Design, Synthesis and Pharmacological Evaluation of Certain GABA_B Agonists

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TO MY MOTHER AND MY WIFE
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1 Introduction

The discipline of medicinal chemistry is devoted to the discovery and development of new agents for treating diseases. Hundreds of thousands of new organic chemicals are prepared annually throughout the world and many of them are entered into the random pharmacological screening to determine whether they have a useful biological activity.\(^{(1)}\)

The neutral amino acid \(\gamma\)-aminobutyric acid (GABA) is present in every region of the mammalian central nervous system (CNS). Present estimates indicate that more than 40% of all inhibitory synaptic activity is mediated through this neutral amino acid. It never ceases to amaze me that GABA, which has such a simple structure, should play such an important neurotransmitter role within the mammalian brain. This provided a major impetus for further studies in neurochemistry, pharmacology, medicinal chemistry, as well as electrophysiology, which together have now substantiated its physiological role.\(^{(2)}\)

\[
\text{GABA}
\]

GABA is involved in the control of many physiological mechanisms, including the regulation of prolactin secretion and other hormones, including the growth hormone. Furthermore, GABA plays a role in the regulation of cardiovascular functions, such as blood pressure and heart rate and is involved in the sensation of pain and anxiety.\(^{(3)}\)

The growing interest in the pharmacology of GABA has been stimulated by the findings that GABA is apparently involved in the development of certain neurological and psychiatric diseases such as epilepsy, Huntington’s chorea, parkinson’s disease and perhaps, schizophrenia.\(^{(3)}\)

The specific aim of this work is the design and synthesis of a series of GABA analogues and homologues to be pharmacologically evaluated for GABA\(_B\) receptor (GABA\(_B_R\)) agonist activity.
2 Neurotransmitter Receptors

The diversity of neurotransmitters is extensive but their receptors can be grouped into two broad classes: ligand-gated ion channels (Table 1\(^4\)) and G protein-coupled receptors (Table 2).\(^4\) Activation of ligand-gated ion channel receptors induces rapid changes, within a few milliseconds, in the permeability and potential of the postsynaptic membrane. In contrast, the postsynaptic responses triggered by activation of G protein-coupled receptors occur much more slowly, over seconds or minutes, because these receptors regulate opening and closing of ion channels indirectly.\(^4\)

<table>
<thead>
<tr>
<th>Function type</th>
<th>Ligand</th>
<th>Ion channel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excitatory</td>
<td>Glutamate (non-NMDA class receptors)</td>
<td>Na(^+)/K(^+)</td>
</tr>
<tr>
<td></td>
<td>Glutamate (NMDA class receptors)</td>
<td>Na(^+)/K(^+) and Ca(^{2+})</td>
</tr>
<tr>
<td></td>
<td>Acetylcholine (nicotinic receptor)</td>
<td>Na(^+)/K(^+)</td>
</tr>
<tr>
<td></td>
<td>Serotonin (5HT(_3) class receptors)</td>
<td>Na(^+)/K(^+)</td>
</tr>
<tr>
<td>Inhibitory</td>
<td>GABA(_{A/C}) and glycine receptors</td>
<td>Cl(^-)</td>
</tr>
</tbody>
</table>

**Table 1**
Ligand-gated ion channel receptors

**Table 2**
Some neurotransmitter G protein-coupled receptors

- Glutamate receptors (metabotropic receptors)
- Acetylcholine (muscarinic receptors)
- Epinephrine, norepinephrine
- Serotonin (5HT\(_1\), 5HT\(_2\), 5HT\(_4\), receptors)
- GABA\(_B\) receptors
- Dopamine receptors
- Histamine receptors
2.1 Ligand-Gated Ion Channel Receptors

2.1.1 GABA<sub>A</sub> Receptors

The discovery of GABA in the brain tissue was made independently by J. Awapara et al.<sup>(5)</sup> and E. Roberts et al.<sup>(6)</sup> in early fifties. GABA is synthesized in a single step catalyzed by the enzyme glutamate decarboxylase (GAD). This enzyme converts L-glutamate to GABA and CO₂, using pyridoxal phosphate as a cofactor. The breakdown of GABA is catalyzed by GABA aminotransferase (GABA-T). This enzyme transfers the amino group from GABA to α-ketoglutarate (α-KG). The products of the reaction are succinic semialdehyde and glutamate. The succinic semialdehyde formed by GABA-T is quickly oxidized to succinate by the mitochondrial enzyme succinic semialdehyde dehydrogenase (SSADH) (scheme 1). Hence, the net effect of synthesizing and metabolizing a molecule of GABA is to convert a molecule of α-KG to succinate (since the glutamate used in the initial step is subsequently regenerated).<sup>(7)</sup>

![Chemical diagram of GABA synthesis and metabolism](image)

Blocking GAD by interfering with the cofactor pyridoxal phosphate leads to reduction in the brain GABA. Although the drugs available for this purpose are not entirely specific because many enzymes utilize this cofactor. The compounds allylglycine 1, L-glutamate-γ-hydrazide 2 and 3-mercaptopropionic acid 3 are considered somewhat selective for GAD inhibition. These drugs reduce GABA availability invariably and cause convulsions when given at sufficient doses.<sup>(7)</sup>
On the other hand, GABA levels can be elevated by drugs that block GABA-T. Aminooxyacetic acid 4 (AOAA), 5-amino-1,3-cyclohexadienecarboxylic acid 5 (gabaculine) and \( \gamma \)-vinyl GABA ((S)-vigabatrin) 6 are all well-known GABA-T inhibitors. GABA-T inhibitors exhibit sedative and anticonvulsant properties and vigabatrin 6 has recently been introduced clinically for the treatment of epilepsy.\(^{(7)}\)

In the same vein, 4-amino-5-fluoro-pentanoic acid\(^{(8)}\) 7 was rationally designed as inactivator of GABA-T and it has been shown to be more potent GABA-T inhibitor than its conformationally restricted analogue 8.\(^{(9)}\)

GABA is the major inhibitory neurotransmitter in the central and peripheral nervous systems where it exerts its effects through different classes of receptors consisting of the ionotropic GABA\(_A\) and GABA\(_C\) receptors and the metabotropic GABA\(_B\) receptors.\(^{(10)}\) The overall activity of the brain is basically determined by two superior functions: 1- excitation by the major excitatory amino acid transmitter, Glu, which depolarizes neurons through a large number of receptor subtypes and 2- inhibition by GABA, which hyperpolarizes neurons through multiple receptors.\(^{(11)}\) At the majority of vertebrate GABAergic synapses, the effect of the transmitter is to increase Cl\(^-\) conductance across the membrane.\(^{(12)}\)

In recent years, the application of sophisticated biochemical molecular biological techniques has led to rapid progress in the structural analysis of the GABA\(_A\) receptor complex. Purified GABA\(_A\) receptors exhibit a pentameric structure analogous to that of
the nicotinic cholinergic receptors, each with four putative transmembrane domains.\textsuperscript{(13)}

Later on, nineteen GABA\textsubscript{A} receptor subunit subtypes, namely \(\alpha\) (1-6), \(\beta\) (1-4), \(\gamma\) (1-3), \(\delta\), \(\epsilon\), \(\pi\) and \(\rho\) (1-3) have been identified and can be organized into myriad heteropentameric combinations.\textsuperscript{(14)}

### 2.1.1.a GABA\textsubscript{A} Receptor Agonists

The basically inhibitory nature of the central GABA neurotransmission prompted the design and development of different structural types of GABA agonists. Conformational restriction of various parts of the molecule of GABA and bioisosteric replacements of the functional groups of this amino acid have led to a broad spectrum of specific GABA\textsubscript{A} agonists.

Muscimol 9, the degradation product of ibotenic acid 10\textsuperscript{(15,16)}, is the classic GABA\textsubscript{A} agonist and has been extensively used as a lead for the design of different classes of GABA analogues.\textsuperscript{(7)} The 3-hydroxyisoxazole moiety of 9 can be replaced by a 3-hydroxyisothiazole or 3-hydroxy-isoxazoline group to give thiomuscimol 11 and dihydromuscimol 12, respectively, without significant loss of GABA\textsubscript{A} receptor agonism.\textsuperscript{(17)} Other specific monoheterocyclic agonists at the GABA\textsubscript{A} binding site include isoguvacine 13 and isonipecotic acid 14.\textsuperscript{(18)}

![Chemical structures](image)

(Figure 3)

### 2.1.1.b GABA\textsubscript{A} Receptor Antagonists

Specific receptor antagonists are essential tools for studies of the physiological role and pharmacological importance of the particular receptors. The classical GABA\textsubscript{A} antagonists bicuculline\textsuperscript{(19)} 15 and its quaternized analogue bicuculline methochloride (BMC)\textsuperscript{(20)} 16 have played a key role in such studies on GABA\textsubscript{A} receptors. In recent years, new structural classes of GABA\textsubscript{A} antagonists have been developed. Whereas the bicyclic 5-isoxazole derivative, Iso-THAZ 17, is a moderately potent GABA\textsubscript{A}
antagonist\(^\text{(21)}\), a series of arylaminopyridazine analogues of GABA, notably gabazine \(\text{18}\), showed very potent and selective GABA\(_A\) antagonist effects.\(^\text{(22)}\)

Bioisosteric substitution of a 2-amino-1,3,4-thiadiazole unit for the 3-aminopyridazine part of \(\text{18}\) gives compound \(\text{19}\) which also showed GABA\(_A\) antagonistic properties though markedly weaker than those of \(\text{18}\).\(^\text{(23)}\) Replacement of the GABA structure element of \(\text{18}\) by a thiomuscimol unit afforded compound \(\text{20}\) which showed the most potent GABA\(_A\) antagonist activity in the arylaminopyridazine series.\(^\text{(24)}\)

It has been appreciated that some GABA antagonists do not interact with the GABA binding site itself, but rather with separate sites on or near the Cl\(^-\) channel. Such noncompetitive antagonists block GABA-mediated Cl\(^-\) flux, and therefore act as potent convulsants just as the competitive blocker \(\text{15}\). One well-known drug of this type is the synthetic CNS stimulant pentylenetetrazole \(\text{21}\) which is still used experimentally to induce seizures in animal subjects. Furthermore, picrotoxinin \(\text{22}\) is a naturally occurring, found in the seeds of the East Indian shrub \textit{Animirta cocculus}, noncompetitive GABA\(_A\) antagonist.\(^\text{(7)}\)
2.1.1.c Partial GABA<sub>A</sub> Agonists

Full GABA<sub>A</sub> agonists or antagonists may be rather difficult to introduce as drugs for practical clinical applicability. Whilst the former class of compounds may induce rapid desensitization of the target receptors after constant activation by systemically administered agonist drugs, antagonists are potential anxiogenics, proconvulsants or frank convulsants.\(^\text{(11)}\)

4,5,6,7-tetrahydroisoxazolo[5,4]-3-pyridinol (THIP)\(^\text{(25)}\) 23 and the heterocyclic GABA bioisosteres imidazole-4-acetic acid (IAA)\(^\text{(26)}\) 24 and piperidine-4-sulphonic acid (PAS)\(^\text{(27)}\) 25 show the characteristics of partial GABA<sub>A</sub> agonists. The nonfused THIP analogue 5-(4-piperidyl)-3-isoxazolol (4-PIOL)\(^\text{(28)}\) 26 was about 200 times less potent as an agonist than isoguvacine 13 and its response was competitively
antagonized by BMC 16. The 3-isothiazolol analogue of 26, Thio-4-PIOL 27, was approximately equieffective with 26 at GABA\textsubscript{A} binding sites, whereas the unsaturated analogue of 27, DH-Thio-4-PIOL 28, was significantly more efficacious.\textsuperscript{(29)} Recently, Frølund et al.\textsuperscript{(30)} synthesized analogues of 26, in which the 4-position of the 3-isoxazolol ring was substituted by different groups. The 3,3-diphenylpropyl and the 2-naphthylmethyl analogues, compounds 29 and 30, respectively, showed a potent GABA\textsubscript{A} antagonist activity (IC\textsubscript{50} = 0.02 µM for 29 and 0.37 µM for 30 in the electrophysiological assay).

2.1.1.1 Allosteric Modulation of GABA\textsubscript{A} Receptors

The GABA\textsubscript{A} receptor complex comprises a large number of binding sites for drugs, notably benzodiazepines (BDZs), barbiturates and steroids.\textsuperscript{(31)} The primary mechanism of such drugs involves allosteric modulation of the GABA\textsubscript{A} receptor complex.\textsuperscript{(12)} Examples of such drugs are shown in Figure 6.\textsuperscript{(11)}
2.1.2 GABA C Receptors

GABA C receptors are non-GABA A, non-GABA B (NANB) ionotropic GABA receptors and they are bicuculline- and baclofen-insensitive receptors. \(^{(32)}\) GABA C receptors share several agonists with GABA A receptors but the sensitivity of GABA C receptors to GABA is much higher than that of GABA A receptors. \(^{(33)}\) The conformationally restricted GABA analogues, namely \textit{cis}-4-amino-crotonic acid (CACA) \(^{35}\) and \textit{cis}-2-aminomethyl-cyclopropane-carboxylic acid (CAMP) \(^{36}\) are GABA-like neuronal depressants which are not sensitive to \(^{16}\) and they bind to a class of GABA receptor sites which do not recognize isoguvacine \(^{13}\) or (\textit{R})-baclofen. \(^{(34)}\) IAA \(^{24}\) has recently been shown to be an antagonist at the retinal GABA receptors, probably of the GABA C type. \(^{(35)}\) Also, 1,2,5,6-tetrahydro-4-pyridinylmethyl-phosphinic acid (TPMPA) \(^{37}\), a methyl-phosphinic acid derivative of \(^{13}\), is a weak antagonist at GABA A receptors and a weak agonist at GABA B receptors but shows a highly potent antagonist effect at GABA C receptors. \(^{(36)}\)
2.2 G Protein-Coupled Receptors

G protein-coupled receptors (GPCRs) are cell-surface receptors containing seven transmembrane α-helical regions with N-terminal segment on the extracellular face and their C-terminal segment on the cytosolic face of the plasma membrane. Ligand binding to these receptors activates their associated trimeric signal transducing G protein, which in turn activates or inhibits an effector enzyme that generates an intracellular specific second messenger or modulates an ion channel, causing a change in membrane potential. (37) This large receptor superfamily has been divided based on their homology into three families: family A (rhodopsin receptor-like), family B (secretin receptor-like) and family C (metabotropic glutamate receptor-like, calcium sensing receptor, GABA_B receptors and several pheromone receptors). (38) Although these receptors are activated by different ligands and may mediate different cellular responses, they all mediate a similar signalling pathway. (37)

2.2.1 Metabotropic Glutamate Receptors (mGluRs)

To date, eight subtypes of mGluRs (and their splice variants) have been identified and classified into three groups, according to structural homology of about 70% within groups and about 40% between groups, signal transduction mechanisms and shared pharmacological properties within a group. (39) **Group I** (mGluR1 and mGluR5) are coupled to the activity of phospholipase C (PLC), and their activation results in an increased phosphoinositide hydrolysis and intracellular calcium mobilization. **Group II** (mGluR2 and mGluR3) and **group III** (mGluR4, mGluR6, mGluR7 and mGluR8) are both negatively coupled to the activity of adenylyl cyclase (AC), but they share a low sequence homology and are endowed with a different pharmacology and a different localization. (40) A potential role for mGluR modulators in the treatment of either chronic or degenerative CNS diseases has long been postulated. (41)

2.2.2 GABA_B Receptors

In 1981, Bowery et al. (42) defined the GABA_B receptors on the basis of pharmacological responses to GABA and related agonists. The effect of GABA on these receptors was not blocked by bicuculline 15, was not mimicked by isoguvacine 13 and was not dependent on Cl^−, all of which are characteristics of the classical GABA_A receptors. A major distinction between GABA_A and GABA_B receptors is that the former are ligand-gated ion channels whereas the latter are coupled to G proteins
and were activated in a stereoselective manner by the clinically employed spasmyotic, 
((R)-baclofen).

The molecular structure of GABA\textsubscript{B} receptors has remained elusive for many years, until a cDNA encoding a putative GABA\textsubscript{B} receptor was cloned in 1997.\textsuperscript{(43)} The cloned GABA\textsubscript{B} receptors showed a significant similarity with mGluRs which in turn is a member of family C of G protein-coupled receptor superfamily. Although the protein product of the cDNA encoding the putative GABA\textsubscript{B} receptor had an overall profile comparable with the endogenous GABA\textsubscript{B} receptor, when expressed in heterologous systems the cloned GABA\textsubscript{B} receptor failed to produce functional activity.\textsuperscript{(44)} Subsequent studies revealed that this GABA\textsubscript{B1} protein is not transported to the plasma membrane but remains associated with the endoplasmic reticulum.\textsuperscript{(45)} Ultimately, the discovery of a second cDNA encoding a GABA\textsubscript{B} receptor termed GABA\textsubscript{B2}, which has 54% similarity and 35% homology with the former GABA\textsubscript{B1} receptor and has many of the structural features of GABA\textsubscript{B1} subunit, including a high molecular weight, seven-transmembrane domains and a long extracellular chain at the N terminus, provided the necessary explanation.\textsuperscript{(46)} Co-expression of GABA\textsubscript{B1} and GABA\textsubscript{B2} receptors in heterologous systems produced functionally active GABA\textsubscript{B} receptors, thus pointing out the GABA\textsubscript{B} receptor as the first example of a G protein-coupled receptor active as heterodimer.\textsuperscript{(47)}

GABA\textsubscript{B1} receptor subunit possesses two domains, a seven transmembrane core and an extracellular domain containing the agonist binding site. This binding domain constituted of two lobes that close upon ligand binding.\textsuperscript{(48)} GABA\textsubscript{B} receptor heterodimerization is mediated via C terminal of the GABA\textsubscript{B1} and GABA\textsubscript{B2} subunits and hence, masking the intracellular retention signal (IRS) located in the C terminal tail of GABA\textsubscript{B1} subunit. It is to be noted that several roles of GABA\textsubscript{B2} receptor subunit have been identified. First, GABA\textsubscript{B2} masks the IRS of GABA\textsubscript{B1}, such that the heterodimer GABA\textsubscript{B1}+ GABA\textsubscript{B2} reaches the cell surface. Second, GABA\textsubscript{B2} increases the agonist affinity on GABA\textsubscript{B1}. Third, GABA\textsubscript{B2} contains all the determinants required for G protein-coupling and plays a pivotal role in G protein activation by the heterodimer.\textsuperscript{(49)}

It is worth mentioning that effector mechanisms associated with neural GABA\textsubscript{B} receptors are the adenylate cyclase system and Ca\textsuperscript{2+} and K\textsuperscript{+} ion channels.\textsuperscript{(50)}

Neurotransmitter Receptors
agonists inhibit basal and forskolin-stimulated neuronal adenylate cyclase in brain slices through a G protein-dependant mechanism that results in a reduced level of intracellular cAMP.\(^{(51)}\) In addition, activation of GABA\(_B\) receptors decreases Ca\(^{2+}\) conductance and increases K\(^{+}\) conductance in neuronal membranes.\(^{(52)}\)

Recently, it has been reported that there are five GABA\(_{B1}\) splice variants and three GABA\(_{B2}\) splice variants\(^{(53)}\) but binding studies of the GABA\(_{B1}\) splice variants expressed alone or in the presence of GABA\(_{B2}\) have not revealed any difference in binding affinity between these splice variants. Furthermore, functional assays are unable to detect any significant pharmacological profile difference between GABA\(_B\) receptor subtype splice variants.\(^{(54)}\)

GABA\(_B\) receptors are widely distributed in the central and peripheral nervous systems. Studies with rat and human cerebellum and spinal cord indicate that GABA\(_{B1(a)}\) is associated with presynaptic sites whereas GABA\(_{B1(b)}\) is located predominantly at postsynaptic sites.\(^{(55)}\) Moreover, photoaffinity-labelling studies suggest that GABA\(_{B1(a)}\) and GABA\(_{B1(b)}\) are differentially distributed in the periphery. Thus, GABA\(_{B1(a)}\) is present in the adrenals, pituitary, spleen and prostate whereas GABA\(_{B1(b)}\) is found in the rat kidney and liver.\(^{(56)}\) Elsewhere in the brain, however, the GABA\(_{B1(a)}\) is in postsynaptic sites and the GABA\(_{B1(b)}\) at presynaptic terminals. For example, GABA\(_{B1(a)}\) subunits appear to be postsynaptic on cell bodies in the thalamocortical circuits.\(^{(57)}\) Thus, it would seem that a functional role, or cellular location, cannot be generally assigned to specific GABA\(_B\) receptor subunit splice variants.

In 2000, Clark et al.\(^{(58)}\) mentioned that although the regional distribution of individual GABA\(_B1\) and GABA\(_B2\) protein subunits is similar to that of the wild type receptor, in some brain areas such as the caudate-putamen, GABA\(_B2\) is not detectable even though GABA\(_B1\) is present. In addition, the GABA\(_B2\) subunit was not always present with GABA\(_B1\) in the peripheral organs such as in uterus and spleen.\(^{(59)}\) These findings support the existence of additional, as yet unidentified, GABA\(_B\) receptor subunits.\(^{(52)}\)
2.2.2.a GABAB Receptor Agonists

The importance of GABA in the central and peripheral nervous systems prompted the researchers to design an analogue which unlike GABA itself could readily access to the brain. By retaining the atomic groups of functional importance whilst introducing a lipophilic substituent, it was hoped that the substance would penetrate the brain and act at GABA receptors. 4-Amino-3-phenyl-butanoic acid was the original product of their labours and because this substance proved capable of inhibiting spinal reflexes after oral administration, other structurally related compounds were synthesized. More active substances were obtained when substituents were introduced in the phenyl nucleus and in particular when the substituent was a halogen in the 4-position of the phenyl ring. Thus, (R)-4-amino-3-(4′-chloro-phenyl)-butanoic acid ((R)-baclofen) emerged as a possible GABA-mimetic which could administered orally and activate GABAB receptors in a stereoselective manner.

Baclofen is the drug of choice to alleviate spasticity associated with tardive dystonia, brain and spinal cord injury, tetanus and multiple sclerosis, although side effects, principally sedation, limit its utility. This action appears due to a baclofen-induced reduction in neurotransmitter release onto motoneurons in spinal cord. There is also suggestion that the antispastic activity effect is due to post- rather than presynaptic action on motoneurons.

Furthermore, baclofen possesses antinociceptive effect and is used to treat migraine headache, musculoskeletal pain and in the pain associated with stroke and spinal cord injury. The general effectiveness of baclofen as analgesic is limited which may be due to rapid desensitization of GABAB receptors.

In 1998 Ling et al. mentioned that baclofen could reduce the craving for cocaine in humans. The importance of this observation in the possible treatment of drug abuse is reinforced by the finding that baclofen reduces craving for a host addictive substances including heroin, alcohol and nicotine.

Baclofen has also displayed antitussive activity in humans and laboratory animals. This effect is mediated through both a direct action on peripheral nerves in the lung as well as receptors in the brain stem controlling the cough reflex. Moreover, baclofen has also been reported to inhibit the growth of mammary cancer cells in mice and humans, and there appears to be a correlation between glandular GABA levels and mammary pathology.
Screening the literature revealed that GABA$_B$ receptors in the hypothalamus modulate sympathetic nerve activity, resulting in an elevation in blood pressure.\textsuperscript{(69)} In addition, baclofen has been shown to have anti-bronchoconstrictor activity through activation of presynaptic receptors on parasympathetic nerve terminals.\textsuperscript{(70)}

Elaboration of baclofen structure was made by Berthelot et al.\textsuperscript{(71)} in 1987. They reported the synthesis and pharmacological evaluation of a series of baclofen analogues in which the compounds 4-amino-3-benzo[b]-2-furanyl-butanoic acid $\text{41}$ and 4-amino-3-(5-methoxy-benzo[b]-2-furanyl)-butanoic acid $\text{42}$ were the most active candidates. Compounds $\text{41}$ and $\text{42}$ showed affinity for the GABA$_B$ receptors with IC$_{50}$ values = 18 and 5.6 µM, respectively, as compared with that of (RS)-baclofen (IC$_{50}$ value = 0.2 µM) for their ability to displace $[^3$H]baclofen from rat brain membranes.

Introduction of methyl group in the 7-position of benzofuran moiety of $\text{41}$ gave 4-amino-3-(7-methyl-benzo[b]-2-furanyl)-butanoic acid $\text{43}$ which showed IC$_{50}$ value = 5.4 µM as compared with that of (RS)-baclofen (IC$_{50}$ value = 0.13 µM) in the displacement of $[^3$H]GABA on rat whole-brain synaptic membranes.\textsuperscript{(72)} Further modification of baclofen structure in the search for new compounds that bind specifically to GABA$_B$ receptors led to the development of 4-amino-3-(5-methyl-2-thienyl)-butanoic acid $\text{44}$ and 4-amino-3-(5-chloro-2-thienyl)-butanoic acid $\text{45}$. The IC$_{50}$ values for the displacement of (R)-$[^3$H]baclofen on rat whole-brain synaptic membranes are 1.34 and 0.61 µM for compounds $\text{44}$ and $\text{45}$, respectively, as compared to that of (RS)-baclofen (IC$_{50}$ value = 0.33 µM).\textsuperscript{(73)}

Also, Mann et al.\textsuperscript{(74)} designed and synthesized a series of baclofen analogues starting from the structural informations contained in the solid state of baclofen, regarded as a possible bioactive conformation, in which the 4-chloro-phenyl ring is perpendicular to the GABA backbone. Only 4-amino-3-(2′,4′-dichloro-phenyl)-butanoic acid $\text{46}$ showed affinity for GABA$_B$ receptors with IC$_{50}$ value = 11 µM as compared with that of baclofen (IC$_{50}$ value = 0.42 µM) in $[^3$H]baclofen binding assay.

It is worth mentioning that the conformationally restricted analogues of baclofen, namely 1-(aminomethyl)-5-chloro-2,3-dihydro-1H-indene-1-acetic acid $\text{47}$\textsuperscript{(74)} and (IR,2S)-2-aminomethyl-2-(4′-chloro-phenyl)-cyclopropane-carboxylic acid $\text{48}$\textsuperscript{(75)} were surprisingly found inactive as GABA$_B$ ligands in the binding assay.
Bioisosteric replacement of the carboxylic acid group of GABA with phosphinic acid group led to formation of 3-aminopropyl-phosphinic acid (3-APPA, CGP27492) and its methyl homologue 3-aminopropyl-methyl-phosphinic acid (3-APMPA, CGP35024) 50. These phosphinic acid derivatives are more potent than the active isomer of baclofen and possess IC\textsubscript{50} values = 2.4 and 6.6 µM for compounds 49 and 50, respectively, as compared with that of (R)-baclofen (IC\textsubscript{50} value = 15 µM) in the inhibition of binding of [\textsuperscript{3}H]baclofen\textsuperscript{(76)}. Furthermore, the fluorine atoms of 3-aminopropyl-(difluoromethyl)-phosphinic acid (CGP47656) 51 may be considered as a bioisosteric replacement of the hydrogen atoms in 50 producing a compound with high affinity to GAB\textsubscript{A}\textsubscript{B} receptors with partial agonist activity\textsuperscript{(76)}.

In the same vein, replacement of the carboxylic group of baclofen by phosphinic acid group gave the phosphinic acid derivative 52 which showed IC\textsubscript{50} value = 0.039\textsuperscript{(77)}. Whereas replacement of the carboxylic group of baclofen with nitro group furnished compound 53 with about one-third the activity of racemic baclofen\textsuperscript{(78)}. 
In 1991, Kristiansen and Fjalland reported that the \((R)-4\)-amino-3-hydroxybutanoic acid (3-OH-GABA) 54 acts as a GABA\(_B\) agonist at ileal GABA\(_B\) receptors\(^{(79)}\).

