developed. Second, these new classes of drugs should preferentially target biochemical pathways that are currently unexploited. **Chapter 2** describes the discovery of a new druggable target for antibiotics. Inhibitors of protein kinase B (PKB/Akt1) are currently pursued as potential anti-tumor agents. The chapter describes the discovery that this protein is required for intracellular survival of several strains of pathogenic bacteria, including *Salmonella*. Targeting host cell kinases is a promising new strategy to treat pathogen bacterial infections. **Chapter 3** describes the synthesis of two series of compounds aimed at optimizing the inhibition profile towards PKB/Akt1. Starting point is the known protein kinase A inhibitor, H-89. Several structural modifications of the H-89 structure

resulted in the identification of two positions on the H-89 skeleton amenable for optimization towards more potent and selective inhibitors. Application of the reduction transimination reduction sequence led to the construction of a library of derivatives with increased structural variation, namely phenyl substitutions, double bond substitutions and double bond configurations. **Chapter 4** describes the parallel synthesis of a 63-member compound library based on Suzuki coupling of the bromine moiety in three suitably protected H-89 derivatives, bearing the bromine on the *ortho*, *meta* and *para* position, with 21 different boronic acids.

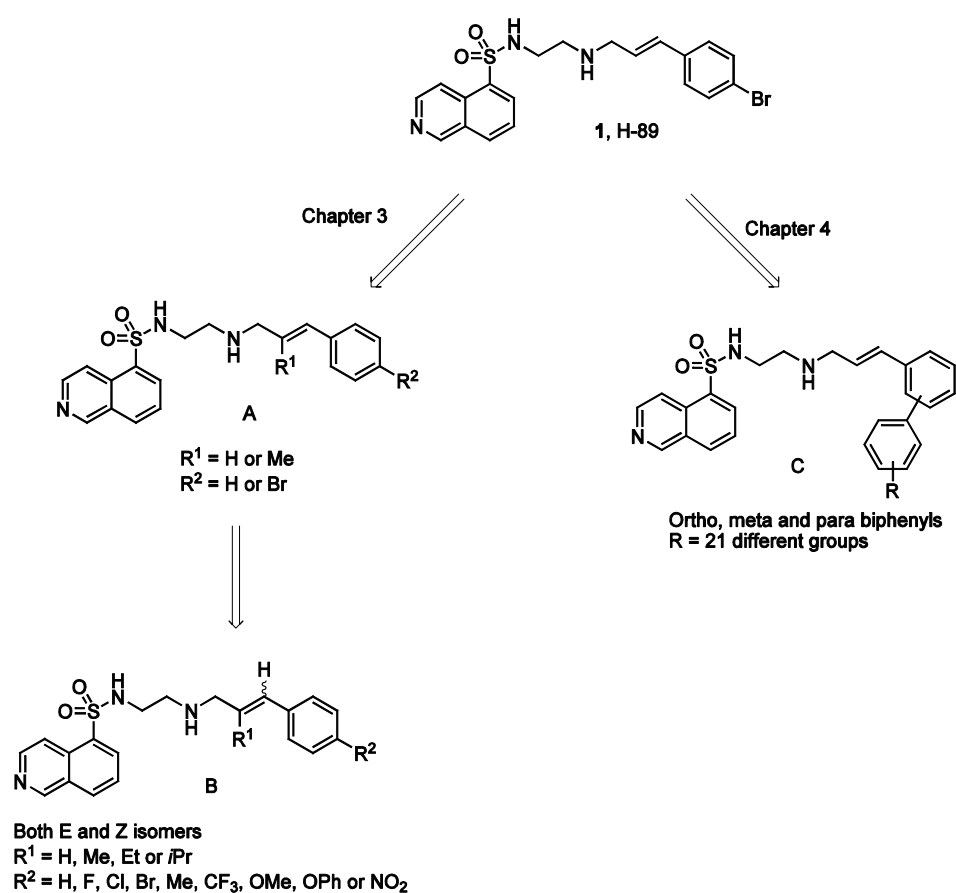


Figure 1; Novel H-89 analogues described in Chapters 3 and 4

Modifications of the H-89 skeleton are by no means limited to the positions described in chapters 3 and 4. In the next section, several strategies are discussed that allow the modification of the H-89 skeleton in the 3 regions depicted in Figure 2.

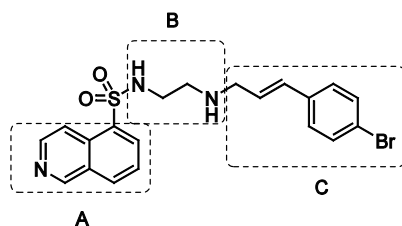
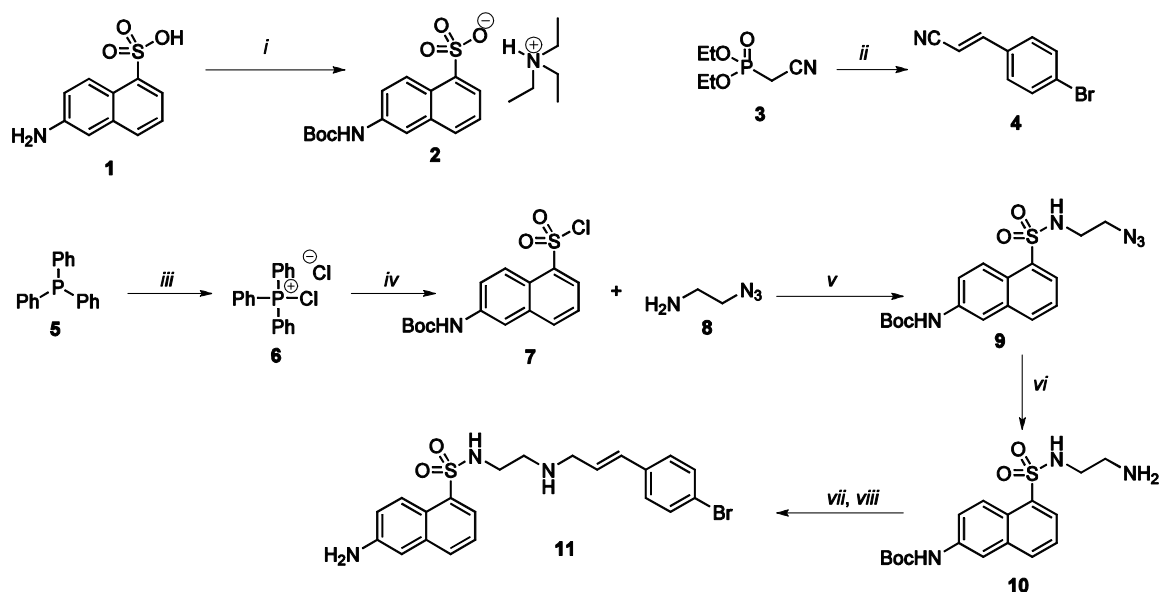