Recently, Karla et al.\(^{(80)}\) elaborated the structure of 54 and described the synthesis and pharmacological evaluation of \((R)-5\)-amino-3-(4'\)-chloro-phenyl)-pentanoic acid 55 and \((S)-5\)-amino-3-(4'\)-chloro-phenyl)-pentanoic acid 56. Compound 55 possesses IC\(_{50}\) value = 7.4 \(\mu\)M (IC\(_{50}\) value of \((R)\)-baclofen = 0.14 \(\mu\)M) in the GABA\(_B\) receptor binding.
assay and EC$_{50}$ value = 150 µM (EC$_{50}$ value of (R)-baclofen = 0.11 µM) in the guinea pig ileum functional assay as GABA$_B$ agonist. On the other hand, compound 56 did not interact significantly with GABA$_B$ receptor sites in the GABA$_B$ receptor binding assay but surprisingly was shown to be one-half as potent as 55 in the guinea pig ileum functional assay as GABA$_B$ agonist (EC$_{50}$ value = 310 µM and that of (S)-baclofen = 3300 µM).

(Figure 9)

2.2.2.b GABA$_B$ Receptor Antagonists

Although there are no reports on the pharmacological actions of GABA$_B$ receptor antagonists in humans, a number of predictions has been done on the basis of results obtained in animals and knowledge of the effects of the GABA$_B$ receptor agonists. The preclinical results indicate that GABA$_B$ receptor antagonists may be of value in treating absence epilepsy, cognitive dysfunction and possibly, pulmonary and intestinal disorders.\(^{(81)}\)

In addition, the design and development of selective GABA$_B$ receptor antagonists with increasing receptor affinity and potency play an important role in establishing the significance and structure of the GABA$_B$ receptor.

In 1996, Kerr and Ong\(^{(82)}\) reported the selective GABA$_B$ receptor antagonist activity of phosphonic acid derivative compound 57. Further modification of the structure of 57, led to the development of the orally active GABA$_B$ receptor antagonist compound 58, which showed IC$_{50}$ value = 38 µM in inhibition of binding of $[^3]$H-49 to GABA$_B$ receptors of rat cerebral cortex membranes.\(^{(83)}\)

In the same vein, the phosphonic and sulphonic acid analogues of baclofen, compounds 59 and 60 were described to possess GABA$_B$ receptor antagonist activity with IC$_{50}$ values = 130 and 26 µM, respectively, in inhibition of binding of $[^3]$H-49 to GABA$_B$ receptors on rat cerebral cortex membranes.\(^{(84)}\)
The most crucial breakthrough in the discovery of antagonists came with the development of compounds with affinities about 10,000 times higher than previous antagonists. This major advance stemmed from the attachment of 3,4-dichlorobenzyl or 3-carboxybenzyl substituents to the existing molecules. Compounds CGP54626 \textbf{61} and CGP56999 \textbf{62} are candidates of this class of compounds having IC$_{50}$ values = 4 and 2 nM, respectively.\textsuperscript{(85)} Furthermore, introduction of the phosphinic acid moiety into the Schering \textit{GABA$_B$} receptor antagonist, SCH 50911 \textbf{63},\textsuperscript{(86)} led to a very potent \textit{GABA$_B$} receptor antagonist, compound \textbf{64}, which shows IC$_{50}$ value = 2 nM.\textsuperscript{(87)}

Finally, two iodinated high-affinity antagonists, i.e., $[^{125}\text{I}]$CGP64213 \textbf{65} (IC$_{50}$ value = 1.6 nM) and $[^{125}\text{I}]$CGP71872 \textbf{66} (IC$_{50}$ value = 2.4 nM) were developed and used for the elucidation of the structure \textit{GABA$_{B1}$} receptors.\textsuperscript{(56, 88)}
2.2.2.1 Allosteric Modulation of GABA<sub>B</sub> Receptors

Allosteric modulators provide a novel means for the pharmacological manipulation of G protein-coupled receptors. They may delay dissociation by stabilizing the bound-agonist state, or act as subtype-selective enhancers of agonist binding, and promote receptor G protein coupling. All these properties suggest that allosteric modulators may
offer a number of potential pharmacological improvements over the use of conventional agonists, particularly in GABA_B receptors where subtype selective enhancers would be a definite advantage. \(^{(89)}\)

In 2001, Urwyler et al.\(^{(90)}\) reported for the first time that 2,6-di-tert-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) 67 was identified as a positive modulator for GABA_B receptor function with EC\(_{50}\) values = 5.37 and 4.6 \(\mu\)M in the native and recombinant GABA_B receptor preparations. This positive allosteric modulator acts synergistically with an agonist, increasing its potency and its maximal activity, but have no intrinsic efficacy on their own.

Recently, Kerr et al.\(^{(89)}\) described the positive modulatory effect of phenylalkylamine derivatives at GABA_B receptors. They reported that \(N\)-(3,3-diphenyl-propyl)-\(\alpha\)-methyl-benzylamine (fendiline) 68 possesses EC\(_{50}\) value = 20 \(\mu\)M for potentiation of baclofen responses, whilst the EC\(_{50}\) value for \(N\)-(3,3-diphenyl-propyl)-\(\alpha\)-methyl-3-methoxy-benzylamine (F551) 69 = 3 \(\mu\)M and that for \(N\)-(3,3-diphenyl-propyl)-\(\alpha\)-methyl-phenylethylamine (prenylamine) 70 = 30 \(\mu\)M.

(Figure 11)
3 Rationale of the Present Investigation

The widespread distribution of GABA_B receptors in both the CNS and the periphery is a clear clue of their physiological and physiopathological importance. In general, presynaptic GABA_B receptors modulate synaptic transmission by depressing neurotransmitter release, including that of GABA itself, through autoreceptors,(91) while postsynaptic GABA_B receptors contribute to the inhibitory control of overall neuronal excitability. Thus, GABA_B receptors play a critical role in fine-tuning the CNS synaptic transmission and are attractive targets for the treatment of epilepsy, anxiety, depression, cognitive deficits, sclerosis and nociceptive disorders.(92)

Indeed, several potential therapeutic applications are associated with pharmacological control of GABA_B receptor. GABA_B agonists, in particular, may be employed as antispastic agents, in respiratory diseases such as asthma, in the pharmacological control of cocaine addiction, in migraine and in pain. On the other hand, GABA_B antagonists, when employed at doses that do not induce convulsion, increase neutrophin expression in the CNS and in the spinal cord and, thus may have therapeutic relevance in neurodegenerative diseases. As a result, the development of GABA_B receptor agonists and antagonists is of great therapeutic interest.

Several selective GABA_BR ligands are nowadays available, either agonists or antagonists (cf. Figures 8, 9 and 10). Furthermore, (R)-4-amino-3-(4′-chloro-phenyl)-butanoic acid (baclofen) 40, the aromatic analogue of GABA, introduced as racemate in 1973 in the therapy of muscle spasticity, is still the classical prototype of the selective GABA_BR agonists. Whereas δ-amino-valeric acid (DAVA) 71, the homologue of GABA, is a nonselective GABA_BR antagonist.(93) In 1999, Karla et al.,(80) in our group, described the synthesis and pharmacological evaluation of the structural hybrid of 40 and 71, namely (R)-5-amino-3-(4′-chloro-phenyl)-pentanoic acid 55 which showed a selective GABA_BR agonist activity.
Based upon the hereabove mentioned rationales and in a search for novel GABA\textsubscript{B}R agonists, a series of (RS)-5-amino-3-aryl-pentanoic acid hydrochlorides (1\textsubscript{a-h}) was designed, synthesized and evaluated for GABA\textsubscript{B}R agonist activity. The general synthetic strategy to achieve compounds 1\textsubscript{a-h} is depicted in Schemes 2 and 3.

![Chemical structure of 1\textsubscript{a-h}](image)

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>1\textsubscript{a}</td>
<td>4-Chloro</td>
</tr>
<tr>
<td>1\textsubscript{b}</td>
<td>2,4-Dichloro</td>
</tr>
<tr>
<td>1\textsubscript{c}</td>
<td>4-Methyl</td>
</tr>
<tr>
<td>1\textsubscript{d}</td>
<td>H</td>
</tr>
<tr>
<td>1\textsubscript{e}</td>
<td>3,4-Dichloro</td>
</tr>
<tr>
<td>1\textsubscript{f}</td>
<td>4-Fluoro</td>
</tr>
<tr>
<td>1\textsubscript{g}</td>
<td>3-Methoxy</td>
</tr>
<tr>
<td>1\textsubscript{h}</td>
<td>4-Methoxy</td>
</tr>
</tbody>
</table>

To investigate the importance of the aromatic moiety in the selectivity of GABA homologues to GABA\textsubscript{B} receptors, we synthesized the aliphatic homologue of baclofen, (RS)-5-amino-3-methyl-pentanoic acid (1\textsubscript{i}), to be evaluated for its GABA\textsubscript{B}R agonist activity. The synthetic route adopted to prepare 1\textsubscript{i} is illustrated in Scheme 4.

![Chemical structure of 1\textsubscript{i}](image)
In the same vein, we describe in Scheme 5 the synthesis of aromatic amino acid derivative, namely 3-amino-methyl-5-chloro-benzoic acid hydrochloride (1j). Incorporation of the \textit{para} chloro-phenyl moiety in the DAVA backbone (compound 1j) will increase lipophilicity of DAVA and may enhance its selectivity at GABA\textsubscript{B} sites.

![Chemical structure of 3-amino-methyl-5-chloro-benzoic acid hydrochloride (1j)](image)

Screening the literature revealed that the electronegativity of the acetylenic function is similar to that of the chloro functionality. In addition, the ethynyl group would be able to act as a hydrogen acceptor or donor in the receptor active site.\(^{(94,95)}\) Consequently, we are convinced to synthesize the \textit{para} acetylene analogue of baclofen, (RS)-4-amino-3-(4′-ethynyl-phenyl)-butanoic acid hydrochloride (1k), as illustrated in Scheme 6.

![Chemical structure of (RS)-4-amino-3-(4′-ethynyl-phenyl)-butanoic acid hydrochloride (1k)](image)

The bioisosteric replacement of functional groups in receptor ligands, enzyme inhibitors or antimetabolities has become a promising strategy for the design of new drug candidates.\(^{(96)}\) Therefore, we describe in Scheme 6 the synthesis of the baclofen analogue, (RS)-4-amino-3-(4′-ido-phenyl)-butanoic acid hydrochloride (1l). Compounds 1k and 1l will be evaluated for their GABA\textsubscript{B}R agonist activity.
Further search for novel GABA$_B$R ligands will be done by docking the synthesized compounds 1a-1 in the available 3D homology model of the GABA$_B$R and subsequently developing a model which could predict the biological activity of new GABA$_B$ ligands.
Scheme 2

5a-c

\[ \text{X} \]

\[ \begin{align*} \text{i) } & (\text{EtO})_2\text{POCH}_2\text{COOEt} / \text{NaH} / \text{dry } 1,2 \text{ dimethoxyethane} / 50 ^\circ \text{C} / 18 \text{h} \\
\text{ii) } & (\text{C}_2\text{H}_5)_4\text{N}^+ \text{CN} / \text{CH}_3\text{CN} / 50 ^\circ \text{C} / 18 \text{h} \\
\text{iii) } & \text{H}_2 / \text{Pd/C or PtO}_2 / 4 \text{ bar} / 95\% \text{ C}_2\text{H}_5\text{OH} / \text{conc. HCl} / 25 ^\circ \text{C} / 18 \text{h} \\
\text{iv) } & 5\text{N HCl} / \text{reflux} / 4 \text{h} \end{align*} \]

\[ \begin{align*} E ( \text{minor}) & \quad Z (\text{major}) \\
4a-c & \quad 3a-c \\
\end{align*} \]
Scheme 3

\[
\begin{align*}
\text{Scheme 3} & \\
\text{i) (EtO)\text{2POCH}_2\text{COOEt} / KO-t\text{-Bu} / dry \text{THF} / reflux / 18h} & \quad \text{ii) NBS / benzoyl peroxide / CCl}_4 / reflux / 24 \text{ or } 6h \\
\text{iii) (C}_2\text{H}_5\text{4N}^+ \text{CN} / \text{CH}_3\text{CN} / 50 \text{°C} / 18h} & \\
\text{iv) H}_2 / \text{Pd/Cor PtO}_2 / 4 \text{ bar} / 95\% \text{ C}_2\text{H}_5\text{OH} / \text{conc. HCl} / 25 \text{°C} / 18h & \quad \text{v) 5N HCl / reflux / 4h}
\end{align*}
\]
Scheme 4

1) 1N HCl / 100 °C / 1.5h

ii) Ammonium acetate / acetic acid / benzene / reflux / 8h

iii) H₂ / Pd/C / conc. HCl / 4bar / 95% ethanol / 25 °C / 18h

iv) 5N HCl / reflux / 4h / reflux

v) 1) Benzyl chloroformate / 4N NaOH / 0 °C / 0.5h 2) HCl

vi) H₂ / Pd/C / 4 bar / 50% 2-propanol / 25°C / 18h
Scheme 5

1) NaNO₂ / H₂SO₄ / 70 °C 2) CH₃COOH / 40 °C / 0.5h 3) CuCl / conc. HCl / 80 °C / 20 min

ii) CH₃OH / H₂SO₄ / reflux / 24h

iii) 1) IN NaOH / CH₃OH / 25 °C / 18h 2) HCl

iv) Borane-methyl sulfide complex / THF / 25 °C / 30h

v) PBr₃ / -30 °C / 0.5h

vi) NaN₃ / acetone / H₂O / reflux / 18h

vii) 1)1N NaOH / CH₃OH / reflux / 2.5h 2) HCl

viii) H₂ / PtO₂ / 4 bar / 95% 2-propanol / 25 °C / 1h
Rationale of the Present Investigation

Scheme 6 (cont.)

i) SOCl₂ / dry THF / reflux / 1h  
ii) NaBH₄ / dioxane / 100 °C / 1.5h  
iii) Pyridinium dichromate / CH₂Cl₂ / 2.5h  

iv) Ph₃P═CHCO₂CH₃ / dry CH₂Cl₂ / 25 °C / 1h  
v) CH₃NO₂ / Triton B / 85 °C / 2h  
vi) 1) CH₃OH / HCl / Zn / 0 °C / 0.5h  
  2) 6N NaOH  

vii) Boc₂O / DMAP / TEA / CH₂Cl₂ / 25 °C / 30h  
viii) PdCl₂(PPh₃)₂ / Cul / TMSA / TEA / 25 °C / 12h  

ix) 1) 1M LiOH / 25 °C / 18h  
    2) 0.5M KHSO₄  
x) 2.5M dry HCl/ethyl acetate / 25 °C / 1h  
xi) 1) 1M LiOH / 25 °C / 2h  
    2) 0.5M KHSO₄
4 Theoretical Discussion

4.1 Synthetic Plan for the Synthesis of (RS)-5-Amino-3-aryl-pentanoic acid hydrochlorides (1a-h).

One of the aims of this work is to synthesize a series of baclofen homologues, namely (RS)-5-amino-3-aryl-pentanoic acid hydrochlorides 1a-h as described in Schemes 2 and 3.

4.1.1 Synthesis of 3-Aryl-4-chloro-2-butenoic acid ethyl esters (4a-c)

4.1.1.1 Horner-Wadsworth-Emmons (HWE) Reaction

HWE is a condensation reaction between resonance-stabilized carbanions 73a-b and carbonyl compounds to produce α,β-unsaturated esters or ketones 76a-b (Scheme 7). It is also considered as a phosphonate modification of Wittig reaction.
Phosphonate carbanions 73a-b are known to be more nucleophilic than the phosphonium ylides employed in the conventional Wittig reaction and hence they react with a wider variety of aldehydes and ketones under milder conditions. Furthermore, the water-soluble phosphate ion formed from the phosphonate allows much easier separation of the olefin from the reaction mixture. \(^{(99)}\)

Screening the literature revealed that HWE reagent can be obtained either by adopting Arbusov reaction \(^{(101)}\) in which trialkyl phosphite 77 was treated with alkyl halide 78 to give \(\alpha\)-(alkoxycarbonyl)-phosphonic acid dialkyl ester 72a (Scheme 8) \(^{(98)}\) or by acylation of a metallated phosphonic acid ester 80 to afford \(\beta\)-ketophosphonic acid dialkyl ester 81 (Scheme 9). \(^{(98)}\)
Mechanism and Stereochemistry

The mechanism of HWE reaction has not been definitively established. The presumably closer analogy to the Wittig-Horner reaction would argue for a two step formation of the oxaphosphetanes. In the first step, the phosphonate carbanions would react with the carbonyl compound reversibly to form an intermediate alkoxides which would then cyclize to give a four-membered cyclic intermediate oxaphosphetanes in the second step. The irreversible decomposition of this heterocycle by a one step [2+2]-cycloreversion (cis elimination) which would finally lead to olefin formation (cf. Scheme 7). The intermediate alkoxide can exist as a diasteromeric mixture and gives either cis olefin or trans olefin via cis elimination. The stereochemistry of the reaction generally favours the thermodynamically stable trans isomer.

3-Aryl-4-chloro-2-butenoic acid ethyl esters have been successfully achieved by adopting HWE reaction on substituted acetophenones in 1,2 dimethoxyethane using triethyl phosphonoacetate and sodium hydride following the procedure cited by Wadsworth and Emmons (Scheme 10). The produced diasteromeric mixtures were separated by column chromatography using the appropriate solvent system (cf. Experimental Part).
$^1$H-NMR spectra where the chemical shifts of 4-H protons appear further downfield when compared with those in the minor product ($(E)$-configuration). Also coupling constants between 2-H and 4-H protons of $4_{a-c}$ have been detected only in the minor product ($(E)$-configuration, $J = 1.23$ Hz) and could not be detected in the major product ($(Z)$-configuration). Furthermore, the $^{13}$C chemical shift differences between C-1, C-3 and in particular C-4 for $(E)$ and $(Z)$-isomers of $4_{a-c}$ are consistent with the observed differences for $(E)$ and $(Z)$-isomers mentioned by Allan and Tran$^{103}$ (cf. Experimental Part).

It is noteworthy that substitution in the ortho position of the phenyl ring in 2-chloro-1-(2′,4′-dichloro-phenyl)-1-ethanone $5_b$ increased the proportion of $(E)$-isomer in the produced diastereomeric mixture 4-chloro-3-(2′,4′-dichloro-phenyl)-2-butenoic acid ethyl ester $4_b$ (Scheme 11) which might be attributed to Jones and Maisey findings.$^{104}$ They found that ortho substitution in the phenyl ring in certain ketones which underwent HWE reaction would increase the proportion of the less thermodynamically stable isomer due to a decrease in conjugative stabilization of the incipient double bond in the transition state. Since conjugative stabilization is usually greater in the transition state leading to the thermodynamically stable isomer, the proportion of this thermodynamically stable isomer will be decreased in the produced diastereomeric mixture.

![Chemical Structure](attachment:image.png)

2-Chloro-1-(4′-methyl-phenyl)-1-ethanone$^{105}$ $5_c$ is the precursor of 4-chloro-3-(4′-methyl-phenyl)-2-butenoic acid ethyl ester $4_c$ and it was achieved by Friedel-Crafts acylation of toluene $82$ with chloroacetyl chloride $83$ in the presence of aluminium chloride following the procedure cited by Huff et al.$^{106}$ to afford $5_c$ in 63% yield (Scheme 12).
On the other hand, our attempt to prepare the bromo analogue, \((Z)-4\)-bromo-3-(4'-methyl-phenyl)-2-butenoic acid ethyl ester 86, by adopting HWE reaction using 2-bromo-1-(4'-methyl-phenyl)-1-ethanone 85 as a starting material did not yield the anticipated compound 86 in detectable yield (Scheme 13).

### 4.1.1.2 Reformatsky Reaction

We tried to synthesize the bromo surrogate \((Z)-4\)-bromo-3-(2',4'-dichloro-phenyl)-2-butenoic acid ethyl ester 91 by adopting Reformatsky reaction using 2,4-dichloro-acetophenone 87 as a starting material in a mixture of tetrahydrofuran/trimethyl borate (1:1) in the presence of ethyl bromoacetate 88 and zinc metal (20 mesh) according to the method developed by Rathke and Lindert.\(^{(108)}\) The reaction mixture was stirred at room temperature for 18 h to yield a mixture of the starting material 87 and the \(\beta\)-hydroxy ester 89. This mixture underwent dehydration according to the procedure mentioned by Berthelot et al.\(^{(71)}\) using \(\text{P}_2\text{O}_5\) in toluene to afford a mixture of the starting material 87 and \((E)-90\). This mixture has been subjected to chemical separation through saponification and extractive working up followed by reesterification to give
pure \((E)\)-90 in an overall yield of 22%. Allylic bromination of \((E)\)-90 using NBS gave the desired product \((Z)\)-91 (Scheme 14).

\[
\begin{align*}
\text{Cl} & \quad \text{O} \\
\text{Cl} & \quad \text{CH}_3 \\
\text{Cl} & \quad \text{O} \\
\text{Cl} & \quad \text{Cl} \\

\text{BrCH}_2\text{OC}_2\text{H}_5 & \quad \text{THF/TMB, Zn} \\
r.t., 18 \text{ h} & \quad \text{Cl} \quad \text{OH} \quad \text{Cl} \\

\text{CH}_3 & \quad \text{O} \\
\text{Cl} & \quad \text{Cl} \\
\text{OC}_2\text{H}_5 & \quad \text{BrCH}_2 \quad \text{OC}_2\text{H}_5 \\

\text{P}_2\text{O}_5 & \quad \text{toluene} \\
\text{reflux} & \quad 3 \text{ h} \\

\text{Cl} & \quad \text{O} \\
\text{Cl} & \quad \text{Cl} \\
\text{OC}_2\text{H}_5 & \quad \text{OC}_2\text{H}_5 \\
\text{Cl} & \quad \text{Cl} \\

\text{Br} & \quad \text{OC}_2\text{H}_5 \\

\text{C}_2\text{H}_5 & \quad \text{O} \\

\text{Cl} & \quad \text{Cl} \\

\text{Cl} & \quad \text{Cl} \\

\end{align*}
\]

(Scheme 14)

It is worth mentioning that the function of trimethyl borate is to neutralize the zinc alkoxides formed in the reaction and hence decreases the base catalyzed side reactions of the starting materials.\(^{108}\)

Moreover, our trials to synthesize \((Z)\)-4-bromo-3-(2′,4′-dichloro-phenyl)-2-butenoic acid ethyl ester 91 by adopting the conventional Wittig reaction but under pressure\(^{109}\) (50 bar) on the corresponding substituted acetophenone 87 gave a very poor yield (~8 % as detected by \(^1\text{H-NMR}\)) of compound \((E)\)-90 after heating at 130 °C in toluene for 48 h (Scheme 15). Also addition of 1.5 equiv. of acetic acid to accelerate Wittig reaction according to Barrett et al.\(^{110}\) observation did not improve the yield.
The low yield of both Reformatsky reaction (22 %) and Wittig reaction (~8 %) for the preparation of \((E)-90\) convinced us to adopt the HWE reaction to achieve the required compounds \(4_{a-c}\) in 40-88 % yield (cf. Experimental Part).

### 4.1.2 Synthesis of 3-Aryl-2-butenoic acid ethyl esters (5e-h)

Substituted acetophenones \(6_{e-h}\) are the starting synthons for the synthesis of 3-aryl-2-butenoic acid ethyl esters \(5_{e-h}\) by adopting HWE reaction according to the procedure mentioned by Nicolas et al.\(^{(11)}\) (cf. Scheme 3). A solution of the appropriate acetophenone derivatives \(6_{e-h}\) in dry tetrahydrofuran was added dropwise to the carbanion produced from triethyl phosphonoacetate and potassium \(t\)-butoxide in dry tetrahydrofuran. The produced reaction mixture was refluxed under stirring for 18 h and the produced diastereomeric mixtures \(5_{e-h}\) were separated by column chromatography.
using the appropriate solvent system (cf. Experimental Part). $^1$H-NMR of the diasteromeric mixtures $5_{e-h}$ revealed the predominance of the thermodynamically stable $(E)$-isomers. Chemical shifts of 4-H protons in $(E)$-isomers of $5_{e-h}$ are shown to be further downfield as compared to those of $(Z)$-isomers. Also coupling constants of 2-H and 4-H protons of $5_{e-h}$ are smaller in $(E)$-isomers ($J = 1.23$ Hz) than that in $(Z)$-isomers ($J = 1.53$ Hz). In addition, the $^{13}$C chemical shift differences between C-4 for $(E)$ and $(Z)$-isomers of $5_{e-h}$ are consistent with the observed differences for $(E)$ and $(Z)$-isomers mentioned by Allan and Tran$^{(103)}$ (cf. Experimental Part).

4.1.3 Synthesis of $(Z)$-3-Aryl-4-bromo-2-butenoic acid ethyl esters ($4_{e-h}$)

4.1.3.1 Wohl-Ziegler Bromination$^{(112)}$

Chemoselective allylic bromination of 3-aryl-2-butenoic acid ethyl esters $5_{e-h}$ (as diasteromeric mixtures) was accomplished by adopting Wohl-Ziegler bromination. Compounds $5_{e-h}$ and a stoichiometric amount of NBS were refluxed in carbon tetrachloride and then a catalytical amount of dibenzoyl peroxide (DBP) was added to the reaction mixture according to the method advocated by Chiefari et al.$^{(113)}$ The reflux was continued until the insoluble NBS on the bottom of the flask disappears and the lighter succinimide floats on the surface to afford $(Z)$-3-aryl-4-bromo-2-butenoic acid ethyl esters $4_{e-h}$ in moderate yields (cf. Experimental Part). We could isolate only the thermodynamically stable isomers which were assigned to be $(Z)$-isomers based on $^1$H-NMR spectral data (cf. Experimental Part). Inclusion of DBP as a free radical generator in the reaction mixture shortens considerably the reaction time and suppresses the formation of by-products.$^{(114)}$ Thermal dissociation of DBP to form a radical which then reacts with the NBS to form the propagation radical.$^{(115)}$
Our attempts to use chloroform instead of carbon tetrachloride as a solvent according of the procedure of Balakumar et al.\textsuperscript{(116)} in the preparation of (Z)-4\textsubscript{e} led to addition of bromine to the double bond and afforded 93 instead of allylic bromination (Scheme 16).

\begin{center}
\includegraphics[width=\textwidth]{Scheme16.png}
\end{center}

4.1.4 Synthesis of (E)-3-Aryl-4-cyano-2-butenoic acid ethyl esters (3\textsubscript{a-c} and 3\textsubscript{e-h})

Our trial to synthesize 3-(3′-chloro-phenyl)-4-cyano-2-butenoic acid ethyl ester 97 as a model for the synthesis of compounds 3\textsubscript{a-c} and 3\textsubscript{e-h} by adopting Knoevenagel condensation in one step using 3-(3′-chloro-phenyl)-3-oxo-propionic acid ethyl ester\textsuperscript{(117)} 96, which has been prepared from 3-chloro-benzoyl chloride 94 and malonic acid monoethyl ester\textsuperscript{(118)} 95, and cyano-acetic acid afforded a very poor yield of the required compound 97 (Scheme 17).
4-Chloro-3-(2',4'-dichloro-phenyl)-2-butenoic acid ethyl ester $4_b$ (as diasteromeric mixture) was subjected to a nucleophilic displacement of the halogen with potassium cyanide in aqueous ethanol by adopting the trivial procedure mentioned by Ives and Sames$^{(120)}$. Unfortunately, the starting material was decomposed and we did not obtain the target compound, $(E)$-4-cyano-3-(2',4'-dichloro-phenyl)-2-butenoic acid ethyl ester $3_b$, in detectable amount (Scheme 18).

Dehmlow and Dehmlow$^{(121)}$ found that with allylic and benzylic halides, hydrolysis may under certain conditions, compete unfavourably with substitution when aqueous cyanides are used. Substitution of aqueous ethanol with absolute ethanol or with acetone led to a very low yield (~3%) of the required compound $(E)$-$3_b$. Addition of a catalytical amount of potassium iodide as a promotor$^{(122)}$ did not improve the yield. These results could be attributed to the insolubility of potassium cyanide in the solvent.
system used in performing the reaction and hence compound $4_b$ could not be in intimate contact with the water soluble potassium cyanide.