Figure 2; The structure of H-89. Highlighted are the three domains that are discussed in the text below.

Modifying region A

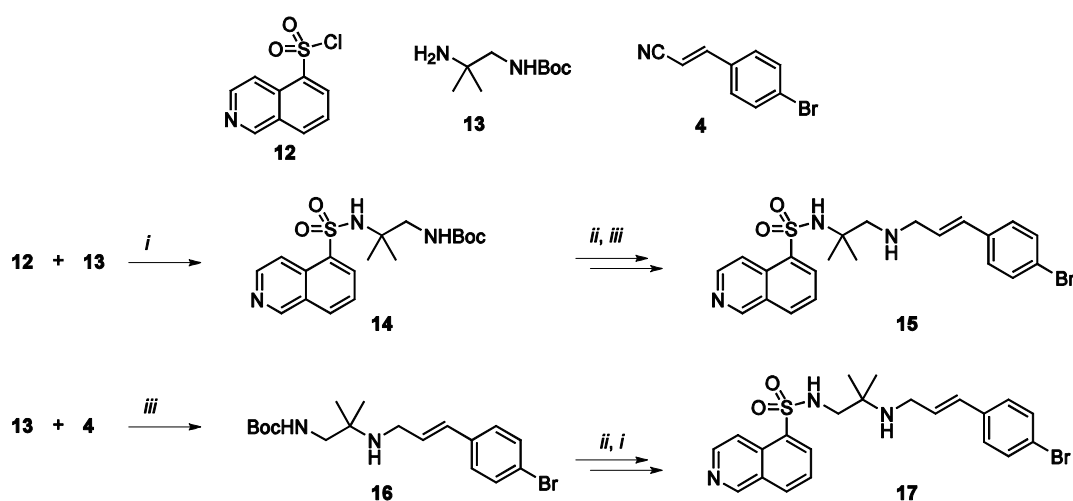
This region of the pharmacophore engages in hydrogen bonding with the so-called hinge region which is located at the bottom of the ATP binding pocket of the kinase. The isoquinoline nitrogen atom serves as a hydrogen bond acceptor in the active site of the kinase. Replacing the isoquinoline moiety with an aminonaphtyl group would make this group a hydrogen bond donating moiety. A synthetic strategy towards this class of compounds is exemplified by the synthesis of 6-aminonaphtyl derivative **11**, (in Scheme 1). Amine protection in 6-aminonaphtalenesulfonic acid using Boc_2O and triethylamine in MeOH should give protected **2**. Transformation of the sulfonic acid into the corresponding sulfonyl chloride requires **6** as mild chlorinating agent. Acylation of **8** followed by Staudinger reduction of the azide in **9** should yield amine **10**. Submitting known **4** and **10** to the reduction transimination reduction protocol as described in chapter 3, followed by acidic deprotection would give **11** as novel H-89 analogue.



Scheme 1; Synthesis of naphthylamino based inhibitors. *Reagents and conditions:* (i) Et_3N , Boc_2O , MeOH. (ii) NaH, *p*-bromobenzaldehyde, DMF, 0°C . (iii) SOCl_2 , DCM. (iv) **2**, DCM. (v) DiPEA, DCM. (vi) PMe_3 , THF/ H_2O . (vii) a: **4**, DiBAL-H, Et_2O , -78°C . b: MeOH, -100°C . c: **9**. d: NaBH_4 . (viii) TFA/DCM.