In the same vein, Friedman and Shechter$^{(123)}$ introduced dimethyl sulfoxide as an excellent solvent to prepare nitriles from the corresponding primary and secondary chlorides or bromides and sodium cyanide. Our trial to use dimethyl sulfoxide as a solvent to prepare $(E)-3_b$ using sodium or potassium cyanide was unsuccessful as we obtained decomposition products and tars after heating the reaction mixture at 50 °C for one hour. The relatively high nucleophilicity of dimethyl sulfoxide probably is the reason of formation of these complex products as dimethyl sulfoxide will compete with alkali cyanide to react with alkyl halide forming O-alkyl or S-alkyl derivatives according to Smith and Winstein findings.$^{(124)}$

### 4.1.4.1 Phase Transfer Catalysis$^{(125,126)}$

Screening the literature indicated that alkyl cyanides have been prepared from the corresponding alkyl halides by phase transfer catalytic methods.$^{(127,128)}$ Phase transfer catalysis permits or accelerates reactions between ionic compounds (ex. alkali cyanides) and organic, water insoluble substrates (ex. alkyl halides) in solvents of low polarity. The basic function of the catalyst is to transfer the anions of the reacting salt into the organic medium in the form of unsolvated and consequently are very reactive ion pairs (Figure 13).$^{(127)}$ The catalysts most commonly used are quaternary ammonium or phosphonium halides which contain a lipophilic cation. It is to be noted that the identity of halide is not necessary but it must be exchangeable with the nucleophile. The chloride ion is readily exchanges with diverse nucleophiles as hydroxide and cyanide.

\[
\begin{align*}
QN_u + R-X &\rightarrow R-Nu + QX & \text{Organic phase} \\
\downarrow & & \uparrow \\
QN_u + MX &\leftrightarrow MN_u + QX & \text{Aqueous phase}
\end{align*}
\]

(Figure 13)

Our attempt to synthesize $(E)-3_b$ from $4_b$ by *in situ* formation of benzyltrimethylammonium cyanide (obtained from sodium cyanide and benzyl trimethylammonium chloride in water) according to the procedure of Sugimoto et al.$^{(129)}$ gave a very low yield (~5%) of the anticipated compound $(E)-3_b$. 


(E)-3-Aryl-4-cyano-2-butenoic acid ethyl esters 3a-c have been successfully achieved by adopting the procedure developed by Simchen and Kobler\textsuperscript{(130)} using a stoichiometric amount of 3-aryl-4-chloro-2-butenoic acid ethyl esters 4a-c (as diasteromeric mixtures) and tetraethylammonium cyanide (TEAC).\textsuperscript{(131)} The reaction mixture was stirred at 50 °C in acetonitrile for 18 h (Scheme 19). The crude 3a-c were purified by column chromatography using the appropriate solvent system to afford mainly (E)-3a-c in 42-66% yield (cf. Experimental Part). We tried to use a catalytical amount of tetraethylammonium cyanide instead of stoichiometric amount to achieve 3a-c but the yield decreased dramatically.

![Scheme 19](image-url)

It has to be pointed out that we could isolate (Z)-4-cyano-3-(2',4'-dichloro-phenyl)-2-butenoic acid ethyl ester 3b in a very low yield (~4%). The chemical shifts of 4-H protons of (E)-3b appear further downfield (δ (ppm) = 3.93) as compared with that of (Z)-3b (δ (ppm) = 3.59). Also chemical shift of 2-H proton of (Z)-3b appears further downfield (δ (ppm) = 5.81) when compared with that of (E)-3b (δ (ppm) = 5.62). In addition, we could detect only the coupling constant between 2-H and 4-H protons for (Z)-3b (J = 1.23 Hz) and we could not detect it for (E)-3b. Furthermore, the $^{13}$C chemical shift difference between C-4 of (E)-3b (δ (ppm) = 40.6) and C-4 of (Z)-3b (δ (ppm) = 42.5) is consistent with the observed differences for (E) and (Z)-isomers mentioned by Allan and Tran.\textsuperscript{(103)}
(E)-3-Aryl-4-cyano-2-butenoic acid ethyl esters $3_{e-h}$ were prepared using (Z)-3-aryl-4-bromo-2-butenoic acid ethyl esters $4_{e-h}$ as a starting materials (Scheme 20) by adopting the aforementioned procedure for the preparation of (E)-3$\alpha$-$c$.

![Scheme 20](image)

4.1.5 Synthesis of (RS)-5-Amino-3-aryl-pentanoic acid ethyl ester hydrochlorides ($2_{a-h}$)

The amino ester (RS)-5-amino-3-aryl-pentanoic acid ethyl ester hydrochlorides $2_{a-h}$ are the starting synthons for our target compounds (RS)-5-amino-3-aryl-pentanoic acid hydrochlorides $1_{a-h}$.

(E)-3-Aryl-4-cyano-2-butenoic acid ethyl esters $3_{a-c}$ and $3_{e-h}$ are multifunctional molecules and we aimed to reduce selectively both nitrile and $\alpha,\beta$-double bond functions without affecting ester function to afford (RS)-5-amino-3-aryl-pentanoic acid ethyl ester hydrochlorides $2_{a-h}$.

Catalytic hydrogenation$^{[132]}$ is one of the most powerful methods in the arsenal of the synthetic medicinal chemist facilitating the chemical synthesis of myriads of bioactive molecules both in research laboratories and industrial settings. Most functional groups can be readily reduced, often under milder conditions, and frequently in high chemo-, regio-, and stereoselectivity by adopting catalytic hydrogenation.
Literature survey showed that addition of dry ammonia in the reaction mixture during hydrogenation of nitriles in the presence of Raney nickel will minimize the formation of secondary amines\(^{133}\). This assumption can be attributed to the reaction of ammonia rather than primary amine with the intermediate imine formed during hydrogenation of nitriles, thus giving rise to the formation of additional primary amine.

Bergeron et al.\(^{134}\) found that sodium hydroxide also suppresses formation of coupling products during hydrogenation of nitriles using Raney nickel to form primary amines exclusively.

In the same vein, addition of concentrated hydrochloric acid in the reaction mixture during hydrogenation of nitriles in the presence of Pd/C and/or PtO\(_2\) will give high yield of primary amines due to removal of the base from the reaction equilibrium by salt formation, thus preventing the possible interaction with the intermediary imine\(^{135}\).

\((RS)-5\text{-Amino-3-aryl-pentanoic acid ethyl ester hydrochlorides} \text{ 2c, d, g, h were synthesized according to the procedure cited by Schwartz et al.}^{136}\). A mixture of 3b, c, g or h, concentrated hydrochloric acid and a catalytical amount of 10\% Pd/C in 95\% ethanol was hydrogenated on a Parr shaker apparatus under 4 bar of H\(_2\) for 18 h at room temperature (cf. Experimental Part).

\((RS)-5\text{-Amino-3-aryl-pentanoic acid ethyl ester hydrochlorides} \text{ 2a, b, e, f were prepared by adopting the procedure mentioned by Secrist and Logue.}^{137}\) A mixture of 3a, b, e or f, concentrated hydrochloric acid and a catalytical amount of PtO\(_2\) in 95\% ethanol was hydrogenated under the aforementioned conditions for preparation of 2c, d, g, h (cf. Experimental Part).

It is noteworthy that when \((E)-4\text{-cyano-3-}(2',4'\text{-dichloro-phenyl)-2-butenoic acid ethyl ester} \text{ 3b was subjected to catalytic hydrogenation using PtO}_2 \text{ afforded} \,(RS)-5\text{-amino-3-}(2',4'\text{-dichloro-phenyl)-pentanoic acid ethyl ester hydrochloride} \text{ 2b whereas by using 10\% Pd/C hydrogenation was accompanied by dehalogenation to give} \,(RS)-5\text{-amino-3-phenyl-pentanoic acid ethyl ester hydrochloride} \text{ 2d (Scheme 21).}
It has to be pointed out that the presence of concentrated hydrochloric acid in the hydrogenation mixture of 3a-c and/or 3e-h has a dual function. It will capture the formed primary amines to form hydrochloride salts, thus preventing intramolecular ring closure and lactam formation (Scheme 22) in addition to preventing the possible interaction with the intermediary imine.
Without further purification the ester function of (RS)-5-amino-3-aryl-pentanoic acid ethyl ester hydrochlorides $2_{a-h}$ was hydrolyzed by refluxing $2_{a-h}$ in 5 N hydrochloric acid for 4 h (Scheme 23). The crude $1_{a-h}$ were recrystallized from the appropriate solvents to afford the target compounds $1_{a-h}$ in 69-85% yields (cf. Experimental Part). The structures of $1_{a-h}$ have been established through microanalytical, IR, $^1$H-NMR, $^{13}$C-NMR, and mass spectral data.
4.2 Synthetic Plan for the Synthesis of (RS)-5-Amino-3-methyl-pentanoic acid (1i).\textsuperscript{(138)}

\[ \text{(RS)-5-Amino-3-methyl-pentanoic acid 1i} \]

(RS)-5-Amino-3-methyl-pentanoic acid 1i is the aliphatic analogue of the synthesized compounds 1a-h. Compound 1i has been successfully achieved by adopting Knoevenagel reaction due to its simplicity as compared with HWE reaction employed in the synthesis of the aromatic analogues 1a-h. The synthetic strategy applied to obtain compound 1i is provided in Scheme 4.

4.2.1 Synthesis of 4-Cyano-3-methyl-2-butenoic acid ethyl ester (5i)\textsuperscript{(139)}

4.2.1.1 Knoevenagel Reaction\textsuperscript{(140,141)}

A Knoevenagel reaction is a condensation reaction between a carbonyl compound and any compound having an active methylene group. The reaction is carried out in mildly basic medium or in neutral solution in the presence of salts such as piperidine acetate or ammonium salts. The superior catalytic activity of salts can be attributed to the findings of Cope.\textsuperscript{(142)} He found that salts as ammonium acetate furnish both the conjugate base, namely acetate ion, which catalyzes enolization of the active methylene compound, and the conjugate acid, namely ammonium ion which catalyzes elimination of water by E1\textsubscript{ch} mechanism (Scheme 24).\textsuperscript{(143)}
It is to be noted that condensation of cyano-acetic acid with carbonyl compounds by way of Knoevenagel condensation is accompanied with decarboxylation to yield $\alpha,\beta$-unsaturated compounds (Scheme 25). The decarboxylation occurs after condensation and can not be avoided.\(^\text{143}\)

4-Cyano-3-methyl-2-butenoic acid ethyl ester 5\text{i} has been successfully achieved by adopting Knoevenagel reaction described by Simchen.\(^\text{139}\) A mixture of cyano-acetic acid\(^\text{144}\), ethyl acetoacetate, ammonium acetate and acetic acid in dry benzene was refluxed for 8 h using Dean-Stark apparatus. The crude 5\text{i} was distilled (100-102 $^\circ$C/5mm) to afford the $\alpha,\beta$-unsaturated diastereomeric mixture 5\text{i} with $E/Z$ ratio = 1.7 (lit.\(^\text{139}\) $E/Z$ ratio = 1.5) as detected by $^1$H-NMR (cf. Experimental Part).

### 4.2.2 Synthesis of (RS)-5-Amino-3-methyl-pentanoic acid ethyl ester hydrochloride (4\text{i})

![Scheme 24](image)

![Scheme 25](image)
Theoretical Discussion

4-Cyano-3-methyl-2-butenoic acid ethyl ester \(5_i\) (as diastereomeric mixture) was subjected to catalytic hydrogenation using 10% Pd/C and concentrated hydrochloric acid in 95% ethanol. The reaction mixture was hydrogenated in Parr shaker apparatus under 4 bar of \(H_2\) for 18 h at room temperature to afford \((RS)-5\text{-amino-3-methyl-pentanoic acid ethyl ester hydrochloride} 4_i\) (cf. Experimental Part).

### 4.2.3 Synthesis of \((RS)-5\text{-Amino-3-methyl-pentanoic acid hydrochloride} (3_i)\)

Without further purification, the crude \(4_i\) was hydrolyzed by reflux in 5 N hydrochloric acid for 4 h to give \((RS)-5\text{-amino-3-methyl-pentanoic acid hydrochloride} 3_i\). It has to be mentioned that our trial to obtain compound \(3_i\) in a sufficient pure form by recrystallization was unsuccessful. This futile result persuaded us to derivatize amino function of compound \(3_i\) with a lipophilic moiety thereby facilitating its purification by a simple acid-base chemical purification.

### 4.2.4 Synthesis of \((RS)-5\text{-Benzyloxycarbonylamino-3-methyl-pentanoic acid} (2_i)\)

An examination of literature revealed that benzyl chloroformate is a suitable protecting group for amino functions of amino acids and it enables us to increase lipophilicity of \(3_i\) and hence facilitating its purification.

\((RS)-5\text{-Benzyloxycarbonylamino-3-methyl-pentanoic acid} 2_i\) has been synthesized by adopting the trivial procedure for protecting amino groups of amino acids cited by Boissonnas and Preitner\(^{145}\). The reaction mixture containing crude \(3_i\) was basified with 4 N sodium hydroxide solution and treated with a stoichiometric amount of
benzyl chloroformate at 0 °C (cf. Experimental Part). The reaction mixture was extracted with diethyl ether to remove all water insoluble matter. The aqueous layer containing sodium salt of (RS)-5-benzyloxycarbonylamino-3-methyl-pentanoic acid 2i was cooled and acidified using concentrated hydrochloric acid and extracted with diethyl ether leaving all water soluble impurities in the aqueous phase. The organic phase was evaporated to give 2i as a viscous pale yellow oil in 65% yield (based on 5i) which was sufficiently pure (as detected by \(^1\)H and \(^{13}\)C-NMR) to be used in the next step without further purification (Scheme 26).

The crude (RS)-5-benzyloxycarbonylamino-3-methyl-pentanoic acid 2i was subjected to catalytic hydrogenation to cleave N-benzyloxycarbonyl protecting group. The reaction mixture containing 2i and 10%Pd/C in 50% 2-propanol (to avoid esterification of the free carboxylic group which probably occurs when using ethanol or methanol as a hydrogenation solvent) was hydrogenated in Parr shaker apparatus under 4 bar of H\(_2\) for 18 h at room temperature. The crude (RS)-5-amino-3-methyl-pentanoic acid 1i was recrystallized from 2-propanol/water to give 1i as a white powder m.p. 164-165 (lit.\(^{138}\) 133-135 °C) in 69% yield (cf. Experimental Part). The structure of 1i has been established through microanalytical, IR, \(^1\)H-NMR, \(^{13}\)C-NMR, and mass spectral data.

It is to be mentioned that a search in the literature revealed that compound 1i has been prepared by Wallach\(^{138}\) as outlined in Scheme 27.
The author mentioned that oxime 100 underwent Beckmann rearrangement and ring enlargement using H₂SO₄ to give a mixture of piperidine 101 and piperidine 102 which upon hydrolysis gave a mixture of (RS)-5-amino-3-methyl-pentanoic acid 1i and (RS)-5-amino-4-methyl-pentanoic acid 103, respectively. This may be the reason of the big difference of the reported melting point of 1i (133-135 °C) and our recorded melting point (164-165 °C).

4.3 Synthetic Plan for the Synthesis of 3-Aminomethyl-5-chloro-benzoic acid hydrochloride (1j).

3-Aminomethyl-5-chloro-benzoic acid hydrochloride 1j is a lipophilic DAVA derivative and it has been synthesized using 5-amino-isophthalic acid 9j as a starting material (Scheme 5).
4.3.1 Synthesis of 5-Chloro-isophthalic acid (8j)

Our trials to prepare 8j by diazodization of 5-amino-isophthalic acid 9j by the procedure cited by Hartman and Brethen or by the procedure cited by Chand et al. using aqueous hydrochloric acid as a solvent were unsuccessful which might be attributed to the insolubility of the formed diazonium salt in the solvent system used in the reaction.

5-Chloro-isophthalic acid 8j has been achieved by diazodization of 5-amino-isophthalic acid 9j by adopting the procedure mentioned by Gunstone and Tucker. Sodium nitrite was dissolved in concentrated sulfuric acid and the resulting solution was added dropwise to the solution of 9j in acetic acid. The resulting diazonium salt was added in portions to a solution of cuprous chloride in concentrated hydrochloric acid and the reaction mixture was heated to 80 °C for 20 min (cf. Experimental Part). The crude 8j was sufficiently pure to be used for the next step without further purification.

4.3.2 Synthesis of 5-Chloro-isophthalic acid dimethyl ester (7j)

The crude 8j was subjected to esterification by adopting the trivial procedure described by Dimick et al. using methanol and a catalytic amount of concentrated sulfuric acid. The crude 5-chloro-isophthalic acid dimethyl ester 7j was dissolved in dichloromethane and washed with sodium bicarbonate solution to remove any unreacted starting material. The organic layer was dried and evaporated to dryness to yield 7j as a white powder (cf. Experimental Part) which was pure enough to be used in the next step without further purification.
4.3.3 Synthesis of 5-Chloro-isophthalic acid monomethyl ester (6j)\(^{(150)}\)

Saponification of the diester 7j using one mole of 1 N sodium hydroxide solution afforded monoester 5-chloro-isophthalic acid monomethyl ester 6j which was purified by acid base purification to give pure 6j in 77% yield as a white powder (cf. Experimental Part).

4.3.4 Synthesis of 3-Chloro-5-hydroxymethyl-benzoic acid methyl ester (5j)\(^{(150)}\)

Borane-dimethyl sulfide complex was used for the selective reduction of the carboxylic function of 6j in the presence of ester function in tetrahydrofuran at ambient temperature to afford 3-chloro-5-hydroxymethyl-benzoic acid methyl ester 5j (Scheme 28). The crude 5j was subjected to acid-base purification to remove unreacted acid and gave a pure 5j (as detected by \(^1\)H and \(^{13}\)C-NMR) in 87% yield (cf. Experimental Part).
4.3.5 Synthesis of 3-Bromomethyl-5-chloro-benzoic acid methyl ester (4j)\textsuperscript{(151)}

\[ \text{Br} \quad \text{H}_3\text{COOC} \quad \text{Cl} \]

\[ 4j \]

Elaboration of the alcohol 5\textsubscript{j} to the corresponding bromo derivative 4\textsubscript{j} was accomplished by addition of phosphorous tribromide to 5\textsubscript{j} without solvent according to the procedure mentioned by Amrollah-Madjdabadi et al.\textsuperscript{(152)} The reaction mixture was stirred at room temperature for 30 min. The crude 3-bromomethyl-5-chloro-benzoic acid methyl ester 4\textsubscript{j} was purified by column chromatography to yield 4\textsubscript{j} as a white powder (cf. Experimental Part).

4.3.6 Synthesis of 3-Azidomethyl-5-chloro-benzoic acid methyl ester (3\textsubscript{j})

\[ \text{H}_3\text{COOC} \quad \text{Cl} \quad \text{N}_3 \]

\[ 3j \]

The azide derivative 3-azidomethyl-5-chloro-benzoic acid methyl ester 3\textsubscript{j} has been synthesized by replacement the bromo function of 4\textsubscript{j} with the azide function. A mixture of 4\textsubscript{j} and sodium azide was refluxed in aqueous acetone for 18 h according to the procedure described by Dimick et al.\textsuperscript{(151)} The crude azide 3\textsubscript{j} was purified by column chromatography to afford 3\textsubscript{j} as a pale yellow oil in 86% yield (cf. Experimental Part).

4.3.7 Synthesis of 3-Azidomethyl-5-chloro-benzoic acid (2\textsubscript{j})

\[ \text{Cl} \quad \text{HOOC} \quad \text{N}_3 \]

\[ 2j \]
The ester function of 3\textsubscript{j} was hydrolyzed by refluxing in methanolic sodium hydroxide solution for 2.5 h. The crude 3-azidomethyl-5-chloro-benzoic acid 2\textsubscript{j} was purified by acid-base chemical purification to give pure 2\textsubscript{j} as a pale yellow powder which was sufficiently pure to be used in the next step without further purification.

Catalytic hydrogenation of the azide function of 2\textsubscript{j} in 95% 2-propanol and concentrated hydrochloric acid in the presence of PtO\textsubscript{2} gave the target compound 3-aminomethyl-5-chloro-benzoic acid hydrochloride 1\textsubscript{j} (Scheme 29). The crude 1\textsubscript{j} was recrystallized from 2-propanol/diethyl ether mixture to afford 1\textsubscript{j} in 82% yield as a white hygroscopic powder. The structure of 1\textsubscript{j} has been established through microanalytical, IR, \textsuperscript{1}H-NMR, \textsuperscript{13}C-NMR, and mass spectral data.

\[
\begin{array}{c}
\text{Cl} \\
\text{HOOC} \\
\text{N}_3 \\
\text{Cl} \\
\text{HOOC}
\end{array}
\xrightarrow{\text{H}_2/\text{PtO}_2, \text{HCl}}
\begin{array}{c}
\text{Cl} \\
\text{HOOC} \\
\text{NH}_3 \text{Cl}
\end{array}
\]

(Scheme 29)

It has to be pointed out that catalytic hydrogenation of 3\textsubscript{j} followed by hydrolysis of the ester function in 5 N hydrochloric acid afforded a mixture of 1\textsubscript{j} and 104 which was not easy to separate by recrystallization.

\[
\begin{array}{c}
\text{Cl} \\
\text{CH}_3\text{O} \\
\text{O} \\
\text{NH}_3 \text{Cl}
\end{array}
\]

4.4 Synthetic Plan for the Synthesis of (RS)-4-Amino-3-(4’-ethynyl-phenyl)-butanoic acid hydrochloride (1\textsubscript{k}).
(RS)-4-Amino-3-(4’-ethynyl-phenyl)-butanoic acid hydrochloride 1k is an aromatic GABA analogue containing para ethynyl group instead of para chloro functionality of baclofen. The synthetic strategy adopted to achieve 1k is illustrated in Scheme 6.

4.4.1 Synthesis of (4-Iodo-phenyl)-methanol (9k)\(^{(153)}\)

\[
\begin{align*}
\text{I} & \quad \text{OH} \\
\text{9}_k
\end{align*}
\]

Sodium borohydride reduction of 4-iodo-benzoyl chloride 10k, which has been prepared from 4-iodo-benzoic acid 11k and thionyl chloride in tetrahydrofuran according to the procedure cited by Sasse et al.\(^{(154)}\), in dioxane afforded the anticipated compound (4-iodo-phenyl)-methanol 9k (Scheme 30) in 73% yield as a white powder (cf. Experimental Part).

\[
\begin{align*}
\text{COOH} & \quad \text{I} \\
\text{11}_k & \quad \text{SOCl}_2, \text{dry THF} \quad \text{reflux, 1 h} \\
\text{Cl} & \quad \text{I} \\
\text{10}_k & \quad \text{NaBH}_4, \text{dioxane} \quad 100 \, ^\circ\text{C}, 1.5 \text{ h} \\
\text{OH} & \quad \text{I} \\
\text{9}_k
\end{align*}
\]

(Scheme 30)

4.4.2 Synthesis of 4-Iodo-benzaldehyde (8k)\(^{(153)}\)

\[
\begin{align*}
\text{I} & \quad \text{O} \quad \text{H} \\
\text{8}_k
\end{align*}
\]
Oxidation of the alcohol function of \( 9_k \) was readily accomplished by using pyridinium dichromate in dichloromethane for 2.5 h (TLC monitoring) to give the required 4-iodo-benzaldehyde \( 8_k \) in 83\% yield as a white powder (cf. Experimental Part). It is to be noted that increase the reaction time more than 2.5 h decreased the yield of the target aldehyde \( 8_k \) due to further oxidation of the aldehyde function to the corresponding acid and gave \( 11_k \) instead of \( 8_k \).

### 4.4.3 Synthesis of \((E)-3-(4'-Iodo-phenyl)-acrylic acid methyl ester \((7_k)\)\(^{(155)}\)

![Chemical Structure](image)

\(-\)

### 4.4.3.1 Wittig Reaction\(^{(100,156)}\)

Wittig reaction is a carbon-carbon olefin synthesis from phosphonium ylides and carbonyl compounds. Stabilized ylides react with aldehydes almost exclusively via trans-oxaphosphetanes. Initially, a small portion of the cis-isomer may still be produced. However, all the heterocyclic material isomerizes very rapidly to the trans-configured four-membered ring through an especially pronounced stereochemical drift. Only after this point does the (2+2)-cycloreversion start. It leads to triphenylphosphine oxide and a trans-configured olefin (Scheme 31).\(^{(156)}\)

\((E)-3-(4'-Iodo-phenyl)-acrylic acid methyl ester \(7_k\) has been successfully achieved by adopting the general trivial procedure of Wittig reaction using 4-iodo-benzaldehyde \(8_k\) and (methoxycarbonylmethylene)-triphenylphosphorane\(^{(157)}\) \(105\), prepared from triphenylphosphine and methyl bromoacetate in toluene according to Isler and collaborators\(^{(157)}\) (Scheme 32), in dichloromethane for one hour (NMR monitoring) at room temperature. The crude \((E)-3-(4'-iodo-phenyl)-acrylic acid methyl ester \(7_k\) was purified by column chromatography to afford \((E)-7_k\) in 93\% yield as a pale yellow powder (cf. Experimental Part).
**Theoretical Discussion**

\[ \text{cis} \rightarrow \text{cis-olefin} \]
\[ \text{trans} \rightarrow \text{trans-olefin} \]

\[ \text{Oxaphosphetanes} \]

\[ K_{\text{cis}} \] is the rate constant for the formation of the cis-oxaphosphetane, \( K_{\text{trans}} \) is the rate constant for the formation of the trans-oxaphosphetane, and \( K_{\text{drift}} \) is the rate constant for the isomerization of cis- to trans-configured oxaphosphetane, which is called stereoc~

\[ \text{(Scheme 31)} \]

\[ \text{Ph} \quad \text{Ph} \quad \text{Ph} + \text{BrCH}_2\text{OMe} \quad \text{Toluene} \quad \text{NaOH} \quad \text{O=PPPh}_3 \quad \text{OMe} \quad 105 \]

\[ \text{(Scheme 32)} \]

**4.4.4 (RS)-Synthesis of 3-(4'-Iodo-phenyl)-4-nitro-butanoic acid methyl ester (6_k)**

\[ 6_k \]
Condensation of nitromethane with \((E)-3-(4`\text{-iodo-phenyl})\text{-acrylic acid methyl ester 7}_k\) in the presence of benzyltrimethylammonium hydroxide (Triton B, \(~40\%\) solution in methanol) was done in methanol according to the procedure described by Berthelot and co-workers.\(^{(73)}\) The crude \((RS)-3-(4`\text{-iodo-phenyl})\text{-4-nitro-butanoic acid methyl ester 6}_k\) which was purified by column chromatography to furnish pure \(6_k\) in 89\% yield as a white powder (cf. Experimental Part).

### 4.4.5 Synthesis of \((RS)-4-(4`\text{-Iodo-phenyl})\text{-2-pyrrolidinone (5}_k\)\(^{(158)}\)

\[(RS)-4-(4`\text{-Iodo-phenyl})\text{-2-pyrrolidinone 5}_k\] is the key intermediate in the synthetic strategy employed to achieve our targets, compounds \(1_k\) and \(1_l\).

Alumina and silica gel have a growing application as catalysts or co-catalysts as well as supports for many different kinds of reactions in organic chemistry.\(^{(159-161)}\) Bladé-Font\(^{(162)}\) developed an efficient, simple and inexpensive procedure for cyclo-dehydration of \(\gamma\)-, \(\delta\)- and \(\varepsilon\)-amino acids to the corresponding lactams.

\((RS)-4-(4`\text{-Chloro-phenyl})\text{-2-pyrrolidinone}^{(162)}\) \(107\) was prepared by refluxing a mixture of \((RS)-4\text{-amino-3-(4`\text{-chloro-phenyl})\text{-butanoic acid}^{(163)}\) (baclofen)\(^{\circ}\) \(106\) and neutral \(\text{Al}_2\text{O}_3\) (activity I) in toluene for 5 h using a Dean-Stark trap to collect the water formed in the reaction (Scheme 33).
Protection of the amide nitrogen of 107 by introduction of tert-butylxycarbonyl group was realized by reaction of 107 with di-tert-butyldicarbonate in dichloromethane in the presence of triethylamine and 4-(dimethylamino)-pyridine according to literature procedures\(^\text{(164)}\) to afford (RS)-1-tert-butylxycarbonyl-4-(4′-chloro-phenyl)-2-pyrrolidinone 108 (Scheme 34).

\[
\begin{align*}
\text{O} & \quad \text{N} & \quad \text{Cl} \\
\text{\hspace{1cm} 107} & \quad \overset{\text{Boc}_2\text{O}, \text{DMAP}}{\text{\hspace{1cm} 108}} & \quad \text{O} & \quad \text{N} & \quad \text{Cl} \\
& & & \text{TEA, CH}_2\text{Cl}_2, \text{r.t.} & & & \text{TEA, CH}_2\text{Cl}_2, \text{r.t.}
\end{align*}
\]

\text{(Scheme 34)}

We tried to synthesize (RS)-4-(4′-ido-phenyl)-2-pyrrolidinone 5\(_k\) and/or (RS)-1-tert-butyloxycarbonyl-4-(4′-ido-phenyl)-2-pyrrolidinone 4\(_k\) by adopting Grignard reaction on 107 and/or 108, respectively, according to the procedure described by Gately et al.\(^\text{(165)}\) The formed Grignard complex was reacted with a saturated solution of iodine in diethyl ether according to the method cited by Kasai et al.\(^\text{(166)}\) to afford the anticipated compounds 5\(_k\) and/or 4\(_k\). Unfortunately, we obtained a complex mixture (as detected by TLC) and we could not isolate the required targets (Scheme 35).

\[
\begin{align*}
\text{O} & \quad \text{N} & \quad \text{Cl} \\
\text{\hspace{1cm} 107} & \quad \overset{1-\text{Grignard}}{\text{\hspace{1cm} 5\(_k\)}} & \quad \text{O} & \quad \text{N} & \quad \text{I} \\
& & & \text{R} & & & \text{R} \\
\text{\hspace{1cm} 108} & \quad \overset{2-\text{I}_2}{\text{\hspace{1cm} 4\(_k\)}} & \quad \text{O} & \quad \text{N} & \quad \text{Cl} \\
& & & \text{R} & & & \text{R}
\end{align*}
\]

\text{(Scheme 35)}

An examination of literature revealed that Guijarro et al.\(^\text{(167)}\) described a regio-selective lithiation of different chloroarenes in the presence of lithium powder.
and a catalytical amount of naphthalene. The formed organolithium compounds can react with different electrophiles to give the expected polyfunctionalized aromatic molecules (Figure 15).\(^{(168)}\)

We studied the direct lithiation of \((RS)-1\text{-}\text{tert}-\text{butyloxycarbonyl}-4\text{-}(4′\text{-chloro-phenyl})\text{-}2\text{-pyrrolidinone}\) \(108\) using the available lithium ribbon or lithium granules in the presence of a catalytical amount (3%) of naphthalene according to the procedure mentioned by Yus and Ramòn\(^{(168)}\) followed by reaction with iodine to yield the expected compound \((RS)-1\text{-}\text{tert}-\text{butyloxycarbonyl}-4\text{-}(4′\text{-iodo-phenyl})\text{-}2\text{-pyrrolidinone}\) \(4k\). Unfortunately, no reaction took place and the starting material was recovered unchanged. This result might be attributed to the low reactivity of lithium ribbon or lithium granules used in our trial as compared with the highly reactive lithium powder which is not available nowadays owing to its hazards.

According to the hereabove negative trials to achieve \(5k\), we are convinced to return to the original synthetic strategy depicted in Scheme 6 to obtain \(5k\) in a reasonable way.

Selective reduction of the nitro function of \(6k\) without dehalogenation has been established by using zinc dust in methanol/concentrated hydrochloric acid mixture at 0 °C for 30 min. The produced amino ester hydrochloride \(109\) was treated with
6 N NaOH solution according to the method cited by Stevens et al.\textsuperscript{(169)} to afford the pivotal lactam ($RS$)-4-(4'-iodo-phenyl)-2-pyrrolidinone 5\textsubscript{k} in 65% yield as a white powder which was pure enough to be used in the next step without further purification (Scheme 36).

\[
\begin{align*}
\text{I} & \quad \text{Zn, CH}_3\text{OH/HCl} \quad 0 \degree \text{C, 30 min} \\
\text{O} & \quad \text{OMe} \\
\text{6k} & \quad \quad \\
\text{NO}_2 & \quad \text{NH}_3 \text{Cl} \\
\text{O} & \quad \text{OMe} \\
\text{109} & \quad \quad \\
\text{N} & \quad \text{O} \\
\text{H} & \quad \text{I} \\
\text{5k} & \quad \quad \\
\end{align*}
\]

(Scheme 36)

It is noteworthy that our attempt to reduce selectively the nitro function of 6\textsubscript{k} using NiCl\textsubscript{2}-NaBH\textsubscript{4} according to the procedure cited by Walz and Sundberg\textsuperscript{(170)} led to lactam formation with dehalogenation and afforded compound 110 (Scheme 37).

\[
\begin{align*}
\text{I} & \quad \text{NiCl}_2\text{-NaBH}_4 \quad \text{CH}_3\text{OH, r.t.} \\
\text{O} & \quad \text{OMe} \\
\text{6k} & \quad \quad \\
\text{NO}_2 & \\
\text{O} & \quad \text{N} \\
\text{H} & \quad \text{110} \\
\end{align*}
\]

(Scheme 37)
4.4.6 Synthesis of \((RS)-1\text{-}\text{tert}-\text{Butyloxy carbonyl}-4\text{-(4′-iodo-phenyl)}\text{-}2\text{-pyrrolidinone} (4_k)\)

Protection of the amide nitrogen of \((RS)-4\text{-(4′-iodo-phenyl)}\text{-}2\text{-pyrrolidinone} 5_k with \text{tert}-\text{butyloxy carbonyl group has been successfully achieved by reaction of} 5_k with \text{di-tert}-\text{butyldicarbonate in dichloromethane in the presence of triethylamine and} 4\text{-}(\text{dimethylamino})\text{-pyridine according to literature procedures.}^{(164)}

4.4.7 Synthesis of \((RS)-1\text{-}\text{tert}-\text{Butyloxy carbonyl}-4\text{-(4′-trimethylsilanyl-ethynyl-phenyl)}\text{-}2\text{-pyrrolidinone} (3_k)\)

4.4.7.1 Sonogashira-Hagihara Coupling\(^{(171,172)}\)

Sonogashira-Hagihara coupling is an arylation reaction of terminal alkynes using aryl halides (bromides or iodides) and a catalytical amount of both Pd(0) and Cu(I). In step 1 of this reaction: a \(\pi\) complex between the catalytically active zero-valent Pd (Pd(0)), which is produced from Pd(II) complex (PdCl\(_2\)(PPh\(_3\))\(_2\)) by reduction under reaction conditions, and the arylating agent. Step 2: formation of Pd(II) complex with
σ-bonded aryl moiety. Step 3: formation of Cu-acetylide by capture of small equilibrium concentration of ammonium acetylide, formed from acetylene and amine used in the reaction in large excess, as copper acetylide. Step 4: transmetallation, the alkynyl-Pd compound is formed from the alkynyl-Cu compound via ligand exchange. Step 5: reduction elimination to form the π complex of the arylated alkyne. Step 6: decomposition of the complex into the coupling product and the unsaturated Pd species which reenters the catalytic cycle anew with step 1 (Scheme 38). \(^{(172)}\)

\begin{center}
\begin{tikzpicture}
\node (A) at (0,0) {$X = \text{Br or I}$};
\node (B) at (2,2) {$\text{Pd}(\text{PPh}_3)_4$};
\node (C) at (2,-2) {$\text{CuX}$};
\node (D) at (4,0) {$\text{PdCl}_2(\text{PPh}_3)_2$};
\node (E) at (6,0) {$\text{R} = \text{C} = \text{C}$};
\node (F) at (8,0) {$\text{R} = \text{C} = \text{C}$};
\node (G) at (10,0) {$\text{R} = \text{C} = \text{C}$};
\node (H) at (12,0) {$\text{R} = \text{C} = \text{C}$};
\node (I) at (1,1) {$\text{in situ}$};
\node (J) at (1,0) {$\text{Pd}^0$};
\node (K) at (1,-1) {$\text{Cu}^0$};
\node (L) at (3,1) {$\text{Pd}^2$};
\node (M) at (3,-1) {$\text{Cu}^0$};
\node (N) at (5,1) {$\text{Pd}^2$};
\node (O) at (5,-1) {$\text{Cu}^0$};
\node (P) at (7,1) {$\text{Pd}^2$};
\node (Q) at (7,-1) {$\text{Cu}^0$};
\node (R) at (9,1) {$\text{Pd}^2$};
\node (S) at (9,-1) {$\text{Cu}^0$};
\node (T) at (11,1) {$\text{Pd}^2$};
\node (U) at (11,-1) {$\text{Cu}^0$};
\node (V) at (13,1) {$\text{Pd}^2$};
\node (W) at (13,-1) {$\text{Cu}^0$};
\node (X) at (0,4) {step 1};
\node (Y) at (2,4) {step 2};
\node (Z) at (4,4) {step 3};
\node (AA) at (6,4) {step 4};
\node (BB) at (8,4) {step 5};
\node (CC) at (10,4) {step 6};
\node (DD) at (2,2) {step 1};
\node (EE) at (4,2) {step 2};
\node (FF) at (6,2) {step 3};
\node (GG) at (8,2) {step 4};
\node (HH) at (10,2) {step 5};
\node (II) at (2,-2) {step 2};
\node (JJ) at (4,-2) {step 3};
\node (KK) at (6,-2) {step 4};
\node (LL) at (8,-2) {step 5};
\node (MM) at (10,-2) {step 6};
\draw (A) -- (B) node[midway, above] {X = Br or I};
\draw (B) -- (C) node[midway, above] {X = Br or I};
\draw (C) -- (D) node[midway, above] {X = Br or I};
\draw (D) -- (E) node[midway, above] {X = Br or I};
\draw (E) -- (F) node[midway, above] {X = Br or I};
\draw (F) -- (G) node[midway, above] {X = Br or I};
\draw (G) -- (H) node[midway, above] {X = Br or I};
\draw (H) -- (I) node[midway, above] {X = Br or I};
\draw (I) -- (J) node[midway, above] {X = Br or I};
\draw (J) -- (K) node[midway, above] {X = Br or I};
\draw (K) -- (L) node[midway, above] {X = Br or I};
\draw (L) -- (M) node[midway, above] {X = Br or I};
\draw (M) -- (N) node[midway, above] {X = Br or I};
\draw (N) -- (O) node[midway, above] {X = Br or I};
\draw (O) -- (P) node[midway, above] {X = Br or I};
\draw (P) -- (Q) node[midway, above] {X = Br or I};
\draw (Q) -- (R) node[midway, above] {X = Br or I};
\draw (R) -- (S) node[midway, above] {X = Br or I};
\draw (S) -- (T) node[midway, above] {X = Br or I};
\draw (T) -- (U) node[midway, above] {X = Br or I};
\draw (U) -- (V) node[midway, above] {X = Br or I};
\draw (V) -- (W) node[midway, above] {X = Br or I};
\draw (W) -- (X) node[midway, above] {X = Br or I};
\draw (X) -- (Y) node[midway, above] {X = Br or I};
\draw (Y) -- (Z) node[midway, above] {X = Br or I};
\draw (Z) -- (AA) node[midway, above] {X = Br or I};
\draw (AA) -- (BB) node[midway, above] {X = Br or I};
\draw (BB) -- (CC) node[midway, above] {X = Br or I};
\draw (CC) -- (DD) node[midway, above] {X = Br or I};
\draw (DD) -- (EE) node[midway, above] {X = Br or I};
\draw (EE) -- (FF) node[midway, above] {X = Br or I};
\draw (FF) -- (GG) node[midway, above] {X = Br or I};
\draw (GG) -- (HH) node[midway, above] {X = Br or I};
\draw (HH) -- (II) node[midway, above] {X = Br or I};
\draw (II) -- (JJ) node[midway, above] {X = Br or I};
\draw (JJ) -- (KK) node[midway, above] {X = Br or I};
\draw (KK) -- (LL) node[midway, above] {X = Br or I};
\draw (LL) -- (MM) node[midway, above] {X = Br or I};
\end{tikzpicture}
\end{center}

\((RS)-1$-$tert$-$Butyloxycarbonyl$-$4$-(4`$-trimethylsilanyl$-$ethynyl$-$phenyl)$-$2$-$pyrroloidinone $3_k$ was readily accomplished by adopting Sonogashira-Hagihara coupling according to the procedure cited by Kayser and co-workers \(^{(173)}\). Trimethylsilylacetylene was added under nitrogen atmosphere to a suspension of $4_k$ in triethylamine in the presence of a catalytical amount of bis (triphenylphosphine)-palladium dichloride and cuprous iodide (Scheme 39). The reaction mixture was stirred at room temperature for 12 h. The crude $3_k$ was purified by column chromatography to give pure $3_k$ in 90% yield as a pale yellow powder (cf. Experimental Part).
It has to be pointed out that we tried to synthesize compound 3k from (RS)-1-tert-butyloxy carbonyl-4-(4′-chloro-phenyl)-2-pyrrolidinone 108, Ni(acac)$_2$, PPh$_3$, and (trimethylsilylethynyl)-magnesium bromide prepared from (trimethylsilyl)-acetylene and magnesium bromide in tetrahydrofuran according to nickel-catalyzed substitution method mentioned by Katz. Unfortunately, the starting material was recovered unchanged (Scheme 40).

Compound 3k was subjected to cleavage of trimethylsilyl group and lactam ring opening under mild basic conditions. The presence of tert-butyloxy carbonyl as a lactam nitrogen protecting group facilitated the lactam ring opening under mild basic conditions. 1 M lithium hydroxide solution was added to a solution of 3k in tetrahydrofuran (Scheme 41). The reaction mixture was stirred at room temperature for 18 h to afford (RS)-4-tert-butyloxy carbonylamino-3-(4′-ethynyl-phenyl)-butanoic acid 2k as a yellow powder in 90% yield (cf. Experimental Part).
The crude $2_k$ was sufficiently pure to be used in the next step without further purification. N-Boc protecting group has been removed cleanly by stirring a solution of $2_k$ in $\sim 2.5$ M dry hydrogen chloride/ethyl acetate at room temperature for one hour$^{173,176}$ (Scheme 42). The crude (RS)-4-amino-3-(4′-ethynyl-phenyl)-butanoic acid hydrochloride $1_k$ was recrystallized from 2-propanol/diethyl ether mixture to furnish $1_k$ as a colourless crystals in 87% yield (cf. Experimental Part). The structure of $1_k$ has been established through microanalytical, IR, $^1$H-NMR, $^{13}$C-NMR, and mass spectral data.

4.5. Synthetic Plan for the Synthesis of (RS)-4-Amino -3-(4′-iodo-phenyl)-butanoic acid hydrochloride ($1_i$)$^{158}$

![Diagram showing the synthetic plan and structures](image-url)
(RS)-4-Amino-3-(4′-iodo-phenyl)-butanoic acid hydrochloride 1₁ is a baclofen analogue containing the iodo functionality as a bioisostere for the chloro functionality in the para position of baclofen. Compound 1₁ has been successfully achieved from the lactam 4ₖ by lactam ring opening and deprotection of the N-Boc functionality as illustrated in Scheme 6.

4.5.1 Synthesis of (RS)-4-tert-Butyloxycarbonylamino-3-(4′-iodo-phenyl)-butanoic acid (2₁)

(RS)-1-tert-Butyloxycarbonyl-4-(4′-iodo-phenyl)-2-pyrrolidinone 4ₖ is the starting material for the synthesis of (RS)-4-tert-butyloxycarbonylamino-3-(4′-iodo-phenyl)-butanoic acid 2₁. 1 M lithium hydroxide was added to a solution of 4ₖ in tetrahydrofuran. The reaction mixture was stirred at ambient temperature for two hours (Scheme 43).

The crude 2₁ was treated with ~2.5 M dry hydrogen chloride/ethyl acetate solution to cleave the N-Boc protecting group and afforded (RS)-4-amino-3-(4′-iodo-phenyl)-
butanoic acid hydrochloride $\mathbf{1}_1$ (Scheme 44). The crude $\mathbf{1}_1$ was recrystallized from 2-propanol/diethyl ether mixture to give $\mathbf{1}_1$ as a white powder m.p. 208-210 °C (Lit.\textsuperscript{158} 190-195 °C) in 83% yield (cf. Experimental Part). Our attempt to recrystallize $\mathbf{1}_1$ from ethanol/diethyl ether mixture as reported by Wakita et al.\textsuperscript{158} led to partial esterification of the free carboxylic group of $\mathbf{1}_1$ as detected by $^1$H-NMR. The structure of $\mathbf{1}_1$ has been established through microanalytical, IR, $^1$H-NMR, $^{13}$C-NMR, and mass spectral data.
5 Pharmacological Evaluation

The synthesized compounds 1a-1 were evaluated for their GABA B R agonist activity on tsA cells transfected with GABA B1b/GABA B2/Gαq-z5 based on Ca²⁺ measurement rather than measurement of inositol phosphate generation as previously published.\(^{(177)}\)

- Materials and methods

a) Materials

GABA was obtained from Sigma (St. Louis, MO, USA). Culture media, serum and antibiotics were obtained from Invitrogen (Paisley, UK). The rat GABA B R plasmids and the Gαq-z5 construct were generous gifts from Dr. Janet Clark (National Institute of Health, Bethesda, MD, USA) and Dr. Bruce Conklin (University of California, San Francisco, CA, USA). The tsA cells were a generous gift from Dr. Penelope S. V. Jones (University of California, San Diego, CA, USA).

b) Methods

TsA cells (a transformed human embryonic kidney (HEK) 293 cell line)\(^{(178)}\) were maintained at 37 °C in a humidified 5% CO₂ incubator in Dulbecco’s modified Eagle medium (DMEM) supplemented with penicillin (100U/ml), streptomycin (100mg/ml) and 10% fetal calf serum. One million cells were split into a 10 cm tissue culture plate and transfected the following day with 0.7 µg GABA B1b-pcDNA3.1, 3.5 µg GABA B2-pcDNA3.1 and 0.7µg Gαq-z5-pcDNA using SuperFect as a DNA carrier according to the protocol by the manufacturer (Qiagen, Hilden, Germany). The day after transfection, cells were split into one poly-D-lysine coated 96-well black-walled – clear-bottomed tissue culture plates in the same medium as mentioned above and incubated overnight. The following day the measurement of intracellular calcium was performed as follows. The media was exchanged with Hanks balanced saline solution containing 1 mM CaCl₂, 1 mM MgCl₂, 20 mM HEPES, 2.5 mM probencid and 4 µM Fluo-4AM (pH = 7.4). The cells were incubated for 1 h at 37 °C in a humidified 5% CO₂ incubator. Cells were then washed twice with the same buffer without Fluo-4AM and finally 100 µl of the buffer was left in the wells.

The cell plate was then transferred to the NovoStar (BMG Labtechnologies, Offenburg, Germany) and the basal fluorescence level was adjusted to ~ 10000
fluorescence units (FU) using excitation/emission wavelengths of 485-520 nm, respectively. Fluorescence readings were measured for 45 s after addition of ligand and response was calculated as peak response minus basal level. Inactive compounds were also tested as antagonists. Twenty min after application of ligand, 10 µM GABA was added to the well and fluorescence was measured as above. All experiments were performed in triplicate and the results are given as mean ± S.E.M of 3-4 experiments.

Results and Discussion

The GABAB agonist activity of the synthesized compounds 1a-l is summarized in Table 3. Compounds 1a, 1e, 1f, 1k and 1l are active as GABAB agonists (EC50 value 32-240 µM, Figure 16) whereas compounds 1b, 1c, 1d, 1g, 1h, 1i and 1j (EC50 > 300 µM) are considered inactive as GABABR agonists in the GABABR subtype used in our assay.

Regarding the structure-activity relationship in the synthesized series 1a-l, it has to be mentioned that mono substitution on the aromatic moiety in the 3 position of both GABA and/or DAVA backbone with a halogen, specially para chloro, is optimum for GABABR agonist activity. The synthesized compounds which evoked GABABR agonist activity have the following decreasing order of activity: 1l > 1a > 1e > 1f > 1k.

On the other hand, substitution in the para position of the aromatic moiety in the 3 position of DAVA backbone with methoxy, methyl or unsubstitution led to loss of GABABR agonist activity. These results are comparable with the previously published results of GABAB agonists. In addition, substitution of the aryl moiety in the 3 position of DAVA backbone with a methyl group led to complete loss of GABABR agonist activity as in compound 1i.

Compounds 1b, 1c, 1d, 1g, 1h, 1i and 1j which showed EC50 > 300 µM as GABABR agonists were evaluated as GABABR antagonists at 1mM concentration against 10 µM GABA. However, none of these compounds were effective as GABABR antagonists.
**Table 3**

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>n</th>
<th>R</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>pEC&lt;sub&gt;50&lt;/sub&gt; ± S.E.M</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&lt;sub&gt;a&lt;/sub&gt;</td>
<td>1</td>
<td>4-Chloro-phenyl</td>
<td>46</td>
<td>4.34 ± 0.1</td>
</tr>
<tr>
<td>1&lt;sub&gt;b&lt;/sub&gt;</td>
<td>1</td>
<td>2,4-Dichloro-phenyl</td>
<td>&gt;300</td>
<td>&lt;3.52</td>
</tr>
<tr>
<td>1&lt;sub&gt;c&lt;/sub&gt;</td>
<td>1</td>
<td>4-Methyl-phenyl</td>
<td>&gt;300</td>
<td>&lt;3.52</td>
</tr>
<tr>
<td>1&lt;sub&gt;d&lt;/sub&gt;</td>
<td>1</td>
<td>Phenyl</td>
<td>&gt;300</td>
<td>&lt;3.52</td>
</tr>
<tr>
<td>1&lt;sub&gt;e&lt;/sub&gt;</td>
<td>1</td>
<td>3,4-Dichloro-phenyl</td>
<td>130</td>
<td>3.89 ± 0.1</td>
</tr>
<tr>
<td>1&lt;sub&gt;f&lt;/sub&gt;</td>
<td>1</td>
<td>4-Fluoro-phenyl</td>
<td>170</td>
<td>3.77 ± 0.3</td>
</tr>
<tr>
<td>1&lt;sub&gt;g&lt;/sub&gt;</td>
<td>1</td>
<td>3-Methoxy-phenyl</td>
<td>&gt;300</td>
<td>&lt;3.52</td>
</tr>
<tr>
<td>1&lt;sub&gt;h&lt;/sub&gt;</td>
<td>1</td>
<td>4-Methoxy-phenyl</td>
<td>&gt;300</td>
<td>&lt;3.52</td>
</tr>
<tr>
<td>1&lt;sub&gt;i&lt;/sub&gt;</td>
<td>1</td>
<td>Methyl</td>
<td>&gt;300</td>
<td>&lt;3.52</td>
</tr>
<tr>
<td>1&lt;sub&gt;j&lt;/sub&gt;</td>
<td>-</td>
<td>-</td>
<td>&gt;300</td>
<td>&lt;3.52</td>
</tr>
<tr>
<td>1&lt;sub&gt;k&lt;/sub&gt;</td>
<td>0</td>
<td>4-Ethynyl-phenyl</td>
<td>240</td>
<td>3.62 ± 0.1</td>
</tr>
<tr>
<td>1&lt;sub&gt;l&lt;/sub&gt;</td>
<td>0</td>
<td>4-Iodo-phenyl</td>
<td>32</td>
<td>4.49 ± 0.1</td>
</tr>
<tr>
<td><strong>GABA</strong></td>
<td>0</td>
<td>H</td>
<td>0.84</td>
<td>6.08 ± 0.1</td>
</tr>
<tr>
<td><strong>(RS)-baclofen</strong></td>
<td>0</td>
<td>4-Chloro-phenyl</td>
<td>5.8</td>
<td>5.24 ± 0.1</td>
</tr>
</tbody>
</table>
Figure 16: Concentration-response curves of compounds 1a, 1e, and 1f (Figure A) and compounds 1k and 1l (Figure B) on wild type GABA<sub>B</sub>R1b co-expressed with GABA<sub>B</sub>R2 and the chimeric G protein G<sub>α</sub>q-z5. The curves are representative for the average pharmacological profile of the agonists. The Ca<sup>2+</sup> measurement assays were performed as described in the materials and methods section.


6 Molecular Modeling Studies

All molecular modeling studies were performed using SYBYL\(^{(179)}\) version 6.9 running on a Silicon Graphics Indigo2 (IRIX 6.5.20) and on a Linux Box\(^{(180)}\)(SUSE LINUX 8.1).

The human 3D model of the GABA\(_{B1}\) receptor extracellular domain used in our studies was kindly provided by J-P. Pin.\(^{(49)}\)

6.1 Docking

Computer Aided Molecular Design (CAMD) has become a focus of attention in assisting the molecular design of some novel drugs following the rapid development of computer technology. The x-ray crystallography of GABA\(_B\) receptors is not yet available, thus design of new ligands modulating GABA\(_B\) receptor activity based on direct docking is not possible. Therefore, we have decided to use the available 3D homology model of GABA\(_{B1}\) receptor extracellular domain to perform our docking studies.

Literature survey indicated that the GABA\(_B\) receptor is composed of two subunits, GABA\(_{B1}\) and GABA\(_{B2}\). These proteins possess two domains, a seven transmembrane core and an extracellular domain containing the ligand binding site.\(^{(48)}\) This binding domain, the so called Venus Flytrap Module (VFTM), is likely to fold like the bacterial periplasmic binding proteins that are constituted of two lobes that close upon ligand binding, like a Venus Flytrap when touched by an insect.\(^{(181)}\)

The 3D model used in our study (Figure 17) was constructed by structural alignment of the VFTM of Leucine/Isoleucine/Valine-binding protein (LIVBP, pdb code: 2liv), the negative regulator of amidase operon (Amic, pdb code: 1pea), metabotropic glutamate receptor 1 (mGlul, pdb code: 1ewk) and natriuretic peptide receptor (NPRA, pdb code: 1dp4 and NPRC, pdb code: 1jdn).\(^{(49)}\)

6.1.1 Docking using FlexX

Prediction of the binding modes of small flexible compounds to macromolecules is a problem of paramount importance in rational drug design. FlexX allows for fast docking of small ligands into protein active sites. The docking algorithm within FlexX is based on an incremental construction strategy, which consists of three phases:

1- Base selection: the first phase of the docking algorithm is the automatical selection of a connected part of the ligand, the base fragment (the ligand core).
2- Base placement: in the second phase, the base fragment is placed into the active site independently of the rest of the ligand.

3- Complex construction: in the last phase, called the construction phase, the ligand is constructed in an incremental way, starting with the different placements of the base fragment.

The conformational flexibility of the ligand is included by generating multiple conformations for each fragment and including all in the ligand building steps. Placement of the ligand is scored on the basis of protein-ligand interactions and the free binding energy $\Delta G$ of the protein-ligand complex is estimated in a way similar to that developed by Böhm$^{(182)}$. Finally the placements are ranked.