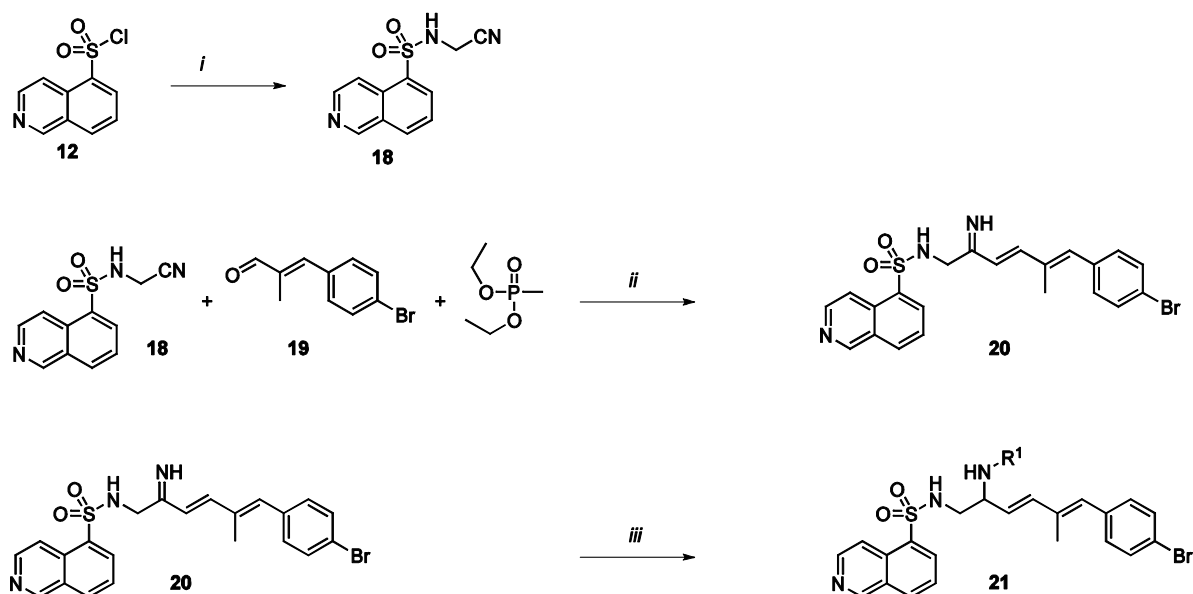
Modifying linker region B

The linker region in H-89 serves two purposes.² It contains a crucial amino functionality which is involved in hydrogen bonding and it enables proper alignment of the isoquinolinesulfonamide moiety and the styrene end group. Reducing the number of rotatable bond by rigidifying this region using a variety of cyclic diamine spacers neither improved potency nor selectivity.³ Incorporating small substituents on the diamine spacer may be a more subtle way to reduce rotatable freedom without locking the structure. The assembly of building blocks **4**, **12** and **13** using chemistry described in chapter 2 enables the synthesis of **15** and **17** as depicted in Scheme 2.



Scheme 2; Introducing rotational constraints in the linker. *Reagents and conditions*: (i) DiPEA, DCM. (ii) TFA/DCM. (iii) a: DiBAL-H, Et₂O, -78°C. b: MeOH, -100°C. c: **13** or deprotected **14**. d: NaBH₄.

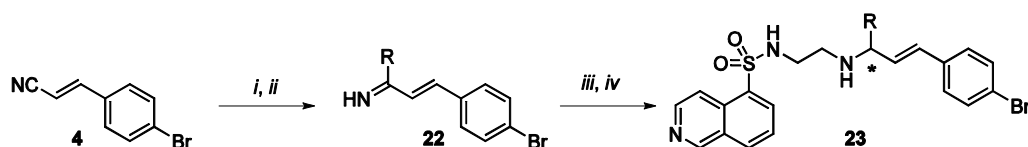
Another way to functionalize the linker is depicted in Scheme 3. Acylation of 2-aminoacetonitrile with isoquinolinesulfonyl chloride **12** gives nitrile **18**. Submitting nitrile **18**, cinnamic aldehyde **19** (chapter 3) and diethyl methylphosphonate to *n*-butyllithium under Horner-Emmons conditions according to the method developed by Orru *et al.* gives unsaturated imine **20**.⁴ This intermediate can be processed in a transimination reaction with a variety of amines to generate derivatives of **21**.



Scheme 3; Linker modifications: *Reagents and conditions*: (i) 2-aminoacetonitril, DMAP, pyridine. (ii) *n*-BuLi, THF. (iii) $R^1CH_2NH_2$, MeOH.