Our ligands, compounds 1a-l, were drawn within SYBYL and then minimized (Tripos Force Field, conjugate gradient, convergence = 0.05 Kcal/mol and Gasteiger-Hueckel charges). Our calculations were done only with the (R)-enantiomers of compounds 1a-l, as we know from our previous studies$^{(177)}$ that these enantiomers are active as GABA$_B$ agonists and (S)-enantiomers are completely inactive. The minimized compounds (R)-1a-l were added to a data base and saved as hitlist to be used by FlexX in the docking experiment.

We used the available 3D model containing the natural ligand, GABA, in its active site (Figure 17) to create the Receptor Description File (RDF). This file contains the

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**Figure 17a**: Three-dimensional model of GABA$_{B1}$ VFTM containing GABA in its active site.

**Figure 17b**: Ligand-binding pocket of the 3D model of GABA$_{B1}$ VFTM containing GABA.
information about the protein, its amino acids, the active site, non-amino acid residues and specific torsion angles. FlexX requires a definition of the active site of the protein. We chose all atoms of the protein with a distance of 6.5 Å from any atom of the natural ligand, GABA, to define the active site. Then FlexX was run with these parameters.

All solutions obtained by FlexX are summarized in Table 4. The third column (FlexX score) corresponds to the lowest predicted free binding energy of the protein-ligand complex which includes both polar (hydrogen bonding and charge-charge) and non-polar (hydrophobic) interactions. It is worth to mention that this FlexX score is a rough approximation of the free energy of binding but Böhm has shown that his scoring function is able to achieve predictions in good agreement with the experimentally observed energies for a wide set of examples. The forth column (G-Score) which is drawn from the work of Willett’s group using the hydrogen bonding, ligand-protein and internal (ligand-ligand) energies. The fifth column (PMF-Score, Potential Mean Force) which is drawn from the work of Muegge and Martin estimates the Helmholtz free energies of interactions for protein-ligand complex. The sixth column (D-Score) which is drawn from the work of Kuntz et al. estimates the charge and van der Waals interactions between the protein and the ligand. The seventh column (Chem-Score) which is based on the work of Eldridge et al. includes hydrogen bonding, lipophilic contact and rotational energies.

Table 4

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>pEC₅₀*</th>
<th>FlexX Score</th>
<th>G-Score</th>
<th>PMF-Score</th>
<th>D-Score</th>
<th>Chem-Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-1a</td>
<td>4.337</td>
<td>-15.0</td>
<td>-189.53</td>
<td>-29.87</td>
<td>-129.18</td>
<td>-19.38</td>
</tr>
<tr>
<td>(R)-1b</td>
<td>&lt;3.523</td>
<td>-15.4</td>
<td>-181.68</td>
<td>-25.01</td>
<td>-132.63</td>
<td>-25.89</td>
</tr>
<tr>
<td>(R)-1c</td>
<td>&lt;3.523</td>
<td>-13.7</td>
<td>-203.14</td>
<td>-30.06</td>
<td>-126.97</td>
<td>-19.16</td>
</tr>
<tr>
<td>(R)-1d</td>
<td>&lt;3.523</td>
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<td>-184.75</td>
<td>-31.15</td>
<td>-119.21</td>
<td>-22.14</td>
</tr>
<tr>
<td>(R)-1e</td>
<td>3.886</td>
<td>-14.3</td>
<td>-194.77</td>
<td>-30.49</td>
<td>-135.65</td>
<td>-24.28</td>
</tr>
<tr>
<td>(R)-1f</td>
<td>3.769</td>
<td>-14.3</td>
<td>-191.79</td>
<td>-16.92</td>
<td>-121.69</td>
<td>-22.15</td>
</tr>
<tr>
<td>(R)-1g</td>
<td>&lt;3.523</td>
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<td>-21.83</td>
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<td>-131.11</td>
<td>-23.08</td>
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<tr>
<td>(R)-1i</td>
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<td>-118.86</td>
<td>-14.77</td>
<td>-106.26</td>
<td>-15.16</td>
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<tr>
<td>1i</td>
<td>&lt;3.523</td>
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<td>-79.06</td>
<td>-24.69</td>
<td>-124.92</td>
<td>-24.62</td>
</tr>
<tr>
<td>(R)-1k</td>
<td>3.62</td>
<td>-14.3</td>
<td>-158.15</td>
<td>-29.96</td>
<td>-116.06</td>
<td>-17.20</td>
</tr>
<tr>
<td>(R)-1l</td>
<td>4.495</td>
<td>-13.8</td>
<td>-174.28</td>
<td>-30.69</td>
<td>-140.11</td>
<td>-17.86</td>
</tr>
<tr>
<td>(R)-baclofen</td>
<td>5.237</td>
<td>-14.7</td>
<td>-125.15</td>
<td>-36.18</td>
<td>-128.16</td>
<td>-15.69</td>
</tr>
<tr>
<td>Correlation</td>
<td>$R^2 = 0.1298$</td>
<td>$R^2 = 0.4359$</td>
<td>$R^2 = 0.4081$</td>
<td>$R^2 = 0.1759$</td>
<td>$R^2 = 0.3511$</td>
<td></td>
</tr>
</tbody>
</table>

* Biological data for racemates
We used the program Origin 4.10\(^{(188)}\) to evaluate the correlation between the experimentally determined pEC\(_{50}\) for the active compounds (pEC\(_{50}\) >3.523) and the estimated scoring functions. As illustrated in Table 4, we did not find a good correlation between the experimentally determined pEC\(_{50}\) and different scoring functions (R\(^2\) = 0.1298 – 0.4359).

After personal contact with Tripos, they advised us to rerun FlexX with a changed definition of the active site (radius 2.5 Å instead of 6.5 Å). Unfortunately, the results did not change significantly as illustrated in Table 5 (R\(^2\) = 0.0073 – 0.4514). These futile results persuaded us to use another docking program to find a good correlation between the estimated scoring function and the experimentally determined pEC\(_{50}\).

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>pEC(_{50})*</th>
<th>FlexX Score</th>
<th>G-Score</th>
<th>PMF-Score</th>
<th>D-Score</th>
<th>Chem-Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-I(_a)</td>
<td>4.337</td>
<td>-12.1</td>
<td>-187.27</td>
<td>-30.52</td>
<td>-139.07</td>
<td>-24.06</td>
</tr>
<tr>
<td>(R)-I(_b)</td>
<td>&lt;3.523</td>
<td>-8.5</td>
<td>-201.45</td>
<td>-40.03</td>
<td>-140.80</td>
<td>-20.63</td>
</tr>
<tr>
<td>(R)-I(_c)</td>
<td>&lt;3.523</td>
<td>-16.1</td>
<td>-177.64</td>
<td>-34.86</td>
<td>-149.28</td>
<td>-18.68</td>
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<tr>
<td>(R)-I(_d)</td>
<td>&lt;3.523</td>
<td>-14.5</td>
<td>-185.06</td>
<td>-35.19</td>
<td>-129.86</td>
<td>-22.74</td>
</tr>
<tr>
<td>(R)-I(_e)</td>
<td>3.886</td>
<td>-11.1</td>
<td>-201.29</td>
<td>-39.79</td>
<td>-134.11</td>
<td>-23.34</td>
</tr>
<tr>
<td>(R)-I(_f)</td>
<td>3.769</td>
<td>-15.6</td>
<td>-194.19</td>
<td>-31.27</td>
<td>-134.17</td>
<td>-21.05</td>
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<tr>
<td>(R)-I(_g)</td>
<td>&lt;3.523</td>
<td>-13.4</td>
<td>-203.33</td>
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<tr>
<td>(R)-I(_h)</td>
<td>&lt;3.523</td>
<td>-13.1</td>
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<td>-41.91</td>
<td>-132.15</td>
<td>-21.55</td>
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<tr>
<td>(R)-I(_i)</td>
<td>&lt;3.523</td>
<td>-10.7</td>
<td>-139.27</td>
<td>-22.13</td>
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<td>-16.10</td>
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<tr>
<td>(R)-I(_j)</td>
<td>&lt;3.523</td>
<td>-17.5</td>
<td>-132.22</td>
<td>-18.34</td>
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<td>-20.86</td>
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<td>(R)-I(_k)</td>
<td>3.62</td>
<td>-8.9</td>
<td>-174.31</td>
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<td>-24.51</td>
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<tr>
<td>(R)-I(_l)</td>
<td>4.495</td>
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<td>-35.01</td>
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<td>-23.10</td>
</tr>
<tr>
<td>(R)-baclofen</td>
<td>5.237</td>
<td>-7.9</td>
<td>-161.25</td>
<td>-37.31</td>
<td>-127.35</td>
<td>-22.26</td>
</tr>
</tbody>
</table>

| Correlation  | R\(^2\) = 0.2343 | R\(^2\) = 0.4514 | R\(^2\) = 0.0073 | R\(^2\) = 0.1935 | R\(^2\) = 0.0389 |

* Biological data for racemates

**6.1.2 Docking using FlexiDock**

As an aid in attempting to obtain structural insight into the possible binding mode and binding energies of the compounds (R)-I\(_{a-l}\) with the putative 3D model of the GABA\(_B\) receptor, we accomplished further docking studies using the program FlexiDock (encoded in SYBYL). FlexiDock works in torsional space, keeping bond lengths and angles constant. The large van der Waals interactions can only relax via bond rotation and the ligand optimization cannot alter chiral centers or bond stereochemistry.
FlexiDock docks flexible ligands into the protein binding sites to predict their bound conformation. It also allows flexibility of both the ligand and the receptor binding pocket during docking so that an induced fit between protein and ligand can be explored. FlexiDock is composed of two main components:

1) A genetic algorithm (GA) component which alters the ligand conformation to determine the optimum ligand geometry. The first step in geometry optimization of the ligand is to produce an initial population of chromosomes. Each chromosome consists of a number of genes, once for each parameter being optimized (the parameters in FlexiDock are torsional angles, translation and rotational angles). Evolution occurs by pairs of chromosomes exchanging genes (crossover) or by random changes in the values of individual genes (mutation). Duplicate checking is used to make each chromosome unique, which increases the solution’s diversity.

2) An energy evaluation function for scoring the resulting interaction. The FlexiDock scoring function is based on the Tripos force field and estimates the energy of the ligand, the receptor binding pocket and the interaction between them. By default, the score is the sum of the van der Waals and the user-selected energy terms (which include electrostatic, torsional, constraint and hydrogen bonding energies). A lower energy in the complexed state suggests better binding.

We defined all single bonds of the minimized compounds (\( R \)-1a-l) rotatable and used these structures in our docking study (FlexiDock). The binding pocket was defined by selecting all atoms within 10 Å radius from C₃ of the natural ligand (GABA). After binding pocket definition, the ligands were pre-positioned in the binding pocket and FlexiDock was run using the scoring function to be the sum of van der Waals, electrostatic and hydrogen bonding energies.

It has to be mentioned that the maximum number of generations (gen-limit) was set to 100,000 generations for all compounds (the default value is 3000 generations). A further increase in the generation number did not show a significant change in the estimated binding energy (BE) and conformation. Table 6 illustrates the estimated BE for compounds (\( R \)-1a-l).
Table 6

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>pEC$_{50}$*</th>
<th>BE (Kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-I$_a$</td>
<td>4.337</td>
<td>-757.1</td>
</tr>
<tr>
<td>(R)-I$_b$</td>
<td>&lt;3.523</td>
<td>-746.1</td>
</tr>
<tr>
<td>(R)-I$_c$</td>
<td>&lt;3.523</td>
<td>-725.2</td>
</tr>
<tr>
<td>(R)-I$_d$</td>
<td>&lt;3.523</td>
<td>-735.0</td>
</tr>
<tr>
<td>(R)-I$_e$</td>
<td>3.886</td>
<td>-750.5</td>
</tr>
<tr>
<td>(R)-I$_f$</td>
<td>3.769</td>
<td>-738.6</td>
</tr>
<tr>
<td>(R)-I$_g$</td>
<td>&lt;3.523</td>
<td>-721.0</td>
</tr>
<tr>
<td>(R)-I$_h$</td>
<td>&lt;3.523</td>
<td>-716.6</td>
</tr>
<tr>
<td>(R)-I$_i$</td>
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<tr>
<td>I$_j$</td>
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<td>-519.9</td>
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<tr>
<td>(R)-I$_k$</td>
<td>3.620</td>
<td>-748.0</td>
</tr>
<tr>
<td>(R)-I$_l$</td>
<td>4.495</td>
<td>-762.6</td>
</tr>
<tr>
<td>(R)-baclofen</td>
<td>5.237</td>
<td>-777.8</td>
</tr>
<tr>
<td>GABA</td>
<td>6.076</td>
<td>-982.6</td>
</tr>
</tbody>
</table>

* Biological data for racemates

We tried to get a correlation between the estimated BE of the (R)-enantiomers of the biologically active (pEC$_{50} >$3.523) aromatic analogues and homologues of baclofen from the synthesized series (RS)-I$_{a-1}$ and their experimentally determined pEC$_{50}$ using the program Origin 4.10. Although the binding energy is a direct measure for pIC$_{50}$, we found a good correlation between the experimentally determined pEC$_{50}$ and the estimated BE of the (R)-enantiomers of the biologically active compounds under investigation ($R^2 = 0.90395$, Table 7 and Figure 18). It has to be pointed out that the pEC$_{50}$, as a measure of the activity in functional assays, is directly proportional to pIC$_{50}$ which is a measure of affinity in binding assays for our type of compounds. Consequently, we could consider the estimated BE of our compounds under study is a direct measure for their experimentally determined pEC$_{50}$ in our performed functional assay.

Moreover, we calculated the BE of the (S)-enantiomers of the biologically active compounds. The estimated BE of (R)-enantiomers are better than that of (S)-enantiomers of the biologically active compounds. These results will support our assumption that the
activity of \((R)\)-enantiomers rather than \((S)\)-enantiomers of this type of compounds as GABA_B agonists (Table 7).

### Table 7

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>pEC&lt;sub&gt;50&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt;</th>
<th>BE&lt;sup&gt;b&lt;/sup&gt; (Kcal/mol)</th>
<th>BE&lt;sup&gt;c&lt;/sup&gt; (Kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&lt;sub&gt;a&lt;/sub&gt;</td>
<td>4.337</td>
<td>-757.1</td>
<td>-348.4</td>
</tr>
<tr>
<td>1&lt;sub&gt;e&lt;/sub&gt;</td>
<td>3.886</td>
<td>-750.5</td>
<td>-305.2</td>
</tr>
<tr>
<td>1&lt;sub&gt;f&lt;/sub&gt;</td>
<td>3.769</td>
<td>-738.6</td>
<td>-337.3</td>
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<td>3.620</td>
<td>-748.0</td>
<td>-352.5</td>
</tr>
<tr>
<td>1&lt;sub&gt;l&lt;/sub&gt;</td>
<td>4.495</td>
<td>-762.6</td>
<td>-145.4</td>
</tr>
<tr>
<td>baclofen</td>
<td>5.237</td>
<td>-777.8</td>
<td>-142.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Biological data for racemates.  
<sup>b</sup> BE for \((R)\)-enantiomers.  
<sup>c</sup> BE for \((S)\)-enantiomers.

\[ pEC_{50} = -0.042112548 \times \text{BE} - 27.60326 \]  

(Figure 18)

From equation 1 we can predict the pEC<sub>50</sub> for certain aromatic baclofen analogues and homologues before their synthesis. Furthermore, we made a regression analysis to calculate the cross validated R<sup>2</sup> for the correlation between the experimentally determined pEC<sub>50</sub> and the estimated BE using the program TSAR<sup>(189)</sup>. We obtained an acceptable cross validated R<sup>2</sup> = 0.7171 (Table 8 and Figure 19). This cross validated R<sup>2</sup> is a direct measure for the predictivity of our model.
Table 8

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Actual pEC$_{50}$*</th>
<th>Predicted pEC$_{50}$</th>
<th>Residual</th>
</tr>
</thead>
<tbody>
<tr>
<td>$(R)$-1$_a$</td>
<td>4.337</td>
<td>4.280</td>
<td>0.057</td>
</tr>
<tr>
<td>$(R)$-1$_e$</td>
<td>3.886</td>
<td>4.002</td>
<td>-0.116</td>
</tr>
<tr>
<td>$(R)$-1$_f$</td>
<td>3.769</td>
<td>3.501</td>
<td>0.268</td>
</tr>
<tr>
<td>$(R)$-1$_k$</td>
<td>3.62</td>
<td>3.897</td>
<td>-0.277</td>
</tr>
<tr>
<td>$(R)$-1$_l$</td>
<td>4.495</td>
<td>4.512</td>
<td>-0.017</td>
</tr>
<tr>
<td>$(R)$-baclofen</td>
<td>5.237</td>
<td>5.152</td>
<td>0.085</td>
</tr>
</tbody>
</table>

* Biological data for racemates

(Figure 19)

It has to be mentioned that the pEC$_{50}$ values of the racemates were used in all our statistical calculations. As mentioned earlier, the biological activity of our type of compounds is enantioselective (the $(R)$-enantiomer is the active one). To consider the case that the other enantiomer should be completely inactive, the EC$_{50}$ values of our compounds were divided by two (according to equations 2-5) and the model derivation was repeated.

\[
EC_{50} \text{ (} (R)\text{-enantiomer} = \frac{EC_{50} \text{ (racemate)}}{2} \quad \text{Eq. 2}
\]

\[
\log EC_{50} \text{ (} (R)\text{-enantiomer} = \log EC_{50} \text{ (racemate)} - \log 2 \quad \text{Eq. 3}
\]

\[
- \log EC_{50} \text{ (} (R)\text{-enantiomer} = - \log EC_{50} \text{ (racemate)} + \log 2 \quad \text{Eq. 4}
\]

\[
pEC_{50} \text{ (} (R)\text{-enantiomer} = pEC_{50} \text{ (racemate)} + 0.3 \quad \text{Eq. 5}
\]
We used equation 5 to calculate the pEC$_{50}$ of the (R)-enantiomers from the experimentally determined pEC$_{50}$ for the racemates. Then we did a new correlation with the estimated BE of (R)-enantiomers (Table 9 and Figure 20).

**Table 9**

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>pEC$_{50}$*</th>
<th>BE (Kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-1a</td>
<td>4.637</td>
<td>-757.1</td>
</tr>
<tr>
<td>(R)-1e</td>
<td>4.186</td>
<td>-750.5</td>
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<tr>
<td>(R)-1f</td>
<td>4.069</td>
<td>-738.6</td>
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<td>(R)-1k</td>
<td>3.920</td>
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<td>(R)-1l</td>
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<td>-762.6</td>
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<tr>
<td>(R)-baclofen</td>
<td>5.537</td>
<td>-777.8</td>
</tr>
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</table>

* The calculated value for (R)-enantiomers

(Figure 20)

\[
pEC_{50} = -0.042112559 \times \text{BE} - 27.303268 \\
R^2 = 0.903952
\]

It is obvious that there are only minor changes within the statistical parameters of equation 6 compared to equation 1. Therefore, we can use the model developed by using the original pEC$_{50}$ values of the racemates for the prediction of the biological activity of certain baclofen analogues and homologues.
In the same vein, we obtained the same cross validated $R^2 = 0.7171$ using the calculated $\text{pEC}_{50}$ for $(R)$-enantiomers (Table 10 and Figure 21) as that obtained by using the original $\text{pEC}_{50}$ values of the racemates.

**Table 10**

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Actual $\text{pEC}_{50}$*</th>
<th>Predicted $\text{pEC}_{50}$</th>
<th>Residual</th>
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</thead>
<tbody>
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<td>$(R)$-1a</td>
<td>4.637</td>
<td>4.580</td>
<td>0.057</td>
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<tr>
<td>$(R)$-1c</td>
<td>4.186</td>
<td>4.302</td>
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<td>$(R)$-1f</td>
<td>4.069</td>
<td>3.801</td>
<td>0.268</td>
</tr>
<tr>
<td>$(R)$-1k</td>
<td>3.920</td>
<td>4.197</td>
<td>-0.277</td>
</tr>
<tr>
<td>$(R)$-1l</td>
<td>4.795</td>
<td>4.812</td>
<td>-0.017</td>
</tr>
<tr>
<td>$(R)$-baclofen</td>
<td>5.537</td>
<td>5.452</td>
<td>0.085</td>
</tr>
</tbody>
</table>

* The calculated value for $(R)$-enantiomers

(Figure 21)

The putative binding modes of $(R)$-baclofen (as an example of the aromatic GABA analogue) and compound $(R)$-1a (as an example of aromatic GABA homologue) are illustrated in Figures 22 and 23, respectively (output from FlexiDock). The carboxylic acid moiety of $(R)$-baclofen is involved in a hydrogen bond network with the hydroxy groups of Ser 247, Ser 269 (corresponds to Ser 246 and Ser 268, respectively, in mouse 3D model of the GABA$_B_1$ receptor extracellular domain) and Tyr 367 (corresponds to Tyr 366 in mouse 3D model). The amino group of $(R)$-baclofen is involved in ionic and hydrogen bonding interactions with Glu 466 (corresponds to Glu 465 in mouse 3D model of the GABA$_B_1$ receptor) and Tyr 396 (corresponds to Tyr 395 in mouse 3D model).
Molecular Modeling Studies

Moreover, the aromatic group of (R)-baclofen is projected into a lipophilic cavity formed by Tyr 367 and Tyr 396.

Figure 22: The putative binding mode of (R)-baclofen.

Figure 23: The putative binding mode of (R)-1a.

This putative binding mode of (R)-baclofen was consistent with its binding mode published in 2001 by Costantino et al.\(^{44}\) One exception is the involvement of ASP 471 in the binding of the amino group of (R)-baclofen (instead of Glu 466). In addition, Costantino et al.\(^{44}\) did not report any role of Ser 269 in the reaction with the carboxylic acid moiety of (R)-baclofen. It is worth mentioning that the aromatic ring of (R)-baclofen may play the role of Ca\(^{2+}\), which is required to enforce the binding and activation of GABAB receptors by GABA.\(^{44}\)

Furthermore, the putative binding mode of (R)-1a is similar to that of (R)-baclofen with the absence of the hydrogen bonding between the carboxylic acid moiety of (R)-1a and both Ser 247 and Tyr 367. This might be the reason of weak activity of compound (RS)-1a (EC\(_{50}\) = 46 µM) as compared with that of (RS)-baclofen (EC\(_{50}\) = 5.8 µM).

The docked conformations of both (R)-baclofen and (R)-1a were minimized in the binding pocket using the conjugate gradient method implemented in SYBYL until the gradient convergence of 0.01 Kcal/mol was reached (Tripos force field, Gasteiger-Hueckel charges). In all cases, the ligand was completely flexible while the protein was completely static. It has to be pointed out that the minimized conformations of both
(R)-baclofen and (R)-1a did not change significantly from the docked conformations produced by FlexiDock.

In a search for the local minimum conformations of both (R)-baclofen and (R)-1a, we run a conformational analysis of both compounds using Multisearch feature in SYBYL with an energy cutoff of 10 Kcal/mol above the lowest energy conformer found in the search (maximum cycles = 2500). The results are summarized in Table 11. Increasing the maximum number of cycles to 10000 has no significant effect on the lowest energy conformer obtained in the search (Table 11). Therefore, we can assume that the obtained local minimum conformation of both compounds is near to each global minima.

Table 11

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Energya (Kcal/mol)</th>
<th>Energyb (Kcal/mol)</th>
<th>Energc (Kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-baclofen</td>
<td>-32.258</td>
<td>-36.6939</td>
<td>-36.6940</td>
</tr>
<tr>
<td>(R)-1a</td>
<td>-32.735</td>
<td>-40.5970</td>
<td>-40.5971</td>
</tr>
</tbody>
</table>

a Energy of the minimized docked conformations. b The lowest energy obtained with maximum number of cycles = 2500. c The lowest energy obtained with maximum number of cycles = 10000.

It is clear from Table 11 that the minimized docked conformation of (R)-baclofen has an energy value only 4.4 Kcal/mol above the local minimum energy conformation of (R)-baclofen and the minimized docked conformation of (R)-1a has an energy value only 7.9 Kcal/mol above the local minimum energy conformation of (R)-1a. The above mentioned results increase the possibility of the docked conformations to be the bioactive conformations for the compounds under study.

6.2 Pharmacophore definition

In an attempt to develop a ligand-binding model which could accommodate the array of compounds that have moderate to high affinity for the GABA_B receptor (if such a single model is possible), the pharmacophore mapping program DISCO (DIStance COmparisons, module within SYBYL) was used. Four compounds were included in our study: (R)-baclofen, (R)-43, (R)-45 and (R)-1a. Each compound represents a class of the biologically active GABA_B agonists.
To elucidate a common alignment model we established databases with representative low-energy conformers for each compound by using the Multisearch component of SYBYL (maximum cycles = 2500, energy cutoff = 10 Kcal/mol). This algorithm limits the large numbers of redundant conformers while at the same time adequately represents conformational space for the flexible molecules under investigation. Once the conformational databases were developed, a master database containing one conformer for each molecule was utilized as a starting point for the DISCO program.

In order to derive a putative pharmacophore model for our set of molecules, we followed the classical DISCO steps. The first step of the DISCO search routine consists of identifying all potential pharmacophoric site points in each molecule of the master database. These site point assignments include aromatic ring centroids and dummies for the carboxylic acid moiety and the amino group of the training set. Then these site points were transferred to every conformer in each of the search databases. Subsequently, we chose (R)-baclofen as reference for site point comparison.

DISCO compared the coordinates of the site points assigned to the reference conformer to the site point coordinates found in all of the other conformers for each compound database. The site point comparison process was then repeated for each reference conformer, resulting in the identification of one conformation for each analogue that could be fitted to the reference molecule within tolerance level 0.5 – 5.0 Å (the maximum allowable RMS (Root Mean Square) deviation for the fitted site points of each conformer to the reference).

The initial DISCO evaluation resulted in a total of 220 models, with one model associated with each conformer of the reference database. We exclude models with tolerance limit > 0.5 Å and the remained models were subjected to visual inspection. The refinement was done by introducing a distance map from the docked (R)-baclofen (Figure 24): distance between carboxylic function and amino nitrogen ($d_1 = 2.869$ Å), distance between carboxylic function and aromatic centroid ($d_2 = 3.900$ Å) and the distance between aromatic centroid and amino nitrogen ($d_3 = 4.472$ Å).
After refinement of the produced DISCO models, we obtained 9 models with minor differences in their statistical parameters (Table 12).

![Figure 24: Intramolecular distances of the docked (R)-baclofen by FlexiDock.](image)

### Table 12

<table>
<thead>
<tr>
<th>Model No.</th>
<th>Size&lt;sup&gt;a&lt;/sup&gt;</th>
<th>ndropped&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Fit score&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Dmean&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>3</td>
<td>0</td>
<td>0.158303</td>
<td>4.10</td>
</tr>
<tr>
<td>57</td>
<td>3</td>
<td>0</td>
<td>0.157091</td>
<td>4.11</td>
</tr>
<tr>
<td>121</td>
<td>3</td>
<td>0</td>
<td>0.156874</td>
<td>4.10</td>
</tr>
<tr>
<td>128</td>
<td>3</td>
<td>0</td>
<td>0.156378</td>
<td>4.12</td>
</tr>
<tr>
<td>133</td>
<td>3</td>
<td>0</td>
<td>0.171152</td>
<td>4.07</td>
</tr>
<tr>
<td>212</td>
<td>3</td>
<td>0</td>
<td>0.156173</td>
<td>4.10</td>
</tr>
<tr>
<td>214</td>
<td>3</td>
<td>0</td>
<td>0.156472</td>
<td>4.10</td>
</tr>
<tr>
<td>218</td>
<td>3</td>
<td>0</td>
<td>0.156921</td>
<td>4.10</td>
</tr>
<tr>
<td>220</td>
<td>3</td>
<td>0</td>
<td>0.156811</td>
<td>4.11</td>
</tr>
</tbody>
</table>

<sup>a</sup>Number of site points found.  
<sup>b</sup>Number of missed molecules.  
<sup>c</sup>RMS deviation of the feature coordinates of the conformers from those of reference.  
<sup>d</sup>The average inter-feature distance (Å).