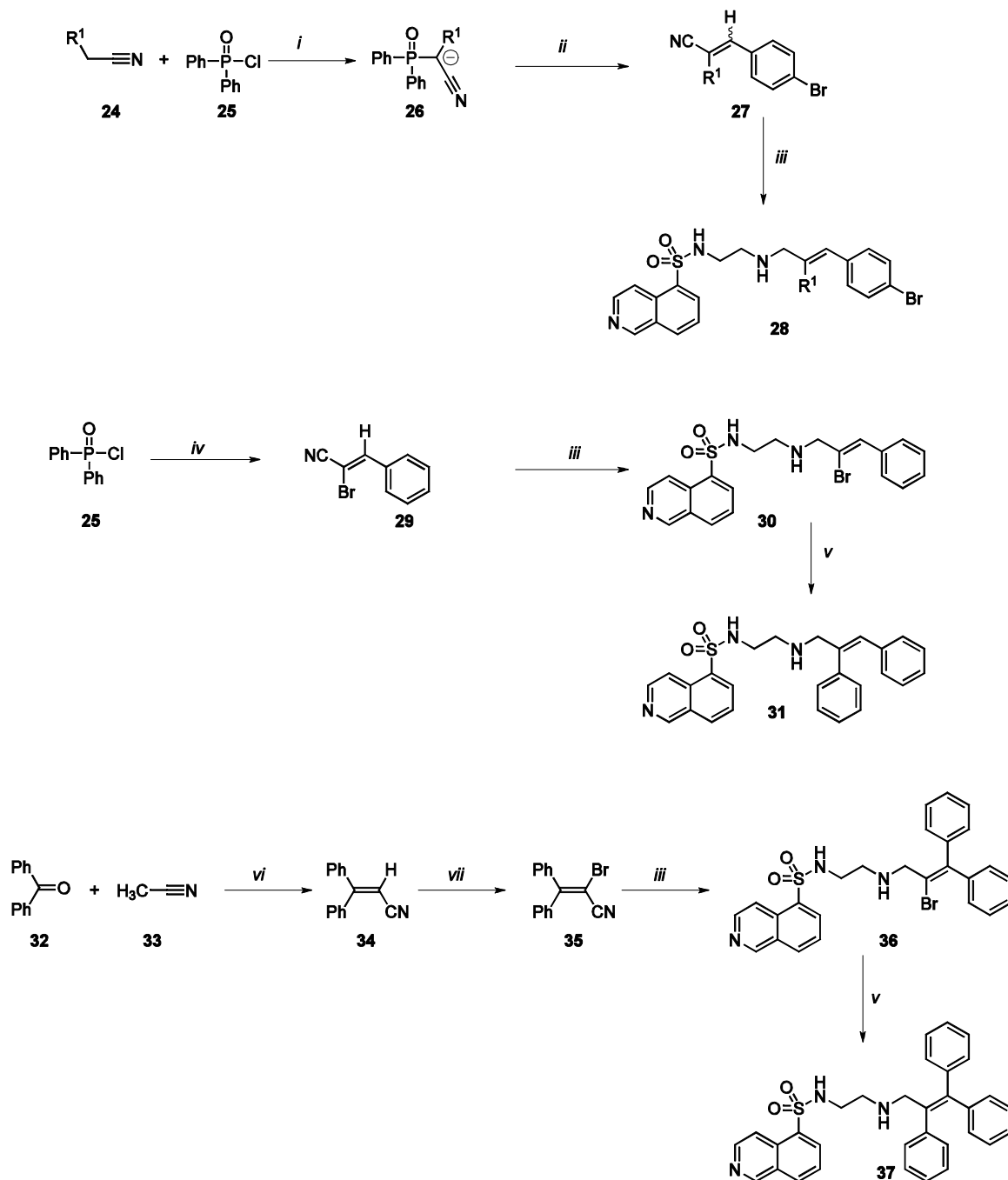
Modification of region C

The reduction-transimination-reduction procedure described in chapter 3 can be modified to introduce α -substituted amines (see Scheme 4).⁵ Treatment of **4** with Grignard reagents in refluxing Et_2O , followed by methanolysis of the intermediate magnesium species affords secondary imine **22**, which after transimination with **12** and $NaBH_4$ reduction affords isoquinolinesulfonamides **23** as racemates.



Scheme 4; Grignard transimination reduction strategy. *Reagents and conditions*: (i) $R-MgBr$, Et_2O , reflux. (ii) MeOH, $-100^\circ C$. (iii) **12**. (iv) $NaBH_4$

As is described in chapter 3, increasing the size of the double bond substituent increases selectivity towards PKB/Akt1. Several strategies can be envisaged to elaborate on these results (Scheme 5). Horner-Wittig reaction between the anion of substituted acetonitriles **24** and diphenylphosphinyl chloride **25** yields anion **26**. Addition to, for instance, *p*-bromobenzaldehyde should result in the formation of isomeric mixtures of α -substituted cinnamic aldehydes **27**. These can be used to extend the range of substituents in **28**. Condensing bromoacetonitrile to **25** using LiHMDS followed by addition of benzaldehyde

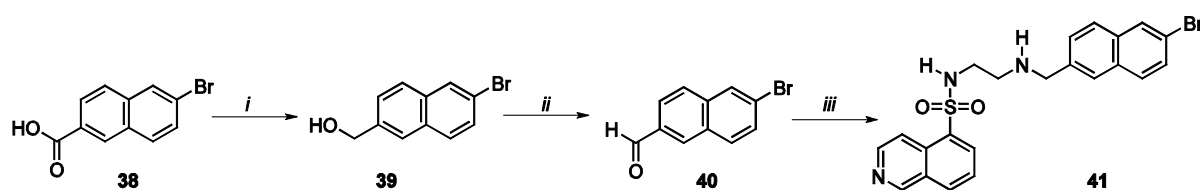


Scheme 5; Novel double bond substitutions. (i) LiHMDS, THF. (ii) LiHMDS, *p*-bromobenzaldehyde, THF. (iii) a: DiBAL-H, Et₂O, -78°C. b: MeOH, -100°C. c: *N*-(2-aminoethyl)isoquinoline-5-sulfonamide. d: NaBH₄. (iv) 2 eq, LiHMDS, bromoacetonitril, THF, -78°C. (v) K₂CO₃, phenylboronic acid, Pd(PPh₃)₄, toluene/H₂O. (vi) KOH. (vii) Br₂, CHCl₃, reflux.

should yield α -bromo-cynnamic aldehyde **29**. Transformation into **30** yields a precursor that, after nitrogen protection, allows for Suzuki type crosscouplings leading to a variety of aryl-substituted analogues like **31**. Similar analogues with an additional aryl-substituent can be obtained starting from a Knoevenagel condensation between benzophenone **32** and acetonitrile **33**. The thus obtained α,β -unsaturated nitrile **34** can be brominated leading to

35 as single isomer, which can be transformed into vinyl bromide **36** that, after protection of the secondary amine group, may serve as precursor for palladium based crosscoupling reactions towards **37**.

The restriction of flexibility in the styrene moiety is exemplified by the synthesis of **41** (Scheme 6). Here commercially available 6-bromo-2-napthoic acid **38** is reduced to **39** and re-oxidized towards the aldehyde **40** followed by reductive amination with the appropriate amine to afford naphtylated isoquinolinesulfonamide **41**.



Scheme 6; constraint analogues; *Reagents and conditions*: (i) LiAlH_4 , Et_2O , 0°C . (ii) Dess-Martin periodane. (ii) a: DiBAL-H , Et_2O , -78°C . b: MeOH , -100°C . c: N -(2-aminoethyl)isoquinoline-5-sulfonamide. d: NaBH_4 .

Cationic antimicrobial peptides

Killing bacteria by disrupting their cell wall membrane with cationic antimicrobial peptides (CAPs) is currently being explored as a new approach in the treatment of multi-drug resistant bacterial infections. The agents used in these approaches are usually very potent, but their lack of selectivity hampers their clinical application to topical use.⁶ **Chapter 5** describes results previously obtained with the cationic peptide Gramicidin S **38** and analogues thereof (**39a** and **39b**, Figure 3). Structural analysis of analogues **40** - **42** revealed a similar β -sheet structure compared to GS with, however, an altered backbone orientation around the SAA amide group and an increased twist angle. Biological evaluation of these analogues revealed a loss in activity for **39a**. Analogue **39b** was shown to be as potent as GS in both the antimicrobial and the hemolytical assay. Chapter 5 describes the synthesis of three novel GS analogues (**40** – **42**) and their structural and biological evaluation. The structure of **40**, containing a furanoid SAA, adopted a similar β -sheet structure with re-oriented backbone conformation in solution compared to **39b**. The solution structure of analogues **41** and **42** resemble the overall structure of GS itself and the backbone re-orientation could not be observed. The results obtained from the antimicrobial and hemolytical assays show a similar profile for **40** compared to GS and analogue **39b**. Whereas the antimicrobial potencies of **41** and **42** are comparable to GS, both show a reduced toxicity for erythrocytes in the hemolytical assay with **41** about half as toxic as GS. The introduction of cyclic furanoid SAAs apparently alters the backbone conformation but does not

influence the biological profile. Linear dipeptide isosters **41** and **42** did not disrupt the backbone conformation and showed an improved biological profile.

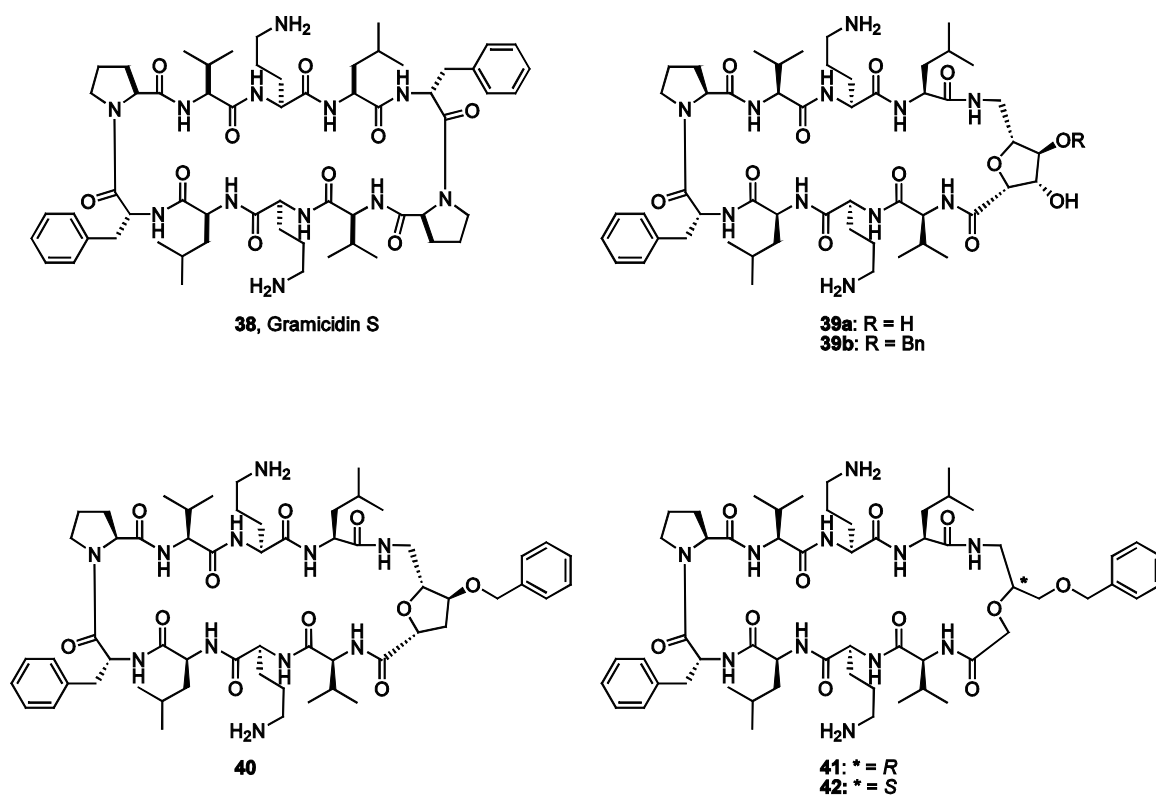


Figure 3; GS, known derivatives **39a** and **b** and newly developed derivative **40 - 42**

Introduction of a second copy of AAA **44** might shed light on the tolerance for less conformationally restricted dipeptide isosters with respect to antimicrobial and hemolytical activity.

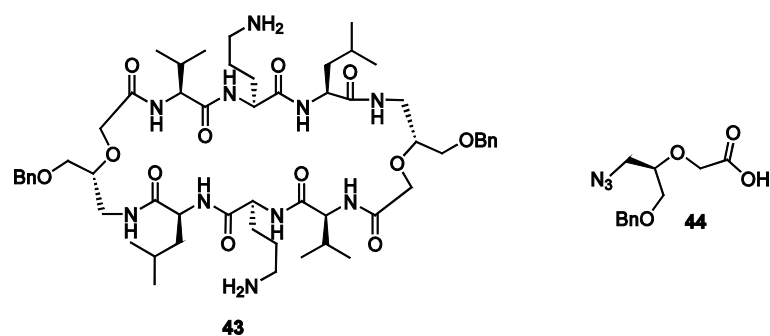


Figure 4; GS analogue with increased flexibility

In **Chapter 6**, a similar strategy, the use of dipeptide isosters, is applied on Loloatin C and the structural and biological consequences of the incorporation of furanoid SAA **46** replacing three different motifs (highlighted in Figure 5). Structural analysis by means of NMR indicated that the solution structure of each analogue differed from the dumbbell-like conformation of Loloatin C. Also, all analogues containing SAA **46** were shown to be less potent in the antimicrobial and the hemolytical assay than the parent compound, Loloatin C. Possibly, incorporating AAA **48** into the Loloatin C allows the compound to adopt the dumbbell-like conformation but still profit from the benefits of non-natural amino acid replacements.