Although the differences in the statistical parameters between DISCO models presented in Table 12 are not large and we did not find significant differences between the nine models by visual inspection, we chose model 212 to be the best model as it possesses the best fit score.

Moreover, further statistical parameters for model 212 (Figure 25) are presented in Table 13 and Table 14.
Figure 25: Pharmacophore model No. 212 derived by DISCO.

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Fit $^a$</th>
<th>Overlap $^b$</th>
<th>$\Delta E^c$</th>
<th>Tolerance $^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-baclofen</td>
<td>0</td>
<td>154.13</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(R)-43</td>
<td>0.1895</td>
<td>103.125</td>
<td>1.77</td>
<td>0.27</td>
</tr>
<tr>
<td>(R)-45</td>
<td>0.1775</td>
<td>107.375</td>
<td>5.67</td>
<td>0.12</td>
</tr>
<tr>
<td>(R)-1a</td>
<td>0.0759</td>
<td>111.250</td>
<td>1.98</td>
<td>0.12</td>
</tr>
</tbody>
</table>

$^a$ The fit is expressed as the RMS deviation of feature coordinates of the selected conformer from those of reference ((R)-baclofen).
$^b$ The overlap volume represents the intersection volume of the selected conformer with the reference compound.
$^c$ The energy difference (Kcal/mol) between the selected conformer and the lowest energy conformer found by a Multisearch analysis.
$^d$ The tolerance is a measure of the deviation of the distances between the features in the ligand and in the reference compound.
Table 14

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>d_1^a</th>
<th>d_2^b</th>
<th>d_3^c</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-baclofen</td>
<td>2.470</td>
<td>4.256</td>
<td>4.436</td>
</tr>
<tr>
<td>Docked (R)-baclofen</td>
<td>2.869</td>
<td>3.900</td>
<td>4.472</td>
</tr>
<tr>
<td>(R)-43</td>
<td>2.615</td>
<td>4.736</td>
<td>4.843</td>
</tr>
<tr>
<td>(R)-45</td>
<td>2.990</td>
<td>4.850</td>
<td>4.029</td>
</tr>
<tr>
<td>(R)-1_a</td>
<td>2.555</td>
<td>4.488</td>
<td>4.620</td>
</tr>
<tr>
<td>Docked (R)-1_a</td>
<td>2.916</td>
<td>5.222</td>
<td>5.347</td>
</tr>
</tbody>
</table>

^a The distance (Å) between the carboxylic function and amino nitrogen. ^b The distance (Å) between the carboxylic function and aromatic centroid. ^c The distance (Å) between aromatic centroid and amino nitrogen.

It has to be pointed out that visual inspection and distance map comparison mentioned in Table 14 for the selected DISCO model revealed that there are no significant differences between the minimized docked conformations for both (R)-baclofen and (R)-1_a and that produced by DISCO in model 212. These findings support our assumption that the docked conformations of (R)-baclofen and (R)-1_a might be the bio-active conformations.

One of the most useful tools in DISCO is to find out if additional compounds can fit to the produced pharmacophore model and hence predicting their activity. The synthesized compounds 1_a-l have been satisfied the pharmacophore requirements in the produced pharmacophore model with exception of compounds 1_i and 1_j. The inactivity of compounds 1_b, 1_c, 1_g and 1_h could be attributed to reasons out of the scope of DISCO such as steric hindrance which can preclude binding of these compounds to the binding pocket of GABA_{B} receptors. Whereas the inactivity of compound 1_d could be due to the absence of halogen substituent in the para position of the phenyl ring, this region seems to be sensitive to steric bulk.\textsuperscript{(72)}
Summary

Synthesis of \((RS\)-5-amino-3-aryl (methyl)-pentanoic acid hydrochlorides \((1_{a-i})\), 3-aminomethyl-5-chloro-benzoic acid hydrochloride \((1_j)\) and \((RS\)-4-amino-3-(4′-ethynyl (iodo)-phenyl)-butanoic acid hydrochlorides \((1_k \text{ and } 1_l)\) have been accomplished. The aim of their synthesis was to evaluate their GABA\(_B\)R agonist activity and to derive a model which will correlate their structure with the observed pEC\(_{50}\).

The GABA\(_B\)R agonist activity of the prepared compounds \(1_{a-i}\) has been determined in functional assay based on calcium measurement \textit{in vitro} using tsA cells transfected with GABA\(_B1\)/GABA\(_B2\)/Gaq-z5.

Reviews on the neurotransmitter receptors (ligand-gated ion channel receptors and G protein-coupled receptors), their agonists and antagonists have been given in the general part of this work.

A detailed discussion on the strategy followed for the synthesis of the designed compounds \(1_{a-i}\) as well as the starting materials and intermediates have been described and illustrated in Schemes 2-6.

The starting materials, intermediates and reagents which have been prepared throughout this work and reported in the literature are:

1- 2-Chloro-1-(4′-methyl-phenyl)-1-ethanone \((5_c)\)
2- Tetraethylammonium cyanide \((111)\).
3- (Methoxycarbonylmethylene)-triphenylphosphorane \((105)\).
4- \((E)\)-4-Cyano-3-(4′-chloro-phenyl)-2-buenoic acid ethyl ester \((E-3_a)\).
5- \((E)\)-3-(4′-Fluoro-phenyl)-2-butenoic acid ethyl ester \((E-5_l)\).
6- \((Z)\)-4-Bromo-3-(4′-fluoro-phenyl)-2-butenoic acid ethyl ester \((Z-4_l)\).
7- \((E)\)-3-(3′-Methoxy-phenyl)-2-butenoic acid ethyl ester \((E-5_g)\).
8- \((Z)\)-4-Bromo-3-(3′-methoxy-phenyl)-2-butenoic acid ethyl ester \((Z-4_g)\).
9- \((E)\)-3-(4′-Methoxy-phenyl)-2-butenoic acid ethyl ester \((E-5_h)\).
10- \((Z)\)-3-(4′-Methoxy-phenyl)-2-butenoic acid ethyl ester \((Z-5_h)\).
11- \((Z)\)-4-Bromo-3-(4′-methoxy-phenyl)-2-butenoic acid ethyl ester \((Z-4_h)\).
12- \((E)\)-4-Cyano-3-(4′-methoxy-phenyl)-2-butenoic acid ethyl ester \((E-3_h)\).
13- Cyano-acetic acid \((6_i)\).
14- 4-Cyano-3-methyl-2-butenoic acid ethyl ester \((5_i)\).
15- 5-Chloro-isophthalic acid \((8_j)\).
16- 5-Chloro-isophthalic acid dimethyl ester (7j).
17- 5-Chloro-isophthalic acid monomethyl ester (6j).
18- 3-Chloro-5-hydroxymethyl-benzoic acid methyl ester (5j).
19- 3-Bromomethyl-5-chloro-benzoic acid methyl ester (4j).
20- 4-Iodo-benzoyl chloride (10k).
21- (4-Iodo-phenyl)-methanol (9k).
22- 4-Iodo-benzaldehyde (8k).
23- (E)-3-(4'-Iodo-phenyl)-acrylic acid methyl ester ((E)-7k).
24- (RS)-4-(4'-Iodo-phenyl)-2-pyrrolidinone (5k).

This study comprises the preparation of the following new intermediates:

1- (E)-4-Chloro-3-(4' chloro-phenyl)-2-butenoic acid ethyl ester ((E)-4a).
2- (Z)-4-Chloro-3-(4' chloro-phenyl)-2-butenoic acid ethyl ester ((Z)-4a).
3- (RS)-5-Amino-3-(4' chloro-phenyl)-pentanoic acid ethyl ester hydrochloride (2a).
4- (E)-4-Chloro-3-(2',4' dichloro-phenyl)-2-butenoic acid ethyl ester ((E)-4b).
5- (Z)-4-Chloro-3-(2',4' dichloro-phenyl)-2-butenoic acid ethyl ester ((Z)-4b).
6- (E)-4-Cyano-3-(2',4' dichloro-phenyl)-2-butenoic acid ethyl ester ((E)-3b).
7- (RS)-5-Amino-3-(2',4' dichloro-phenyl)-pentanoic acid ethyl ester hydrochloride (2b).
8- (E)-4-Chloro-3-(4' methyl-phenyl)-2-butenoic acid ethyl ester ((E)-4c).
9- (Z)-4-Chloro-3-(4' methyl-phenyl)-2-butenoic acid ethyl ester ((Z)-4c).
10- (E)-4-Cyano-3-(4' methyl-phenyl)-2-butenoic acid ethyl ester ((E)-3c).
11- (RS)-5-Amino-3-(4' methyl-phenyl)-pentanoic acid ethyl ester hydrochloride (2c).
12- (RS)-5-Amino-3-phenyl-pentanoic acid ethyl ester hydrochloride (2d).
13- (E)-3-(3',4' dichloro-phenyl)-2-butenoic acid ethyl ester ((E)-5e).
14- (Z)-3-(3',4' dichloro-phenyl)-2-butenoic acid ethyl ester ((Z)-5e).
15- (Z)-4-Bromo-3-(3',4' dichloro-phenyl)-2-butenoic acid ethyl ester ((Z)-4e).
16- (E)-4-Cyano-3-(3',4' dichloro-phenyl)-2-butenoic acid ethyl ester ((E)-3e).
17- (RS)-5-Amino-3-(3',4' dichloro-phenyl)-pentanoic acid ethyl ester hydrochloride (2e).
18- (Z)-3-(4' Fluoro-phenyl)-2-butenoic acid ethyl ester ((Z)-5f).
19- (E)-4-Cyano-3-(4' fluoro-phenyl)-2-butenoic acid ethyl ester ((E)-3f).
20- (RS)-5-Amino-3-(4' fluoro-phenyl)-pentanoic acid ethyl ester hydrochloride (2f).
21- (Z)-3-(3'-Methoxy-phenyl)-2-butenoic acid ethyl ester ((Z)-5g).
22- (E)-4-Cyano-3-(3'-methoxy-phenyl)-2-butenoic acid ethyl ester ((E)-3g).
23-(RS)-5-Amino-3-(3’-methoxy-phenyl)-pentanoic acid ethyl ester hydrochloride (2g).
24-(RS)-5-Amino-3-(4’-methoxy-phenyl)-pentanoic acid ethyl ester hydrochloride (2h).
25-(RS)-5-Amino-3-methyl-pentanoic acid ethyl ester hydrochloride (4i).
26-(RS)-5-Amino-3-methyl-pentanoic acid hydrochloride (3i).
27-(RS)-5-Benzzyloxycarbonylamino-3-methyl-pentanoic acid (2i).
28-3-Azidomethyl-5-chloro-benzoic acid methyl ester (3j).
29-3-Azidomethyl-5-chloro-benzoic acid (2j).
30-(RS)-3-(4’-Iodo-phenyl)-4-nitro-butanoic acid methyl ester (6k).
31-(RS)-1-tert-Butyloxycarbonyl-4-(4’-iodo-phenyl)-2-pyrrolidinone (4k).
32-(RS)-1-tert-Butyloxycarbonyl-4-(4’-trimethylsilanylethynyl-phenyl)-2-pyrrolidinone (3k).
33-(RS)-4-tert-Butyloxycarbonylamino-3-(4’-ethynyl-phenyl)-butanoic acid (2k).
34-(RS)-4-tert-Butyloxycarbonylamino-3-(4’-iodo-phenyl)-butanoic acid (2l).

The structures of these new intermediates have been established through IR, 1H-NMR, and 13C-NMR.

The target compounds 1a-l are:
1-(RS)-5-Amino-3-(4’-chloro-phenyl)-pentanoic acid hydrochloride (1a).
2-(RS)-5-Amino-3-(2’,4’-chloro-phenyl)-pentanoic acid hydrochloride (1b).
3-(RS)-5-Amino-3-(4’-methyl-phenyl)-pentanoic acid hydrochloride (1c).
4-(RS)-5-Amino-3-phenyl-pentanoic acid hydrochloride (1d).
5-(RS)-5-Amino-3-(3’,4’-chloro-phenyl)-pentanoic acid hydrochloride (1e).
6-(RS)-5-Amino-3-(4’-fluoro-phenyl)-pentanoic acid hydrochloride (1f).
7-(RS)-5-Amino-3-(3’-methoxy-phenyl)-pentanoic acid hydrochloride (1g).
8-(RS)-5-Amino-3-(4’-methoxy-phenyl)-pentanoic acid hydrochloride (1h).
9-(RS)-5-Amino-3-methyl-pentanoic acid (1i).
10-3-Aminomethyl-5-chloro-benzoic acid hydrochloride (1j).
11-(RS)-4-Amino-3-(4’-ethynyl-phenyl)-butanoic acid hydrochloride (1k).
12-(RS)-4-Amino-3-(4’-iodo-phenyl)-butanoic acid hydrochloride (1l).

All the target compounds 1a-l gave compatible microanalytical combustion values. Their IR, 1H-NMR, 13C-NMR and mass spectral data were corresponding to the assigned structures.
Compounds 1a-d were synthesized from the corresponding ω-chloro-acetophenone derivatives 5a-c by employing Horner-Wadsworth-Emmons (HWE) reaction using sodium hydride in 1,2 dimethoxyethane to give mixtures of diasteroisomers 4a-c in which (Z)-isomers predominate. These diasteromeric mixtures were converted to the corresponding nitrile derivatives 3a-c through nucleophilic substitution reaction using tetraethylammonium cyanide in acetonitrile. These nitrile derivatives 3a-c were catalytically hydrogenated using PtO₂ (for compounds 2a and 2b) and/or Pd/C (for compounds 2c and 2d) in the presence of concentrated hydrochloric acid at 4 bar in Parr shaker apparatus to yield compounds 2a-d as hydrochlorides which were hydrolyzed in 5 N HCl to give the target compounds 1a-d in good yields (Scheme 2).

Compounds 1e-h were synthesized by adopting HWE reaction on the substituted acetophenones 6e-h using potassium tert-butoxide in tetrahydrofuran to give mixtures of diasteroisomers 5e-h in which (E)-isomers predominate. These mixtures were subjected to allylic bromination using NBS in CCl₄ and benzoyl peroxide as radical starter to give compounds 4e-h in moderate yields. These bromo derivatives 4e-h were converted to the target compounds 1e-h by following the synthetic sequence and reaction conditions identical with those described for the synthesis for compounds 1a-d (Scheme 3).

The aliphatic analogue of compounds 1a-h, compound 1i, was successfully achieved by adopting Knoevenagel reaction using cyano-acetic acid, ethyl acetoacetate, ammonium acetate and acetic acid in dry benzene. The produced diasteromeric mixture was subjected to catalytical hydrogenation followed by acid hydrolysis of the ester functionality to yield the target compound as hydrochloride salt. This hydrochloride salt was derivatized with a benzyloxy carbonyl moiety which facilitates its chemical purification and was finally cleaved by catalytical hydrogenation to yield the amino acid 1i in very pure form (Scheme 4).

The aromatic amino acid derivative 1j was prepared from 5-chloro-isophthalic acid 8j which was esterified followed by selective reduction of one carboxylic group to the corresponding alcohol 5j. This alcohol was brominated and subjected to nucleophilic substitution to afford the azide derivative 3j which was hydrolyzed and hydrogenated to give 1j in good yield (Scheme 5).

Compound 1k was synthesized from the aldehyde 8k which was subjected to Wittig reaction followed by nitromethane addition to yield the nitroester 6k which was reduced
using Zn/HCl and cyclized under basic condition to the pivotal lactam $5_k$. The amide nitrogen of $5_k$ was protected with the Boc functionality and underwent a Sonogashira-Hagihara coupling with trimethylsilylacetylene in the presence of Cu(I) and Pd(0) to afford the trimethylsilylacetylene derivative $3_k$ which gave the target compound after removal of the protecting groups and lactam ring opening (Scheme 6).

The iodo analogue of baclofen, compound $1_l$, was obtained directly from the lactam $5_k$ after lactam ring opening and deprotection of the $N$-boc functionality (Scheme 6).

Compounds $1_{a-l}$ were evaluated for their GABA$_B$R agonist activity. Furthermore, compounds $1_{a-l}$ were docked in the available 3D homology model of GABA$_B$R using the program FlexiDock implemented in SYBYL software. Subsequently, we derived a predictive model which correlates the experimentally determined pEC$_{50}$ with the calculated binding energy of certain baclofen analogues and homologues. In addition, we used the program DISCO (DIStan ce COmparisons) implemented in SYBYL software to find the pharmacophore features of GABA$_B$ agonists.
Zusammenfassung

Ziel dieser Arbeit war die Synthese von (RS)-5-Amino-3-aryl(methyl)-pentansäure Hydrochloride (1a-i), 3-Aminomethyl-5-chlor-benzolsäure Hydrochlorid (1j) und (RS)-4-Amino-3-(4'-ethynyl(jod)-phenyl)-butansäure Hydrochloride (1k-l) und die Testung der pharmakologischen Aktivität dieser Verbindungen.

Die synthetisierten Verbindungen 1a-l wurden als GABA-B-Rezeptor Agonisten, in einem auf Ca^{2+}-Messungen basierenden Funktional-Assay (in vitro tsA Zellen mit GABA_{B1b}/GABA_{B2}/Gaq-z5 transfektiert), getestet und daraus ein Struktur-Aktivitäts Modell abgeleitet.

Im allgemein Teil dieser Arbeit wird ein Überblick, über die Neurotransmitter-Rezeptoren (Liganden gesteuerte Ionen-Kanal-Rezeptoren und G Protein-gekoppelte Rezeptoren) des zentralen Nervensystems und deren Agonisten und Antagonisten, gegeben.

Eine ausführliche Diskussion zur Synthesestrategie der Verbindungen 1a-l, der Zwischenstufen und der Ausgangsmaterialien wird in den Schemata 2-6 beschrieben.

Die ω-Chloracetophenonderivate 5a-c wurden über eine HWE-Reaktion (Horner-Wadsworth-Emmons) mit Natriumhydrid in 1,2-Dimethoxyethan eingesetzt. Es wurden Diastereomerengemische von 4a-c mit jeweiligen Übergewicht des (Z)-Ismers erhalten.

Die Verbindungen 4a-c wurden mit Tetraethylammoniumcyanid in Acetonitril zu den Nitrilderivaten 3a-c umgewandelt. Die anschließende Hydrierung von 3a-c mit Wasserstoff, Pd/C oder PtO2 und Salzsäure bei 4 bar lieferte die Aminosäureethylester 2a-d als Hydrochloride. Diese wurden letztendlich zu den Zielverbindungen 1a-d hydrolysiert (Schema 2).


Die Knoevenagelreaktion mit Cyanessigsäure, Acetessigsäureethylester, Ammoniumacetat und Essigsäure in abs. Benzol lieferte ein Diastereomerengemisch 5i, welches durch Hydrierung mit Pd/C zu Verbindung 4i reduziert wurde. Diese wurde zur Aminosäure 3i als Hydrochlorid hydrolysiert. Derivatisierung von 3i zu 2i mit
Zusammenfassung

Benzylchloroformiat erleichterte die Abtrennung von Verunreinigungen durch Säure- bzw. Basenextraktion. Anschließend wurde \( \text{2}_i \) zur Zielaminosäure \( \text{1}_i \) hydriert (Schema 4).

5-Chlorisophthalsäure \( \text{8}_i \) ist das Ausgangsmaterial um ein aromatisches Aminosäure-Derivat herzustellen. \( \text{8}_j \) wurde zur Verbindung \( \text{7}_j \) verestert, welche durch selektive Reduktion zur Verbindung \( \text{5}_j \) reduziert wurde. Der Alkohol \( \text{5}_j \) wurde zum Bromderivat \( \text{4}_j \) umgewandelt, und anschließend Brom durch Azid substituiert (3j). Diese wurde zur Verbindung \( \text{2}_j \) hydrolysiert und letztendlich zu der Zielverbindung \( \text{1}_j \) hydriert (Schema 5).

Der Aldehyde \( \text{8}_k \) wurde über eine Wittigreaktion zu \( \text{7}_k \) umgesetzt und anschließend Nitromethan addiert um den Nitroester \( \text{6}_k \) zu erhalten. Reduktion von \( \text{6}_k \) mit Zn/HCl und basische Aufarbeitung lieferte unter Ringschluß das Laktam \( \text{5}_k \). Dieses wurde mit der Boc-Gruppe geschützt, mit Trimethylsilylacetylen gekoppelt (Sonogashira-Hagihara Kopplung) um Verbindung \( \text{3}_k \) zu gewinnen. Abspaltung der Boc-Schutzgruppe und Laktam-Ringöffnung von \( \text{3}_k \) zur Zielverbindung \( \text{1}_k \) bereitete hingegen keine Schwierigkeiten. Abspaltung der Boc-Schutzgruppe und Laktam-Ringöffnung von \( \text{5}_k \) lieferte in analoger Weise \( \text{1}_l \) (Schema 6).

Verbindungen \( \text{1}_a-l \) wurden als GABA\(_B\) Agonisten geprüft. Zusätzlich wurden \( \text{1}_a-l \) im 3D Homologie Model mit FlexiDock Programm gedockt. Daraus wurde ein Modell zur Voraussage der Aktivität von Analogen und Homologen des Baclofens abgeleitet. Letztendlich wurde ein Pharmakophor-Modell für GABA\(_B\) Agonisten mit DISCO (DIStance COmparisons) Programm erstellt.
8 Experimental Part

- All melting points were determined with büchi 510 capillary melting point apparatus and were uncorrected.

- Infrared (IR) spectra were recorded as thin layer films (for oils) or as pellets (for solids) with BIO-RAD spectrometer and values are represented in cm⁻¹.

- NMR (1H-NMR and 13C-NMR) spectra were carried out on AC 250 Bruker spectrophotometer (250 MHz for 1H-NMR and 63 MHz for 13C-NMR) and chemical shift values were recorded in ppm on δ scales. All samples were measured at room temperature. The 1H-NMR data are represented as follows: chemical shifts, multiplicity and number of protons.

- The mass spectra were run on Finnigan Mat 8200 spectrometer (70 eV for EI and 150 eV for CI (NH₃)).

- Elemental analyses were carried out at microanalysis laboratory, Institute of Inorganic Chemistry, University of Würzburg.

Silica gel 60 F₂₅₄ plates for TLC (E-Merck) were used for thin layer chromatography. Visualisation was performed by illumination with UV-light source (254 nm).

- Column chromatography was performed with silica gel 60 (0.063-0.200) for gravity columns. The solvent systems used in column and/or TLC elution were:

  (A) petroleum ether (40-60 °C) : diethyl ether (9 : 1).
  (B) petroleum ether (40-60 °C) : diethyl ether (8 : 2).
  (C) petroleum ether (40-60 °C) : diethyl ether (1 : 1).
  (D) petroleum ether (40-60 °C) : ethyl acetate : methanol (7 : 4 : 1).
2-Chloro-1-(4'-methyl-phenyl)- 1-ethanone ($5_c$)$^{(105)}$

![Image of molecule $5_c$]

To a cold (0 °C) suspension of aluminum chloride (5.70 g, 43 mmol) in 1,2 dichlorethane (20 ml) was added toluene (3.50 g, 4.00 ml, 38 mmol) in one portion. Chloroacetyl chloride (5.10 g, 3.60 ml, 45 mmol) was added dropwise to the reaction mixture over a period of 10 min. The reaction mixture was warmed to ambient temperature and stirred for further 4 h at the same temperature. The reaction was quenched by the slow addition of water (40 ml), the layers were separated, the organic layer was washed with 1 N hydrochloric acid (2 x 10 ml), water (2 x 10 ml), dried (Na$_2$SO$_4$), filtered and evaporated under reduced pressure. The residue was crystallized from 2-propanol to afford 4 g (63 %) of $5_c$ as a pale yellow powder m.p. 57-58 °C (lit.$^{(105)}$ 58.5-59 °C).

TLC of $5_c$: $R_f \sim 0.32$ using system (A).

IR (neat) of $5_c$: $\nu$ (cm$^{-1}$) = 1695, 1605, 1402, 1219, 1204.

$^1$H-NMR (CDCl$_3$) of $5_c$: $\delta$ (ppm) = 2.44 (s, 3H, 4'–CH$_3$), 4.70 (s, 2H, 2-H), 7.31 (d, J$_{AB} = 8.25$ Hz, 2H, H$_{arom.}$), 7.87 (d, J$_{AB} = 8.25$ Hz, 2H, H$_{arom.}$).

$^{13}$C-NMR (CDCl$_3$) of $5_c$: $\delta$ (ppm) = 22.2 (4'–CH$_3$), 46.4 (C-2), 129.0, 129.9, 132.2, 145.5 (C$_{arom.}$), 191.1 (C-1).

Tetraethylammonium cyanide (111)$^{(131)}$

![Image of tetraethylammonium cyanide (111)]
To a stirred solution of tetraethylammonium chloride (20 g, 121 mmol) in dry methanol (40 ml) was added a solution of sodium cyanide (10 g, 204 mmol) in dry methanol (300 ml) under nitrogen atmosphere. The resulting reaction mixture was filtered and the filtrate was evaporated under reduced pressure. Acetonitrile (200 ml) was added to the residue and the mixture was filtered. The filtrate was concentrated under vacuum till the crystals of the product started to appear, the mixture was cooled (0 °C) for 2 h, filtered and the solid was dried under vacuum to give 12 g (64%) of pure tetraethylammonium cyanide as a white crystals.

(Methoxycarbonylmethylene)-triphenylphosphorane (105)$^{(157)}$

(Wittig reagent)

\[
\text{O} \quad \text{CH}_2 \quad \text{PPh}_3
\]

To a cold (0 °C) solution of triphenylphosphine (50 g, 190 mmol) in toluene (100 ml) was added dropwise a solution of methyl bromoacetate (29.1 g, 190 mmol) in toluene (100 ml) during one hour. The reaction mixture was stirred for 18 h at ambient temperature and the precipitated solid was removed by filtration. The solid was dissolved in a mixture of water (500 ml) and toluene (300 ml) and the mixture was adjusted to pH = 9 using 2 N sodium hydroxide solution and phenolphthalein as indicator. The mixture was extracted with dichloromethane (3 x 300 ml), the combined organic layer was dried (Na$_2$SO$_4$) and concentrated to its half under reduced pressure. Petroleum ether (40-60 °C) was added dropwise to the organic layer, the precipitated solid was removed by filtration and dried under vacuum to give 51.9 g (82%) of (methoxycarbonylmethylene)-triphenylphosphorane as a white powder m.p.168-170 °C (lit.$^{(157)}$ 162-163 °C).

IR (neat) of 105: $\nu$ (cm$^{-1}$) = 1614, 1479, 1434, 1344, 1101.

$^1$H-NMR (CDCl$_3$) of 105: $\delta$ (ppm) = 2.94 (s, 1H, HC=P), 3.53 (s, 3H, OCH$_3$), 7.42-7.71 (m, 15H, H$_\text{arom}$).

$^{13}$C-NMR (CDCl$_3$) of 105: $\delta$ (ppm) = 29.1/31.3 (HC=P), 50.2 (OCH$_3$), 129.1, 129.3, 132.4, 133.3, 133.5 (C$_\text{arom}$), 172.1/172.2 (carbonyl ester).
General procedure for the preparation of
3-Aryl-4-chloro-2-butenoic acid ethyl esters (4a-c)

Triethyl phosphonoacetate (2.92 g, 13 mmol) was added dropwise to a cold (5–10 °C)
stirred slurry of 60 % sodium hydride (0.52 g, 13 mmol) in dry 1,2 dimethoxyethane (20 ml). After complete addition, the reaction mixture was stirred at ambient temperature for 30 min or until gas evolution was ceased. A solution of the appropriate ketones 5a-c (10 mmol) in dry 1,2 dimethoxyethane (10 ml) was added dropwise to the resulting solution. The reaction mixture was heated under stirring at 50 °C for 18 h. The reaction mixture was cooled to room temperature, poured into water (100 ml) and extracted with diethyl ether (3 x 50 ml). The organic extract was dried (Na$_2$SO$_4$), filtered and evaporated under vacuum to afford viscous oils which were purified by column chromatography using system (A) to give compounds 4a-c in 40-88 % yields (cf. Table 15).