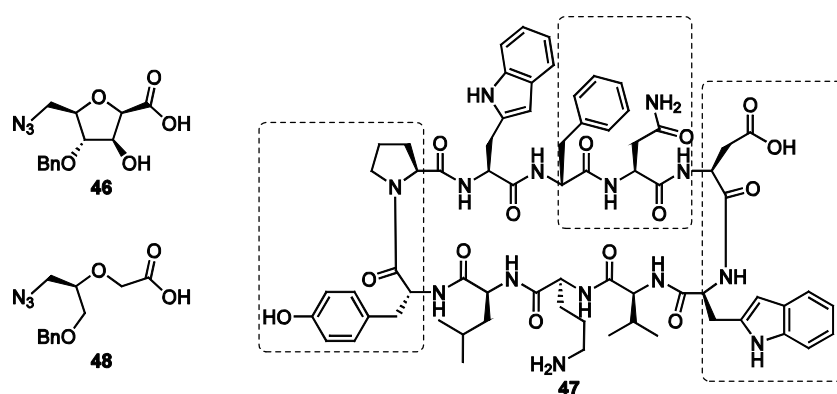
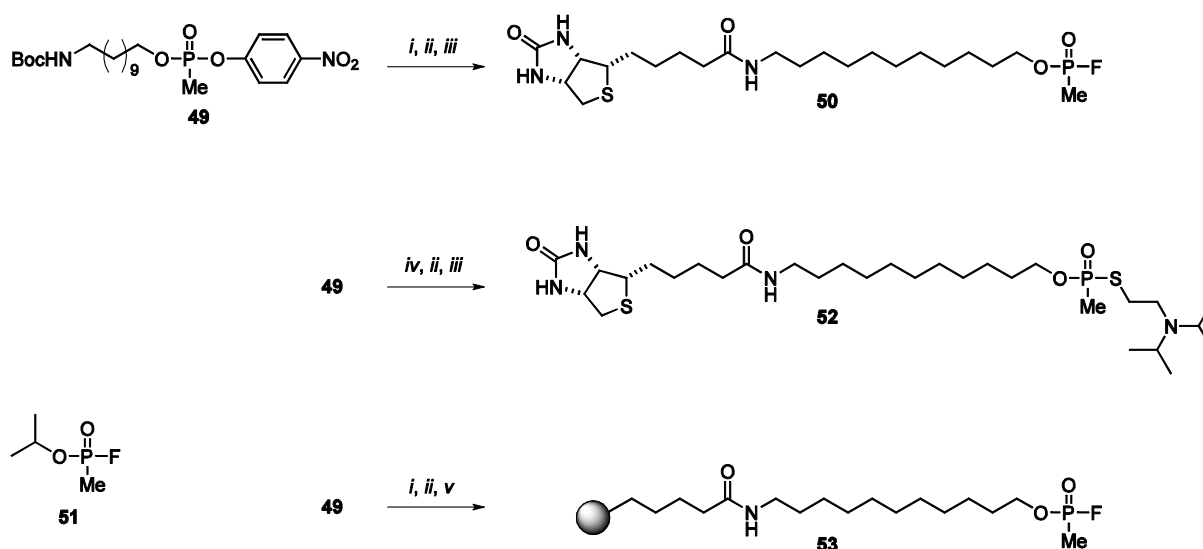


Figure 5; Structures of SAA **46** and Loloatin C, **47**. Highlighted motif have been replaced by SAA **46**.

Biotinylated methylfluorophosphate as biochemical probe

In **Chapter 7**, the synthesis of biotinylated methylfluorophosphate **50** has been described. The compounds were applied to study the biological effects of intoxication with toxins like sarin **51** and the isolation of butyrylcholinesterase from human plasma. Competition experiments between **50** and **51** in monkey liver homogenates, intact and lysed A549 lung cells identified 8 proteins that were inhibited by **51** in a competitive manner. The synthesis of **50** from precursor **49** allows the synthesis of organophosphonate bearing a different leaving group (**52**) or a different reporter group (**53**). In **52**, the fluoride is replaced by a 2-(diisopropylamino)ethanethiol moiety that is also found in the nerve agent VX which enables a detailed study on the inhibition profile of the agent. The introduction of a solid support into the construct may open the way for an alternative isolation procedure.



Scheme 7; Structures and proposed synthesis of biotinylated methylfluorophosphonate **50** and analogue bearing a different leaving group (**52**) or tag (**53**). *Reagent and conditions*: (i) TBAT, THF. (ii) TFA/DCM. (iii) biotin-OSu, DiPEA, DMF. (iv) 2-(diisopropylamino)ethanethiol, DBU, THF. (v) Sepharose-OSu, DiPEA, THF

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Samenvatting

Er is een steeds groter aantal bacteriestammen dat resistent wordt tegen meerdere antibiotica. Deze multi-drug resisistente bacteriën vormen een grote bedreiging voor de volksgezondheid. Om deze bacteriën ook in de toekomst te kunnen bestrijden moeten er een aantal stappen worden genomen. Ten eerste, naast het verbeteren van de huidige antibiotica, moet er geïnvesteerd worden in het ontwikkelen van nieuwe typen antibiotica. Daarnaast moet er worden gezocht naar mogelijke nieuwe doelen voor antibiotica. **Hoofdstuk 2** van dit proefschrift beschrijft de ontdekking van een nieuw doel voor antibiotica. Remmers van het enzym “proteïne kinase B” (PKB/Akt1) worden op dit moment ontwikkeld als anti-tumor medicijn. Ditzelfde enzym blijkt ook nodig te zijn voor sommige pathogene bacteriën, zoals *Salmonella*, om in de gastheer cel te kunnen overleven. Remmers van dit enzym zijn dus niet alleen te gebruiken als anti-tumor medicijn, maar mogelijk ook als antibiotica. **Hoofdstuk 3** beschrijft de synthese van een aantal verbindingen die analoog zijn van een bekende remmer van proteïne kinase A (H-89), met als doel een actieve en selectieve remmer voor PKB/Akt1 te maken. Verschillende modificaties aan de structuur van deze verbinding onthulde twee posities die gebruikt konden worden om uitgaande van deze PKA

remmer, een remmer voor PKB te maken. Vervolgens wordt beschreven hoe een serie nieuwe verbindingen werd gesynthetiseerd en getest op hun potentie om PKB/Akt1 te remmen en zodoende de groei van *Salmonella* in de cel tegen te gaan. Dit leidde tot een nieuwe verbinding die, in vergelijking met H-89, een vergelijkbare activiteit heeft voor PKB/Akt1 en een sterk verminderde activiteit voor PKA. Deze resultaten leidden tot nieuwe inzichten in de structuur-activiteits relatie van dit type verbinding. **Hoofdstuk 4** laat zien hoe de serie potentiële remmers voor PKB/Akt1 verder werd uitgebreid met een bibliotheek van 63 verbindingen die op een parallelle manier gemaakt werden.

Kationische antimicrobiële peptides zijn antibiotica die werken op het membraan van bacteriën. Een voorbeeld van zo'n peptide is het natuurlijk voorkomende Gramicidine S (GS). Deze verbinding is echter ook hemolitisch, dat wil zeggen toxisch voor rode bloedcellen. In **Hoofdstuk 5** van dit proefschrift wordt het onderzoek beschreven dat gaat over het modificeren van GS met als doel de bijwerkingen te verminderen. GS is een cyclisch decapeptide dat is opgebouwd uit twee β -strands, bestaande uit een leucine, ornithine en een valine residu, die aan elkaar gekoppeld zijn door twee type II' β -turns die elk bestaan uit een D-phenylalanine en een proline residu. Middels het gebruik van suiker aminozuren als vervangers voor één van de turns kunnen GS analoga met nieuwe eigenschappen verkregen worden. Eerder onderzoek leerde dat het gebruik van een furanoïde suiker aminozuur leidde tot een GS analoog met een andere secundaire structuur in vergelijking met GS zelf, maar met een vergelijkbare antimicrobiële en hemolitische activiteit. Dit hoofdstuk beschrijft de synthese van drie nieuwe suiker aminozuren, één furanoïde en twee lineaire, en hun toepassing als β -turn vervangers in nieuwe GS analoga. De structuur van deze analoga werd onderzocht met behulp van 1D en 2D NMR analyse. Hieruit bleek dat de structuur van het GS analoog met de furanoïde suiker aminozuur vergelijkbaar was met die van de eerder gemaakte GS analoog. De structuur van de analoga met de lineaire suiker aminozuren kwam weer overeen met die van natuurlijk GS. De antimicrobiële activiteit van alle drie de analoga waren vergelijkbaar met die van GS. Echter, de hemolitische activiteit van één van de analoga met een lineair suiker aminozuur was sterk verminderd. De reden van deze verminderde toxiciteit moet nog nader worden onderzocht. Het gebruik van suiker aminozuren als vervangers voor een dipeptide motief van een ander kationisch antimicrobiële peptide, Loloatine C wordt in **hoofdstuk 6** beschreven. De biologisch active conformatie is een flexibele haltervormige structuur. De synthese van drie suiker amino zuur

bevattende Loloatine C analoga alsmede de bepaling van hun structuur in oplossing en hun biologische activiteit worden in dit hoofdstuk beschreven. Afhankelijk van de positie van het suiker aminozuur in de cyclische peptides werd een meer of minder gedefiniëerde structuur gevonden. De biologische activiteit van de nieuwe analoga was beduidend lager dan die van Loloatine C zelf. Ook worden in dit hoofdstuk drie Loloatine C derivaten beschreven waarbij alleen de stereochemie van bepaalde residuen is veranderd met als doel te kijken of er hybride structuren tussen GS en Loloatine C gemaakt konden worden met gunstige biologische eigenschappen. Uit NMR analyse bleek één analoog een vergelijkbare structuur te hebben als Loloatine C. Helaas bleek geen van de hybride structuren biologisch actief.

Hoofdstuk 7 beschrijft de synthese van een gebiotinyleerd methylfluorofosfonaat. Dit reagens werd voor twee studies gebruikt. In de eerste studie werd gezocht naar secundaire doel eiwitten die geremd worden door het zenuwgas sarin. Competitie experimenten tussen de gebiotinyleerde probe en sarin in lever homogenaten, longcellen en longcel lysaten resulteerde in de identificatie van 8 eiwitten die competitief geremd werden door sarin. In een tweede studie werd het methylfluorofosfonaat gebruikt voor de isolatie van butyrylcholinesterase uit plasma. Dit enzym kan preventief gebruikt worden ter bescherming tegen blootstelling aan zenuwgassen. De methode die in dit hoofdstuk beschreven wordt is een goed uitgangspunt voor het optimaliseren van de huidige, arbeidsintensieve, isolatie procedure voor dit enzym.

Het werk beschreven in dit proefschrift concentreert zich op de ontwikkeling van nieuwe antibiotica. In de hoofdstukken 2 – 4 worden de ontwikkeling van selectievere remmers van PKB/Akt1 en hun toepassing als antibioticum beschreven. In **hoofdstuk 8** worden nieuwe mogelijkheden gepresenteerd die toegepast kunnen worden in de ontwikkeling van nog potentere en selectievere remmers. Ook worden nieuwe voorstellen gedaan voor de toepassing van de kennis die is opgedaan naar aanleiding van de resultaten met suiker amino zuren als dipetide vervangers die beschreven zijn in de hoofdstukken 5 en 6. Tenslotte worden suggesties gedaan die kunnen helpen met het optimaliseren van de isolatieprocedure van butyrylcholinesterase uit plasma.