TLC of (Z)-4a: R$_f$ ~ 0.39 using system (A).
IR (neat) of (Z)-4a: $\nu$ (cm$^{-1}$) = 1711, 1628, 1492, 1176, 1160.
$^1$H-NMR (CDCl$_3$) of (Z)-4a: $\delta$ (ppm) = 1.15 (t, J = 7.33 Hz, 3H, CH$_3$–CH$_2$–), 4.08 (q, J = 7.33 Hz, 2H, –CH$_2$–CH$_3$), 4.88 (s, 2H, 4-H), 6.03 (s, 1H, 2-H), 7.20 (d, J$_{AB}$ = 8.85 Hz, 2H, H$_{arom.}$), 7.30 (d, J$_{AB}$ = 8.85 Hz, 2H, H$_{arom.}$).
$^{13}$C-NMR (CDCl$_3$) of (Z)-4a: $\delta$ (ppm) = 14.6 (CH$_3$–CH$_2$–), 39.4 (C-4), 61.1 (–CH$_2$–CH$_3$), 121.0 (C-2), 128.5, 129.4, 136.2, 137.0 (C$_{arom.}$), 151.8 (C-3), 165.7 (C-1).

TLC of (E)-4a: R$_f$ ~ 0.21 using system (A).
IR (neat) of (E)-4a: $\nu$ (cm$^{-1}$) = 1720, 1651, 1491, 1225, 1163.
$^1$H-NMR (CDCl$_3$) of (E)-4a: $\delta$ (ppm) = 1.16 (t, J = 7.03 Hz, 3H, CH$_3$–CH$_2$–), 4.07 (q, J
1H-NMR (CDCl₃) of \((E)-4a\): \(\delta\) (ppm) = 1.14 (t, J = 7.03 Hz, 3H, \(\text{CH}_3\–\text{CH}_2–\)), 4.06 (q, J = 7.03 Hz, 2H, \(-\text{CH}_2–\text{CH}_3\)), 4.31 (d, J = 1.23 Hz, 2H, 4-H), 6.26 (t, J = 1.23 Hz, 1H, 2-H), 7.13-7.48 (m, 3H, Harom.).

13C-NMR (CDCl₃) of \((E)-4b\): \(\delta\) (ppm) = 14.3 (C\(\text{H}_3–\text{CH}_2–\)), 47.4 (C-4), 60.9 (\(-\text{CH}_2–\text{CH}_3\)), 123.4 (C-2), 127.4, 129.7, 130.9, 132.8, 134.8, 135.2, (Carom.), 149.3 (C-3), 164.8 (C-1).

TLC of \((E)-4c\): Rf ~ 0.27 using system (A).

IR (neat) of \((E)-4c\): \(\nu\) (cm\(^{-1}\)) = 1703, 1607, 1512, 1404, (Carom.), 153.0 (C-3), 166.0 (C-1).

TLC of \((Z)-4c\): Rf ~ 0.42 using system (A).

IR (neat) of \((Z)-4c\): \(\nu\) (cm\(^{-1}\)) = 1710, 1607, 1512, 1225, 1163.
Experimental Part

(t, J = 1.23 Hz, 1H, 2-H), 7.16 (d, J_AB = 8.25 Hz, 2H, H_arom.), 7.23 (d, J_AB = 8.25 Hz, 2H, H_arom.).

13C-NMR (CDCl₃) of (E)-4_c: δ (ppm) = 14.4 (CH₃–CH₂–), 21.8 (4’–CH₃), 48.9 (C-4), 120.5 (C-2), 127.9, 129.3, 134.2, 138.9 (C_arom.), 152.6 (C-3), 165.9 (C-1).

**Table 15**

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>X</th>
<th>E / Z ratio*</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>4_a</td>
<td>4-Chloro</td>
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<td>88</td>
</tr>
<tr>
<td>4_b</td>
<td>2,4-Dichloro</td>
<td>0.7</td>
<td>82</td>
</tr>
<tr>
<td>4_c</td>
<td>4-Methyl</td>
<td>0.1</td>
<td>40</td>
</tr>
</tbody>
</table>

*as detected by ¹H-NMR
Experimental Part

General procedure for the preparation of 3-Aryl-2-butenoic acid ethyl esters (5e-h)

To a cold (5-10 °C) solution of potassium t-butoxide (1.46 g, 13 mmol) in dry tetrahydrofuran (20 ml) was added dropwise triethyl phosphonoacetate (2.92 g, 13 mmol). The resulting solution was stirred at room temperature for 30 min. A solution of the appropriate ketones 6e-h (10 mmol) in dry tetrahydrofuran (10 ml) was added dropwise to the resulting solution. The reaction mixture was refluxed under stirring for 18 h. The reaction mixture was concentrated under vacuum, diluted with water (100 ml) and extracted with diethyl ether (3 x 50 ml). The combined organic extracts were dried (Na2SO4), filtered and evaporated under reduced pressure to give viscous oils which were purified by column chromatography using system (A) to afford compounds 5e-h in good yields (cf. Table 16).

TLC of (E)-5e: Rf ~ 0.47 using system (A).

IR (neat) of (E)-5e: \(\nu\) (cm\(^{-1}\)) = 1711, 1630, 1469, 1277, 1169.

\(^1\)H-NMR (CDCl\(_3\)) of (E)-5e: \(\delta\) (ppm) = 1.21 (t, \(J = 7.03\) Hz, 3H, \(\text{CH}_3-\text{CH}_2-\)), 2.42 (d, \(J = 1.23\) Hz, 3H, 4-H), 4.12 (q, \(J = 7.03\) Hz, 2H, –CH\(_2–\text{CH}_3\)), 5.99 (q, \(J = 1.23\) Hz, 1H, 2-H), 7.10-7.44 (m, 3H, H\(_\text{arom.}\)).

\(^13\)C-NMR (CDCl\(_3\)) of (E)-5e: \(\delta\) (ppm) = 14.7 (CH\(_3–\text{CH}_2–\)), 18.1 (C-4), 60.5 (–CH\(_2–\text{CH}_3\)), 118.8 (C-2), 125.9, 128.7, 130.8, 133.2, 133.4, 142.5 (C\(_\text{arom.}\)), 152.9 (C-3), 166.7 (C-1).

TLC of (Z)-5e: Rf ~ 0.33 using system (A).

IR (neat) of (Z)-5e: \(\nu\) (cm\(^{-1}\)) = 1717, 1644, 1472, 1229, 1165.

\(^1\)H-NMR (CDCl\(_3\)) of (Z)-5e: \(\delta\) (ppm) = 1.16 (t, \(J = 7.00\) Hz, 3H, \(\text{CH}_3–\text{CH}_2–\)), 2.17 (d, \(J = 1.53\) Hz, 3H, 4-H), 4.07 (q, \(J = 7.00\) Hz, 2H, –CH\(_2–\text{CH}_3\)), 5.96 (q, \(J = 1.53\) Hz, 1H,}
Experimental Part

1\textsuperscript{H}-NMR (CDCl\textsubscript{3}) of (Z)-5\textsubscript{f}: \(\delta\) (ppm) = 1.32 (t, J = 7.03 Hz, 3H, \text{CH}_3–\text{CH}_2–), 2.57 (d, J = 1.23 Hz, 3H, 4-H), 4.22 (q, J = 7.03 Hz, 2H, \text{–CH}_2–\text{CH}_3), 6.10 (q, J = 1.23 Hz, 1H, 2-H), 7.02-7.11 (m, 2H, H_{arom.}), 7.43-7.49 (m, 2H, H_{arom.}).

13C-NMR (CDCl\textsubscript{3}) of (Z)-5\textsubscript{f}: \(\delta\) (ppm) = 14.7 (C\text{H}_3–\text{CH}_2–), 18.4 (C-4), 60.3 (C\text{H}_2–\text{CH}_3), 115.3 (d, J_{C-3', F& C-5’, F} = 21.98 Hz, C-3’ and C-5’), 118.5 (C-2), 129.2 (d, J_{C-2', F& C-6’, F} = 7.60 Hz, C-2’ and C-6’), 136.0 (d, J_{C-1’, F} = 2.87 Hz, C-1’), 154.7 (C-3), 162.8 (d, J_{C-4’, F} = 247.41 Hz, C-4’), 166.2 (C-1).

TLC of (Z)-5\textsubscript{f}: \(R_f \sim 0.39\) using system (A).

IR (neat) of (Z)-5\textsubscript{f}: \(\nu\) (cm\textsuperscript{-1}) = 1718, 1638, 1603, 1509, 1226, 1153.

1\textsuperscript{H}-NMR (CDCl\textsubscript{3}) of (E)-5\textsubscript{g}: \(\delta\) (ppm) = 1.35 (t, J = 7.03 Hz, 3H, \text{CH}_3–\text{CH}_2–), 2.59 (d, J = 1.23 Hz, 3H, 4-H), 3.85 (s, 3H, OCH\textsubscript{3}), 4.25 (q, J = 7.03 Hz, 2H, \text{–CH}_2–\text{CH}_3), 6.16 (q, J = 1.23 Hz, 1H, 2-H), 6.19-7.34 (m, 4H, H_{arom.}).

13C-NMR (CDCl\textsubscript{3}) of (E)-5\textsubscript{g}: \(\delta\) (ppm) = 14.7 (C\text{H}_3–\text{CH}_2–), 18.4 (C-4), 55.7 (OCH\textsubscript{3}), 60.3 (–\text{CH}_2–\text{CH}_3), 112.5 (C-2), 114.7, 117.7, 119.2, 129.9, 144.2 (C_{arom.}), 155.8 (C-3), 160.0 (C_{arom.}), 167.2 (C-1).
Experimental Part

TLC of \((Z)-5_g\): Rf ~ 0.18 using system (A).

IR (neat) of \((Z)-5_g\): ν (cm\(^{-1}\)) = 1724, 1599, 1578, 1213, 1151.

\(^1\)H-NMR (CDCl\(_3\)) of \((Z)-5_g\): \(δ (ppm) = 1.13 (t, J = 7.00 Hz, 3H, \text{CH}_3–\text{CH}_2–), 2.20 (d, J = 1.53 Hz, 3H, 4-H), 3.83 (s, 3H, OCH\(_3\)), 4.04 (q, J = 7.00 Hz, 2H, \(-\text{CH}_2–\text{CH}_3\)), 5.93 (q, J = 1.53 Hz, 1H, 2-H), 6.77-7.33 (m, 4H, \text{H}_{arom.}).

\(^{13}\)C-NMR (CDCl\(_3\)) of \((Z)-5_g\): \(δ (ppm) = 14.4 (\text{CH}_3–\text{CH}_2–), 27.5 (\text{C}-4), 55.6 (\text{OCH}_3), 60.2 (\text{CH}_2–\text{CH}_3), 113.1 (\text{C}-2), 113.4, 118.3, 119.7, 129.4, 142.7 (\text{C}_{arom.}), 155.3 (\text{C}-3), 159.6 (\text{C}_{arom.}), 166.3 (\text{C}-1).

TLC of \((E)-5_h\): Rf ~ 0.25 using system (A).

IR (neat) of \((E)-5_h\): ν (cm\(^{-1}\)) = 1707, 1603, 1512, 1250, 1153.

\(^1\)H-NMR (CDCl\(_3\)) of \((E)-5_h\): \(δ (ppm) = 1.34 (t, J = 7.03 Hz, 3H, \text{CH}_3–\text{CH}_2–), 2.59 (d, J = 1.23 Hz, 3H, 4-H), 3.84 (s, 3H, OCH\(_3\)), 4.23 (q, J = 7.03 Hz, 2H, \(-\text{CH}_2–\text{CH}_3\)), 6.14 (q, J = 1.23 Hz, 1H, 2-H), 6.91 (d, \(J_{AB} = 8.85 Hz, 2H, \text{H}_{arom.}\)), 7.48 (d, \(J_{AB} = 8.85 Hz, 2H, \text{H}_{arom.}\)).

\(^{13}\)C-NMR (CDCl\(_3\)) of \((E)-5_h\): \(δ (ppm) = 14.8 (\text{CH}_3–\text{CH}_2–), 18.0 (\text{C}-4), 55.7 (\text{OCH}_3), 60.1 (\text{CH}_2–\text{CH}_3), 114.2 (\text{C}_{arom.}), 115.7 (\text{C}-2), 128.1, 134.7 (\text{C}_{arom.}), 155.2 (\text{C}-3), 160.8 (\text{C}_{arom.}), 167.5 (\text{C}-1).

TLC of \((Z)-5_h\): Rf ~ 0.14 using system (A).

IR (neat) of \((Z)-5_h\): ν (cm\(^{-1}\)) = 1711, 1606, 1511, 1229, 1156.

\(^1\)H-NMR (CDCl\(_3\)) of \((Z)-5_h\): \(δ (ppm) = 1.17 (t, J = 7.00 Hz, 3H, \text{CH}_3–\text{CH}_2–), 2.20 (d, J = 1.53 Hz, 3H, 4-H), 3.84 (s, 3H, OCH\(_3\)), 4.07 (q, J = 7.00 Hz, 2H, \(-\text{CH}_2–\text{CH}_3\)), 5.91 (q, J = 1.53 Hz, 1H, 2-H), 6.91 (d, \(J_{AB} = 8.85 Hz, 2H, \text{H}_{arom.}\)), 7.23 (d, \(J_{AB} = 8.85 Hz, 2H, \text{H}_{arom.}\)).

\(^{13}\)C-NMR (CDCl\(_3\)) of \((Z)-5_h\): \(δ (ppm) = 14.5 (\text{CH}_3–\text{CH}_2–), 27.5 (\text{C}-4), 55.6 (\text{OCH}_3), 60.1 (\text{CH}_2–\text{CH}_3), 113.6 (\text{C}_{arom.}), 117.5 (\text{C}-2), 128.9, 133.1 (\text{C}_{arom.}), 155.3 (\text{C}-3), 159.8 (\text{C}_{arom.}), 166.5 (\text{C}-1).
Experimental Part

Table 16

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>X</th>
<th>E / Z ratio*</th>
<th>Yield %</th>
</tr>
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<td>5e-h</td>
<td>3,4-Dichloro</td>
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<td>84</td>
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<tr>
<td>5f</td>
<td>4-Fluoro</td>
<td>7</td>
<td>79</td>
</tr>
<tr>
<td>5g</td>
<td>3-Methoxy</td>
<td>9</td>
<td>91</td>
</tr>
<tr>
<td>5h</td>
<td>4-Methoxy</td>
<td>19</td>
<td>75</td>
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</table>

* as detected by $^1$H-NMR.

General procedure for the preparation of

(Z)-3-Aryl-4-bromo-2-butenoic acid ethyl esters (4e-h)

A mixture of 3-aryl-2-butenoic acid ethyl esters 5e-h (9 mmol) and N-bromosuccinimide (1.69 g, 10 mmol) was refluxed with stirring. Benzoyl peroxide (0.02 g) was added to the reaction mixture and refluxing was continued for further 24 h. The reaction mixture
was chilled and the solid succinimide was filtered off. The filtrate was dried (Na$_2$SO$_4$), filtered and evaporated under reduced pressure to give viscous oils which were purified by column chromatography using system (A) to yield mainly (Z)-3-aryl-4-bromo-2-butenoic acid ethyl esters 4e-h as light brown viscous oils in moderate yields (cf. Table 17).

TLC of 4e: $R_f \sim 0.31$ using system (A).
IR (neat) of 4e: $\nu$ (cm$^{-1}$) = 1711, 1626, 1474, 1290, 1178.
$^1$H-NMR (CDCl$_3$) of 4e: $\delta$ (ppm) = 1.36 (t, $J = 7.03$ Hz, 3H, CH$_3$–CH$_2$–), 4.29 (q, $J = 7.03$ Hz, 2H, –CH$_2$–CH$_3$), 4.93 (s, 2H, 4-H), 6.19 (s, 1H, 2-H), 7.38-7.65 (m, 3H, H$_{arom.}$).
$^{13}$C-NMR (CDCl$_3$) of 4e: $\delta$ (ppm) = 14.6 (CH$_3$–CH$_2$–), 26.3 (C-4), 61.2 (–CH$_2$–CH$_3$), 121.4 (C-2), 126.3, 129.0, 131.2, 133.6, 134.3, 138.9 (C$_{arom.}$), 151.1 (C-3), 165.5 (C-1).

TLC of 4f: $R_f \sim 0.43$ using system (A).
IR (neat) of 4f: $\nu$ (cm$^{-1}$) = 1709, 1626, 1610, 1510, 1234, 1162.
$^1$H-NMR (CDCl$_3$) of 4f: $\delta$ (ppm) = 1.36 (t, $J = 7.03$ Hz, 3H, CH$_3$–CH$_2$–), 4.29 (q, $J = 7.03$ Hz, 2H, –CH$_2$–CH$_3$), 4.98 (s, 2H, 4-H), 6.19 (s, 1H, 2-H), 7.04-7.17 (m, 2H, H$_{arom.}$), 7.52-7.60 (m, 2H, H$_{arom.}$).
$^{13}$C-NMR (CDCl$_3$) of 4f: $\delta$ (ppm) = 14.6 (CH$_3$–CH$_2$–), 26.9 (C-4), 61.0 (–CH$_2$–CH$_3$), 116.3 (d, $J_{C-3', F} & C-5'; F = 21.57$ Hz, C-3’ and C-5’), 120.1 (C-2), 129.0 (d, $J_{C-2', F} & C-6'; F = 8.30$ Hz, C-2’ and C-6’), 134.9 (d, $J_{C-1', F} = 3.43$ Hz, C-1’), 152.5 (C-3), 163.9 (d, $J_{C-4', F} = 239.36$ Hz, C-4’), 165.9 (C-1).

TLC of 4g: $\sim 0.39$ using system (A).
IR (neat) of 4g: $\nu$ (cm$^{-1}$) = 1709, 1625, 1579, 1224, 1161.
$^1$H-NMR (CDCl$_3$) of 4g: $\delta$ (ppm) = 1.37 (t, $J = 7.00$ Hz, 3H, CH$_3$–CH$_2$–), 3.87 (s, 3H, OCH$_3$), 4.27 (q, $J = 7.00$ Hz, 2H, –CH$_2$–CH$_3$), 4.98 (s, 2H, 4-H), 6.23 (s, 1H, 2-H), 6.96-7.39 (m, 4H, H$_{arom.}$).
$^{13}$C-NMR (CDCl$_3$) of 4g: $\delta$ (ppm) = 14.6 (CH$_3$–CH$_2$–), 27.1 (C-4), 55.8 (OCH$_3$), 60.9 (–CH$_2$–CH$_3$), 112.9 (C-2), 115.5, 119.4, 120.4, 130.2, 140.4 (C$_{arom.}$), 153.5 (C-3), 160.2 (C$_{arom.}$), 165.9 (C-1).
TLC of 4h: ~ 0.62 using system (B).
IR (neat) of 4h: ν (cm⁻¹) = 1701, 1603, 1512, 1250, 1169.
¹H-NMR (CDCl₃) of 4h: δ (ppm) = 1.36 (t, J = 7.03 Hz, 3H, CH₃–CH₂–), 3.86 (s, 3H, OCH₃), 4.28 (q, J = 7.03 Hz, 2H, –CH₂–CH₃), 5.01 (s, 2H, 4-H), 6.21 (s, 1H, 2-H), 6.96 (d, JAB = 9.15 Hz, 2H, Harom.), 7.55 (d, JAB = 9.15 Hz, 2H, Harom.).
¹³C-NMR (CDCl₃) of 4h: δ (ppm) = 14.7 (CH₂–CH₂–), 26.8 (C-4), 55.8 (OCH₃), 60.8 (–CH₂–CH₃), 118.1 (C-2), 114.6, 128.4, 130.8 (Carom.), 152.3 (C-3), 161.4 (Carom.), 166.2 (C-1).

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<th>Compound No.</th>
<th>X</th>
<th>Yield %</th>
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<td>4e</td>
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<tr>
<td>4f (192)</td>
<td>4-Fluoro</td>
<td>67</td>
</tr>
<tr>
<td>4g a (195)</td>
<td>3-Methoxy</td>
<td>73</td>
</tr>
<tr>
<td>4h a,b (196)</td>
<td>4-Methoxy</td>
<td>71</td>
</tr>
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</table>

a) reaction time 6 h. b) m.p. 82-84 °C.
General procedure for the preparation of 
\((E)\)-3-Aryl-4-cyano-2-butenoic acid ethyl esters \((3_{a-c} \text{ and } 3_{e-h})\)

A solution of tetraethylammonium cyanide 111 (0.78 g, 5 mmol) in acetonitrile (5 ml) was added dropwise to a stirred solution of 3-aryl-4-chloro-2-butenoic acid ethyl esters 4_{a-c} and/or \((Z)\)-3-aryl-4-bromo-2-butenoic acid ethyl esters 4_{e-h} (5 mmol) in acetonitrile (10 ml) under nitrogen atmosphere. After complete addition, the reaction mixture was heated at 50 °C for 18 h. The reaction mixture was cooled, diluted with diethyl ether (30 ml) and washed with water (3 x 20 ml). The organic layer was dried (\(\text{Na}_2\text{SO}_4\)) and evaporated under reduced pressure to give dark red viscous oils which were purified by column chromatography using system (B) to afford mainly \((E)\)-3-aryl-4-cyano-2-butenoic acid ethyl esters 3_{a-c} and/or 3_{e-h} as pale yellow viscous oils in moderate yields (cf. Table 18).

TLC of 3_{a}: ~ 0.32 using system (B).
IR (neat) of 3_{a}: \(\nu\) (cm\(^{-1}\)) = 2217, 1731, 1591, 1493, 1176, 1162.
\(^1\)H-NMR (CDCl\(_3\)) of 3_{a}: \(\delta\) (ppm) = 1.21 (t, \(J = 7.03\) Hz, 3H, CH\(_3\)-CH\(_2\)-), 3.88 (s, 2H, 4-H), 4.15 (q, \(J = 7.03\) Hz, 2H, -CH\(_2\)-CH\(_3\)), 5.79 (s, 1H, 2-H), 7.39 (s, 4H, Harom.).
\(^{13}\)C-NMR (CDCl\(_3\)) of 3_{a}: \(\delta\) (ppm) = 14.4 (CH\(_3\)-CH\(_2\)-), 39.7 (C-4), 62.0 (CH\(_2\)-CH\(_3\)), 99.9 (C-2), 116.9 (C\(=\text{N}\)), 127.9, 129.9, 135.7, 137.1 (C\(=\text{arom.}\)), 154.9 (C-3), 168.8 (C-1).

TLC of 3_{b}: ~ 0.42 using system (B).
IR (neat) of 3_{b}: \(\nu\) (cm\(^{-1}\)) = 2223, 1733, 1585, 1472, 1180.
\(^1\)H-NMR (CDCl\(_3\)) of 3_{b}: \(\delta\) (ppm) = 1.26 (t, \(J = 7.03\) Hz, 3H, CH\(_3\)-CH\(_2\)-), 3.93 (s, 2H, 4-H), 4.16 (q, \(J = 7.03\) Hz, 2H, -CH\(_2\)-CH\(_3\)), 5.62 (s, 1H, 2-H), 7.26-7.49 (m, 3H, Harom.).
\(^{13}\)C-NMR (CDCl\(_3\)) of 3_{b}: \(\delta\) (ppm) = 14.4 (CH\(_3\)-CH\(_2\)-), 40.6 (C-4), 61.9 (CH\(_2\)-CH\(_3\)), 105.2 (C-2), 115.8 (C\(=\text{N}\)), 127.9, 130.3, 131.8, 132.8, 134.4, 136.1 (C\(=\text{arom.}\)), 155.4 (C-3), 168.4 (C-1).
TLC of 3c: ~ 0.28 using system (B).
IR (neat) of 3c: ν (cm\(^{-1}\)) = 2214, 1733, 1603, 1314, 1175, 1159.
\(^1\)H-NMR (CDCl\(_3\)) of 3c: δ (ppm) = 1.22 (t, J = 7.03 Hz, 3H, CH\(_3\)–CH\(_2\)–), 2.40 (s, 3H, 4′-CH\(_3\)), 3.90 (s, 2H, 4-H), 4.15 (q, J = 7.03 Hz, 2H, –CH\(_2\)–CH\(_3\)), 5.78 (s, 1H, 2-H), 7.23 (d, J\(_{AB}\) = 8.23 Hz, 2H, H\(_{arom.}\)). 7.38 (d, J\(_{AB}\) = 8.23 Hz, 2H, H\(_{arom.}\)).
\(^13\)C-NMR (CDCl\(_3\)) of 3c: δ (ppm) = 14.4 (C\(_\text{H}_3\)–CH\(_2\)–), 21.7 (4′-CH\(_3\)), 39.7 (C-4), 61.9 (–CH\(_2\)–CH\(_3\)), 98.3 (C-2), 117.5 (C≡N), 126.4, 130.1, 134.3, 141.4 (C\(_{arom.}\)), 155.9 (C-3 ), 169.1 (C-1).

TLC of 3e: ~ 0.24 using system (B).
IR (neat) of 3e: ν (cm\(^{-1}\)) = 2219, 1732, 1550, 1472, 1179.
\(^1\)H-NMR (CDCl\(_3\)) of 3e: δ (ppm) = 1.05 (t, J = 7.03 Hz, 3H, CH\(_3\)–CH\(_2\)–), 3.68 (s, 2H, 4-H), 3.98 (q, J = 7.03 Hz, 2H, –CH\(_2\)–CH\(_3\)), 5.61 (s, 1H, 2-H), 7.08-7.37 (m, 3H, Harom.).
\(^13\)C-NMR (CDCl\(_3\)) of 3e: δ (ppm) = 14.4 (CH\(_3\)–CH\(_2\)–), 39.6 (C-4), 62.2 (–CH\(_2\)–CH\(_3\)), 101.1 (C-2), 116.5 (C≡N), 125.8, 128.5, 131.4, 133.9, 135.2, 137.3, (C\(_{arom.}\)),153.9 (C-3), 168.5 (C-1).

TLC of 3f: ~ 0.67 using system (C).
IR (neat) of 3f: ν (cm\(^{-1}\)) = 2217, 1732, 1601, 1511, 1237, 1162.
\(^1\)H-NMR (CDCl\(_3\)) of 3f: δ (ppm) = 1.22 (t, J = 7.00 Hz, 3H, CH\(_3\)–CH\(_2\)–), 3.89 (s, 2H, 4-H), 4.16 (q, J = 7.00 Hz, 2H, –CH\(_2\)–CH\(_3\)), 5.76 (s, 1H, 2-H), 7.07-7.16 (m, 2H, H\(_{arom.}\)), 7.43-7.51 (m, 2H, H\(_{arom.}\)).
\(^13\)C-NMR (CDCl\(_3\)) of 3f: δ (ppm) = 14.4 (CH\(_3\)–CH\(_2\)–), 39.6 (C-4), 62.0 (–CH\(_2\)–CH\(_3\)), 99.4 (C-2), 116.5 (d, J\(_{C-3′, F\& C-5′, F} = 21.95\) Hz, C-3′ and C-5′), 117.1 (C≡N), 128.6 (d, J\(_{C-2′, F\& C-6′, F} = 8.57\) Hz, C-2′ and C-6′), 133.5 (d, J\(_{C-1′, F} = 3.82\) Hz, C-1′), 155.1 (C-3), 164.4 (d, J\(_{C-4′, F} = 252.23\) Hz, C-4′), 168.9 (C-1).