List of publications

An expeditious route towards pyranopyran sugar amino acids

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Curriculum Vitae

Adriaan Willem Tuin werd op 1 april 1979 geboren in Monster. Na het behalen van het HAVO-diploma aan de Gereformeerde Scholengemeenschap Rotterdam te Rotterdam in 1996 werd in september van dat jaar begonnen aan de studie Organische Chemie aan de Hogeschool Rotterdam & Omstreken te Delft. In het kader van het afstudeeronderzoek werd van september 1999 tot juni 2000 onderzoek verricht bij Byk Gulden te Konstanz (Dld). In deze periode werden verschillende gesubstitueerde tetrahydro-indazolen gesynthetiseerd die als uitgangsstof dienden voor de geautomatiseerde synthese van een bibliotheek van verbindingen op een combinatoriële manier. De opleiding werd in 2000 met succes afgerond waarna in september van dat jaar een aanvang werd gemaakt met de studie Scheikunde aan de Universiteit Leiden. In de periode van september 2001 tot april 2003 werd de hoofdvakstage uitgevoerd bij de vakgroep Bio-organische Synthese onder leiding van prof. dr. J.H. van Boom en prof. dr. G.A. van der Marel. Het onderzoek was gericht op de synthese van verscheidene bicyclische suiker aminozuren die als dipeptide isosteren konden worden ingebouwd in het kationsche antimicrobiële peptide Gramacidine S. Hierna werd in de periode van mei tot en met juli van dat jaar een zomerstage gedaan bij Organon te Oss onder leiding van dr. M. de Kort en dr. R. Buijsman. In deze periode werd bijgedragen aan de ontwikkeling van een nieuwe, op ionische vloeistof gebaseerde drager en de toepassing daarvan in de meerstapssynthese van een potent analogon van het anti-trombose medicijn tirofiban. Het doctoraal examen werd in 2003 behaald.

Van augustus 2003 tot augustus 2008 werd als assistent-in-opleiding het in dit proefschrift beschreven onderzoek uitgevoerd bij de vakgroep Bio-organische Synthese onder leiding van prof. dr. H.S. Overkleeft en dr. M. Overhand in samenwerking met dr. D. Noort van TNO Defense, Security and Safety.

Sinds september 2008 wordt in samenwerking met de groep van prof. dr. R.P.H. Bischoff onderzoek verricht naar de synthese van MMP en ADAM remmers.

Nawoord

Dit proefschrift heeft niet tot stand kunnen komen zonder de inbreng, in verschillende vorm, van een heel aantal personen. Allereerst wil ik mijn ouders bedanken voor hun steun en interesse gedurende mijn opleiding en mijn AIO-schap. Mijn broers en zussen hebben altijd geduldig geluisterd naar al mijn verhalen over de stofjes die ik weer bij elkaar had gegoooid.

Een bijzonder woord van waardering gaat uit naar al de studenten die als onderdeel van hun studie hun bijdrage hebben geleverd aan verschillende hoofdstukken van dit proefschrift. Alex Poot, Gerbrand van der Heden, Arnoud Mol, Adem Göl, Sergio Satimin en Wessel Heldeweg, jullie inzet, geduld en doorzettingsvermogen waren fantastisch. Het was een plezier om met jullie samen te werken. Dimitrios “Jimmy” Palachanis, your eagerness to learn, the progress you have made and the joy you had after the successful oxidation of that stubborn alcohol are unforgettable. En niet te vergeten de vele eerste- en tweedejaars studenten die in het kader van hun “Leren Onderzoeken” hebben bijgedragen aan de synthese van vele kinase remmers.

De (oud-)collega's van de vakgroep Bio-organische Synthese droegen bij aan een fijne sfeer op het lab. Ik heb de contacten met Jimmy, Bas, Paul van S., Michiel, Tom, Jasper, Gijsbert, Mattie, Kimberly, Farid, Karen, Martijn V., Carlo, Henrik, Ali, Vicky, Paul G., Varsha, Martijn R., Matthijs, Martin, Christoph, Clara, Ulrik, Wouter van der L., Wouter H., Boudewijn, Marian, Marthe, Alphert, Jeroen, Annemiek en Nico zeer op prijs gesteld. Er zijn een aantal mensen die een speciale vermelding verdienen. Leendert, onze vriendschap gaat al ver terug, en ik hoop van harte dat hij nog lang zo blijft. Peter, het wekelijks squashen heeft een niet-te-onderschatten invloed gehad op mijn fysieke en mentale gesteldheid de afgelopen tijd. Heerlijk om 45 minuten lang die bal te meppen. Rian, jouw bijdrage aan de synthese van de eerste serie kinase remmers word zeer gewaardeerd. De hulp van Hans bij het zuiveren en analyseren van vele verbindingen waren onmisbaar. Van de NMR afdeling wil ik Fons en Kees bedanken voor hun assistentie bij het opnemen van NMR spectra. De technische ondersteuning door Arnold, Marco en Henny werd zeer gewaardeerd.

De samenwerking met de collega's bij TNO, Defense Safety and Security was altijd een aangename afwisseling. Alex Fidler, bedankt voor jouw bijdrage aan het maken van de fluorfosfonaten. De biochemische expertise van Marijke Mol en Roland van den Berg werd

zeer op prijs gesteld. Bij het uitvoeren van de experimenten met butyrylcholinesterase kon ik altijd rekenen op Marcel van der Schans. Voor de hemolitische experimenten kon ik altijd rekenen op Roos Mars. Maarten Tromp, Annemiek van Zuylen, Duurt Alkema en Martijn de Koning zorgden voor een erg leuke sfeer in het lab.

Han de Neeling en Emile Spalburg van het RIVM in Bilthoven ben ik zeer erkentelijk voor het uitvoeren van de antibacteriële experimenten.

I really appreciated the collaboration with Nigel Savage from the Tom Ottenhof group at the LUMC. Looking into the microscope to see *Salmonella* bacteria move around inside a cell was a priceless moment. De samenwerking met Coen Kuijl van de groep van Sjaak Neefjes op het NKI heb ik als zeer plezierig en leerzaam ervaren. Het is altijd leuk om de stofjes die je maakt ook zelf (samen met jou) te testen.

Corine, de laatste maanden van mijn promotie waren de eerste samen met jou. Jij hebt deze tijd bijzonder aangenaam gemaakt en ik hoop van harte dat de toekomst net zo zal zijn.