TLC of 3g: ~ 0.39 using system (C).
IR (neat) of 3g: ν (cm\(^{-1}\)) = 2216, 1733, 1599, 1577, 1229, 1177.
\(^1\)H-NMR (CDCl\(_3\)) of 3g: δ (ppm) = 1.22 (t, J = 7.03 Hz, 3H, CH\(_3\)–CH\(_2\)–), 3.84 (s, 3H, OCH\(_3\)), 3.89 (s, 2H, 4-H), 4.16 (q, J = 7.03 Hz, 2H, –CH\(_2\)–CH\(_3\)), 5.79 (s, 1H, 2-H), 6.97-7.37 (m, 4H, H\(_{arom.}\)).
\(^13\)C-NMR (CDCl\(_3\)) of 3g: δ (ppm) = 14.4 (CH\(_3\)–CH\(_2\)–), 39.8 (C-4), 55.8 (OCH\(_3\)), 61.9.
Experimental Part

(−CH₂−CH₃), 99.7 (C-2), 112.4, 116.2 (C₉H₈), 117.2 (C≡N), 118.9, 130.5, 138.7 (C₉H₈), 156.2 (C-3), 160.3 (C₉H₈), 168.9 (C-1).

TLC of 3₉: ~ 0.42 using system (C).

IR (neat) of 3₉: ν (cm⁻¹) = 2213, 1732, 1599, 1514, 1251, 1179.

1H-NMR (CDCl₃) of 3₉: δ (ppm) = 1.21 (t, J = 7.03 Hz, 3H, CH₃–CH₂–), 3.84 (s, 3H, OCH₃), 3.88 (s, 2H, 4-H), 4.15 (q, J = 7.03 Hz, 2H, −CH₂–CH₃), 5.72 (s, 1H, 2-H), 6.92 (d, J_AB = 8.85 Hz, 2H, H₉), 7.43 (d, J_AB = 8.85 Hz, 2H, H₉). 13C-NMR (CDCl₃) of 3₉: δ (ppm) = 14.4 (C₉H₈), 39.6 (C-4), 55.8 (OCH₃), 61.9 (−CH₂–CH₃), 96.9 (C-2), 117.7 (C≡N), 155.3 (C-3), 114.8, 128.1, 129.4, 161.9 (C₉H₈), 169.2 (C-1).

Table 18

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>X</th>
<th>Yield %</th>
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<tr>
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<td>3₉b</td>
<td>2,4-Dichloro</td>
<td>46</td>
</tr>
<tr>
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<tr>
<td>3₉e</td>
<td>3,4-Dichloro</td>
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<td>3₉f</td>
<td>4-Fluoro</td>
<td>48</td>
</tr>
<tr>
<td>3₉g</td>
<td>3-Methoxy</td>
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</tr>
<tr>
<td>3₉h⁽¹⁹⁷⁾</td>
<td>4-Methoxy</td>
<td>45</td>
</tr>
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General procedure for the preparation of
(RS)-5-Amino-3-aryl-pentanoic acid hydrochlorides (1a-h)

To a solution of (E)-3-aryl-4-cyano-2-butenoic acid ethyl esters 3a-c and/or 3e-h (2 mmol) in 95% ethanol (10 ml) and concentrated hydrochloric acid (1 ml) was added 10% Pd/C (0.10 g) for compounds 3b, c, g, h or PtO₂ (0.05 g) for compounds 3a, b, e, f. The mixture was hydrogenated on a Parr shaker apparatus under 4 bar of H₂ for 18 h at room temperature. The catalyst was removed by filtration and the solvent was evaporated under reduced pressure to give (RS)-5-amino-3-arylpentanoic acid ethyl ester hydrochlorides 2a-h which were dissolved in 5 N hydrochloric acid (15 ml) and washed with diethyl ether (2 x 10 ml). Without further purification, the aqueous layer was refluxed with stirring for 4 h. The reaction mixture was evaporated under vacuum to give (RS)-5-amino-3-aryl-pentanoic acid hydrochlorides 1a-h which were recrystallized from the appropriate solvents (cf. Table 19).

C₁₁H₁₅Cl₂NO₂ (264.15) of 1a: calcd. C 50.02, H 5.72, N 5.30; found C 49.93, H 5.72, N 5.36.

IR (neat) of 1a: ν (cm⁻¹) = 3200-2727 and 1726.

¹H-NMR (D₂O) of 1a: δ (ppm) = 1.81-2.05 (m, 2H, 4-H), 2.50-2.87 (m, 4H, 2-H and 5-H), 2.99-3.12 (m, 1H, 3-H), 7.17 (d, J₀ₐᵣᵐ = 8.55 Hz, 2H, H₀ₐᵣᵐ.), 7.26 (d, J₀ₐᵣᵐ = 8.55 Hz, 2H, H₀ₐᵣᵐ.).

¹³C-NMR (D₂O) of 1a: δ (ppm) = 33.2 (C-4), 38.0 (C-2), 39.1 (C-3), 41.1 (C-5), 129.2, 129.4, 132.7, 140.9 (C₀ₐᵣᵐ.), 176.6 (C-1).

MS (EI), m/z (%) of 1a: 209 (100), 181 (30), 138 (64), 97 (56), 43 (43).

MS (CI), m/z (%) of 1a: 227 [(100), M⁺].

C₁₁H₁₄Cl₃NO₂ (298.59) of 1b: calcd. C 44.25, H 4.73, N 4.69; found C 44.10, H 4.76, N 4.79.
IR (neat) of 1b: $\nu$ (cm$^{-1}$) = 3200-2700 and 1728.

$^1$H-NMR (D$_2$O) of 1b: $\delta$ (ppm) = 1.87-2.12 (m, 2H, 4-H), 2.57-2.98 (m, 4H, 2-H and 5-H), 3.58-3.70 (m, 1H, 3-H), 7.20-7.32 (m, 3H, H$_{arom.}$).

$^{13}$C-NMR (D$_2$O) of 1b: $\delta$ (ppm) = 32.6 (C-4), 35.0 (C-2), 37.8 (C-3), 39.7 (C-5), 128.3, 129.3, 129.7, 133.1, 134.6, 138.5 (C$_{arom.}$), 176.3 (C-1).

MS (EI), m/z (%) of 1b: 243 (37), 208 (72), 172 (49), 97 (100), 43 (46).

MS (CI), m/z (%) of 1b: 261 [(100), M$^+$ -1].

C$_{12}$H$_{18}$ClNO$_2$ (243.73) of 1c: calcd. C 59.14, H 7.44, N 5.75; found C 58.75, H 7.39, N 5.76.

IR (neat) of 1c: $\nu$ (cm$^{-1}$) = 3200-2720 and 1726.

$^1$H-NMR (D$_2$O) of 1c: $\delta$ (ppm) = 1.80-2.03 (m, 2H, 4-H), 2.19 (s, 3H, 4$^\prime$-CH$_3$), 2.50-2.86 (m, 4H, 2-H and 4-H), 2.96-3.08 (m, 1H, 3-H), 7.12 (s, 4H, H$_{arom.}$).

$^{13}$C-NMR (D$_2$O) of 1c: $\delta$ (ppm) = 20.5 (4$^\prime$-CH$_3$), 33.3 (C-4), 38.1 (C-2), 39.3 (C-3), 41.3 (C-5), 127.8, 129.9, 137.8, 139.2 (C$_{arom.}$), 176.9 (C-1).

MS (CI), m/z (%) of 1c: 207 [(100), M$^+$].

C$_{11}$H$_{16}$ClNO$_2$ (229.71) of 1d: calcd C 57.52, H 7.02, N 6.09; found C 57.12, H 7.13, N 5.99.

IR (neat) of 1d: $\nu$ (cm$^{-1}$) = 3200-2690 and 1724.

$^1$H-NMR (D$_2$O) of 1d: $\delta$ (ppm) = 1.83-2.06 (m, 2H, 4-H), 2.54-2.86 (m, 4H, 2-H and 5-H), 3.00-3.12 (m, 1H, 3-H), 7.18-7.33 (m, 5H, H$_{arom.}$).

$^{13}$C-NMR (D$_2$O) of 1d: $\delta$ (ppm) = 33.3 (C-4), 38.1 (C-2), 39.7 (C-3), 41.2 (C-5), 127.8, 129.4, 142.3 (C$_{arom.}$), 176.9 (C-1).

MS (EI), m/z (%) of 1d: 194 [(10) M$^{++}$ + 1], 175 (95), 104 (100), 91 (41), 43 (42).

C$_{11}$H$_{14}$Cl$_3$NO$_2$ (298.59) of 1e: calcd C 44.25, H 4.73, N 4.69; found C 44.04, H 4.99, N 4.72.

IR (neat) of 1e: $\nu$ (cm$^{-1}$) = 3200-2700 and 1715.

$^1$H-NMR (D$_2$O) of 1e: $\delta$ (ppm) = 1.81-2.05 (m, 2H, 4-H), 2.51-2.95 (m, 4H, 2-H and 5-H), 3.00-3.12 (m, 1H, 3-H), 7.09-7.39 (m, 3H, H$_{arom.}$).

$^{13}$C-NMR (D$_2$O) of 1e: $\delta$ (ppm) = 32.9 (C-4), 37.9 (C-2), 38.9 (C-3), 40.9 (C-5), 127.9, 129.8, 130.7, 131.1, 132.4, 142.9 (C$_{arom.}$), 176.4 (C-1).

MS (CI), m/z (%) of 1e: 261 [(100), M$^+$].
C_{11}H_{15}ClFNO_{2} (247.69) of 1f: calcd. C 53.34, H 6.10, N 5.66; found C 53.17, H 6.34, N 5.66.

IR (neat) of 1f: \( \nu \) (cm\(^{-1}\)) = 3200-2700 and 1724.

\(^1\)H-NMR (D\(_2\)O) of 1f: \( \delta \) (ppm) = 1.81-2.05 (m, 2H, 4-H), 2.49-2.87 (m, 4H, 2-H and 5-H), 3.00-3.12 (m, 1H, 3-H), 6.96-7.03 (m, 2H, H\(_{\text{arom.}}\)), 7.17-7.23 (m, 2H, H\(_{\text{arom.}}\)).

\(^{13}\)C-NMR (D\(_2\)O) of 1f: \( \delta \) (ppm) = 33.3 (C-4), 38.0 (C-2), 38.9 (C-3), 41.3 (C-5), 115.9 (d, J\(_{C-3', F\& C-5'} = 21.38 \text{ Hz, C-3' and C-5'}\)), 129.5 (d, J\(_{C-2', F\& C-6'} = 8.17 \text{ Hz, C-2' and C-6'}\)), 137.9 (d, J\(_{C-1', F = 3.02 \text{ Hz, C-1'}\})), 162.0 (d, J\(_{C-4', F = 242.87 \text{ Hz, C-4'}\})), 176.8 (C-1).

MS (CI), m/z (%) of 1f: 211 [(100), M\(^+\)].

C_{12}H_{18}ClNO_{3} (259.73) of 1g: calcd. C 55.49, H 6.99, N 5.39; found C 55.20, H 7.01, N 5.33.

IR (neat) of 1g: \( \nu \) (cm\(^{-1}\)) = 3200-2700 and 1722.

\(^1\)H-NMR (D\(_2\)O) of 1g: \( \delta \) (ppm) = 1.82-2.04 (m, 2H, 4-H), 2.52-2.87 (m, 4H, 2-H and 5-H), 2.98-3.10 (m, 1H, 3-H), 3.69 (s, 3H, OCH\(_3\)), 6.77-7.25 (m, 4H, H\(_{\text{arom.}}\)).

\(^{13}\)C-NMR (D\(_2\)O) of 1g: \( \delta \) (ppm) = 33.2 (C-4), 38.1 (C-2), 39.7 (C-3), 41.1 (C-5), 55.7 (OCH\(_3\)), 113.1, 113.6, 120.6, 130.6, 144.2, 159.6 (C\(_{\text{arom.}}\)), 176.8 (C-1).

MS (CI), m/z (%) of 1g: 223 [(100), M\(^+\)].

C_{12}H_{18}ClNO_{3} (259.73) of 1h: calcd. C 55.49, H 6.99, N 5.39; found C 55.23, H 7.07, N 5.35.

IR (neat) of 1h: \( \nu \) (cm\(^{-1}\)) = 3200-2721 and 1724.

\(^1\)H-NMR (D\(_2\)O) of 1h: \( \delta \) (ppm) = 1.79-2.03 (m, 2H, 4-H), 2.49-2.86 (m, 4H, 2-H and 5-H), 2.96-3.08 (m, 1H, 3-H), 3.68 (s, 3H, OCH\(_3\)), 6.86 (d, J\(_{AB} = 8.85 \text{ Hz, 2H, H\(_{\text{arom.}}\)}\)), 7.15 (d, J\(_{AB} = 8.85 \text{ Hz, 2H, H\(_{\text{arom.}}\)}\)).

\(^{13}\)C-NMR (D\(_2\)O) of 1h: \( \delta \) (ppm) = 33.4 (C-4), 38.1 (C-2), 38.9 (C-3), 41.4 (C-5), 55.8 (OCH\(_3\)), 114.7, 129.0, 134.8, 158.2 (C\(_{\text{arom.}}\)), 176.9 (C-1).

MS (CI), m/z (%) of 1h: 223 [(100), M\(^+\)].
### Table 19

<table>
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<tr>
<th>Compound No.</th>
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<th>Crystallization Solvent (m.p. °C)</th>
<th>Yield %</th>
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<td>2,4-Dichloro</td>
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<td>1c</td>
<td>4-Methyl</td>
<td>2-propanol (204-206)</td>
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<td>1d**</td>
<td>H</td>
<td>2-propanol (195-196)</td>
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<td>3,4-Dichloro</td>
<td>2-propanol / acetonitrile / ether (201-203)</td>
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<td>1g</td>
<td>3-Methoxy</td>
<td>2-propanol (182-184)</td>
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<tr>
<td>1h</td>
<td>4-Methoxy</td>
<td>2-propanol (194-195)</td>
<td>76</td>
</tr>
</tbody>
</table>

* prepared from (E)-3b using PtO₂.
**prepared from (E)-3b using Pd/C.
Cyano-acetic acid (6i)\(^{(144)}\)

\[ \begin{align*}
\text{HO} \quad \text{C=NN} \\
6_i
\end{align*} \]

A mixture of ethyl cyanoacetate 7\(_i\) (10 g, 88 mmol) and 1 N hydrochloric acid (35 ml) was heated at 100 °C for 1.5 h. The reaction mixture was evaporated under reduced pressure to give 7.5 g (100%) of 6\(_i\) as a colorless crystals m.p. 63-65 °C (lit.\(^{(144)}\) 66 °C) which was pure enough to be used in the next step without further purification.

IR (neat) of 6\(_i\): \(\nu\) (cm\(^{-1}\)) = 3300-2973, 2269, 1725, 1388, 1183.

\(^1\)H-NMR (DMSO-d\(_6\)) of 6\(_i\): \(\delta\) (ppm) = 3.28 (s, 2H, 2-H), 8.1-8.7 (br.s, 1H, COOH).

\(^{13}\)C-NMR (DMSO-d\(_6\)) of 6\(_i\): \(\delta\) (ppm) = 25.5 (C-2), 116.3 (C≡N), 166.5 (C-1).

4-Cyano-3-methyl-2-butenolic acid ethyl ester (5i)\(^{(139)}\)

\[ \begin{align*}
\text{Z} & \quad \text{5}_i \\
\text{E} & \quad \text{C}_2\text{H}_5\text{O} \quad \text{C}_2\text{H}_5\text{O} \quad \text{C}_2\text{H}_5\text{O} \quad \text{C}_2\text{H}_5\text{O} \\
\end{align*} \]

A mixture of cyano-acetic acid 6\(_i\) (4.51 g, 53 mmol), ethyl acetoacetate (6.51 g, 50 mmol), ammonium acetate (0.77 g, 10 mmol) and acetic acid (1.58 g, 1.5 ml, 26.3 mmol) in benzene (15 ml) was refluxed for 8 h using a Dean-Stark apparatus. The reaction mixture was evaporated under reduced pressure, water (10 ml) was added to the residue and extracted with diethyl ether (3 x 15 ml). The organic layer was separated, dried (Na\(_2\)SO\(_4\)) and evaporated under vacuum. The residue was distilled under vacuum to yield 5.2 g (68%) of 5\(_i\) as a colorless oil b.p. 100-102 °C/5mm (lit.\(^{(139)}\) 130 °C/20mm) with E/Z ratio = 1.7 as detected by \(^1\)H-NMR.

IR (neat) of 5\(_i\): \(\nu\) (cm\(^{-1}\)) = 2221, 1733, 1636, 1175, 1161.

\(^1\)H-NMR (CDCl\(_3\)) of 5\(_i\): \(\delta\) (ppm) = 1.24-1.31 (2 x t, 3H, CH\(_3\)-CH\(_2\)-), 2.01 (d, J = 1.53 Hz, 3H, (Z)-3-CH\(_3\)), 2.13 (d, J = 0.93 Hz, 3H, (E)-3-CH\(_3\)), 3.18 (d, J = 0.90 Hz, 2H, (E)- 4-H), 3.42 (s, 2H, (Z)-4-H), 4.12-4.22 (2 x q, 2H, –CH\(_2\)-CH\(_3\)), 5.29-5.32 (m, 1H, 2-H).
Experimental Part


(RS)-5-Benzylxycarbonylamino-3-methyl-pentanoic acid (2$_i$)

\[
\text{RS} \quad \text{5-Benzyloxycarbonylamino-3-methyl-pentanoic acid (2$_i$)}
\]

To a solution of 4-cyano-3-methyl-2-butenoic acid ethyl ester 5$_i$ (0.77 g, 5 mmol) in 95% ethanol (25 ml) was added concentrated hydrochloric acid (1 ml) and 10% Pd/C (0.26 g). The reaction mixture was hydrogenated on a Parr shaker apparatus under 4 bar of H$_2$ for 18 h at room temperature. The catalyst was removed by filtration and the solvent was evaporated under vacuum to give (RS)-5-amino-3-methyl-pentanoic acid ethyl ester hydrochloride 4$_i$ which was dissolved in 5 N hydrochloric acid (10 ml) and extracted with diethyl ether (3 x 10 ml). Without further purification the aqueous layer was refluxed under stirring for 4 h. The reaction mixture containing (RS)-5-amino-3-methyl-pentanoic acid hydrochloride 3$_i$ was cooled (0–5 °C) and basified using 4 N sodium hydroxide solution (14 ml). To this basic solution was added simultaneously in portions and under cooling (0 °C) benzyl chloroformate (0.85 g, 5 mmol) and 4 N sodium hydroxide solution (1.25 ml) during 30 min. The reaction mixture was extracted with diethyl ether (3 x 10 ml), the aqueous layer was cooled (0–5 °C) and acidified using concentrated hydrochloric acid. The reaction mixture was extracted with diethyl ether (3 x 10 ml), dried (Na$_2$SO$_4$) and evaporated under reduced pressure to give 0.86 g (65%) of 2$_i$ as a viscous pale yellow oil which was used in the next step without further purification.

IR (neat) of 2$_i$: ν (cm$^{-1}$) = 3066-2588, 1699, 1528, 1454, 1523.

$^1$H-NMR (CDCl$_3$) of 2$_i$: δ (ppm) = 1.02 (d, J = 6.1 Hz, 3H, 3-CH$_3$), 1.39-1.50 (m, 1H, 4-H$_a$), 1.53-1.67 (m, 1H, 4-H$_b$), 1.98-2.15 (m, 1H, 3-H), 2.21-2.55 (m, 2H, 2-H), 3.25 (m, 2H, 5-H), 5.03 (br.s 1H, N–H), 5.13 (s, 2H, –CH$_2$–C$_6$H$_5$), 7.37 (s, 5H, H$_{arom}$), 10.27 (br.s, 1H, COOH).
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Experimental Part

13C-NMR (CDCl3) of 2i: δ (ppm) = 19.9 (3-CH3), 27.9 (C-3), 36.8 (C-4), 39.3 (C-5), 41.7 (C-2), 67.2 (–CH2–C6H5), 127.5, 128.6, 128.9, 136.9 (C arom.), 157 (O=–N–H), 178.8 (C-1).

(RS)-5-Amino-3-methyl-pentanoic acid (1i)(138)

\[
\begin{array}{c}
\text{O} \\
\text{CH}_3 \\
\text{NH}_3 \\
\end{array}
\]

To a solution of (RS)-5-benzyloxycarbonylamino-3-methyl-pentanoic acid 2i (0.53 g, 2 mmol) in 50% 2-propanol (10 ml) was added 10% Pd/C (0.85 g). The reaction mixture was hydrogenated on a Parr shaker apparatus under 4 bar of H2 for 18 h at room temperature. The catalyst was removed by filtration and the solvent was evaporated under vacuum. The residue was recrystallized (2-propanol/water) to give 0.18 g (69%) of 1i as a white powder m.p. 164-165 °C (lit.(138) 133-135 °C).

C6H13NO2 (131.17) of 1i: calcd. C 54.94, H 9.99, N 10.68; found C 54.64, H 10.11, N 10.60.

IR (neat) of 1i: ν (cm⁻¹) = 3019-2659, 1630, 1528, 1460, 1398.

1H-NMR (D2O) of 1i: δ (ppm) = 0.78 (d, J = 6.73 Hz, 3H, 3-CH3), 1.31-1.58 (m, 2H, 4-H), 1.71-1.85 (m, 1H, 3-H), 1.87-1.96 (m, 1H, 2-Ha), 2.02-2.10 (m, 1H, 2-Hb), 2.77-2.95 (m, 2H, 5-H).

13C-NMR (CDCl3) of 1i: δ (ppm) = 19.1 (3-CH3), 28.5 (C-3), 33.9 (C-4), 37.9 (C-5), 44.7 (C-2), 181.9 (C-1).

MS (CI), m/z (%) of 1i: 149.1 [(100), M⁺+18].

5-Chloro-isophthalic acid (8j)(146)

\[
\begin{array}{c}
\text{Cl} \\
\text{HOOC} \\
\text{8j} \\
\text{COOH} \\
\end{array}
\]

Sodium nitrite (2.10 g, 30 mmol) was added portionwise to stirred concentrated sulfuric acid (22 ml) over a period of 10-15 min at ambient temperature. After the addition was
completed, the temperature was raised to 70 °C and the mixture was stirred until all the sodium nitrite was dissolved. The resulting solution was added dropwise to a cold (5-10 °C) stirred suspension of 5-amino-isophthalic acid 9j (5 g, 28 mmol) in glacial acetic acid (60 ml). After the addition was completed, the solution was stirred at 40 °C for 30 min. The resulting diazonium salt was added in portions over a period of 5 min to a cold (0-5 °C) stirred solution of cuprous chloride (5.60 g, 57 mmol) in concentrated hydrochloric acid (60 ml). After complete addition, the mixture was heated to 80 °C under stirring for 20 min. An equal volume of water was then added and the mixture was cooled in an ice bath. After 2 h, the white crystals were filtered, washed with water and dried under vacuum to give 4.54 g (82 %) of 8j as a white crystals, m.p. 278-280 °C (lit.(146) 277 °C) which was pure enough to be used in the next step without further purification.

IR (neat) of 8j: ν (cm⁻¹) = 3982-2523, 1693, 1605, 1574, 1400, 1271.

¹H-NMR (DMSO-d₆) of 8j: δ (ppm) = 8.06 (d, J = 1.53 Hz, 2H, Hₐrom.), 8.35 (t, J =1.53 Hz, 1H, Hₐrom.).

¹³C-NMR (DMSO-d₆) of 8j: δ (ppm) = 129.2, 133.6, 134.1, 134.6 (Cₐrom.), 166.2 (carbonyl acid).

5-Chloro-isophthalic acid dimethyl ester (7j)¹⁵⁰

A mixture of 5-chloro-isophthalic acid 8j (2.05 g, 10 mmol) and concentrated sulfuric acid (0.52 ml) in methanol (40 ml) was refluxed with stirring for 24 h. The reaction mixture was evaporated under reduced pressure, the residue was dissolved in dichloromethane (25 ml) and washed with saturated sodium bicarbonate solution (2 x 15 ml). The organic layer was dried (Na₂SO₄), evaporated under vacuum to give 2.15 g (92 %) of 7j as a white powder m.p. 76-78 °C which was used in the next step without further purification.

TLC of 7j: Rₜ ~ 0.57 using system (B).

IR (neat) of 7j: ν (cm⁻¹) = 1731, 1582, 1431, 1312, 1248.
$^1$H-NMR (DMSO-d$_6$) of 7j: $\delta$ (ppm) = 3.90 (s, 6H, 2 x CH$_3$–O–), 8.04 (d, $J = 1.23$ Hz, 2H, H$_{arom.}$), 8.24 (t, $J = 1.23$ Hz, 1H, H$_{arom.}$).

$^{13}$C-NMR (DMSO-d$_6$) of 7j: $\delta$ (ppm) = 53.3 (CH$_3$–O–), 128.7, 132.8, 133.7, 134.9 (C$_{arom.}$), 164.9 (carbonyl ester).

5-Chloro-isophthalic acid monomethyl ester (6j)\(^{(150)}\)

![5-Chloro-isophthalic acid monomethyl ester (6j)](image)

1 N NaOH (8.00 ml, 8 mmol) was added to a solution of 5-chloro-isophthalic acid dimethyl ester 7j (2.06 g, 9 mmol) in methanol (75 ml). The reaction mixture was stirred at ambient temperature for 18 h. The solvent was removed under reduced pressure, the residue was dissolved in saturated sodium bicarbonate solution (25 ml) and extracted with dichloromethane (2 x 10 ml). The aqueous layer was cooled (5-10 °C), acidified using concentrated hydrochloric acid and extracted with dichloromethane (3 x 20 ml). The combined organic layers were dried (Na$_2$SO$_4$), evaporated under vacuum to give 1.49 g (77 %) of 6j as a white powder m.p. 172-174 °C which was pure enough to be used in the next step.

IR (neat) of 6j: $\nu$ (cm$^{-1}$) = 3085-2539, 1736, 1703, 1602, 1580, 1309, 1262.

$^1$H-NMR (DMSO-d$_6$) of 6j: $\delta$ (ppm) = 3.91 (s, 3H, CH$_3$–O–), 8.04-8.09 (2 x dd, 2H, H$_{arom.}$), 8.32 (t, $J = 1.23$ Hz, 1H, H$_{arom.}$).

$^{13}$C-NMR (DMSO-d$_6$) of 6j: $\delta$ (ppm) = 53.6 (CH$_3$–O–), 128.9, 132.8, 133.3, 133.9, 134.2, 134.8 (C$_{arom.}$), 165.1 (carbonyl ester), 165.9 (carbonyl acid).
3-Chloro-5-hydroxymethyl-benzoic acid methyl ester (5j)

To a solution of 5-chloro-isophthalic acid monomethyl ester 6j (1.07 g, 5 mmol) in tetrahydrofuran (15 ml) was added dropwise borane-dimethyl sulfide complex (1.01 ml, 10.0 mmol). The reaction mixture was stirred at ambient temperature for 30 h. Methanol (20 ml) was added dropwise and carefully to the reaction mixture and stirring was continued for further 30 min. The reaction mixture was evaporated under reduced pressure, the residue was dissolved in dichloromethane (15 ml) and washed with saturated sodium bicarbonate solution (2 x 10 ml). The organic layer was dried (Na₂SO₄), evaporated under vacuum to afford 0.87g (87 %) of 5j as a pale yellow oil which was used in the next step without further purification.

TLC of 5j: Rf ~ 0.22 using system (C).

IR (neat) of 5j: ν (cm⁻¹) = 3600-3150, 1719, 1582, 1433, 1285.

¹H-NMR (CDCl₃) of 5j: δ (ppm) = 2.88 (br. s, 1H, –OH), 3.69 (s, 3H, CH₃–O–), 4.46 (s, 2H, –CH₂–OH), 7.29 (s, 1H, Hₐrom.), 7.61 (s, 1H, Hₐrom.), 7.64 (t, J = 1.53 Hz, 1H, Hₐrom.).

¹³C-NMR (CDCl₃) of 5j: δ (ppm) = 52.9 (CH₃–O–), 64.2 (–CH₂–OH), 126.2, 128.9, 131.5, 132.1, 135.0, 143.7 (Cₐrom.), 166.4 (carbonyl ester).

3-Bromomethyl-5-chloro-benzoic acid methyl ester (4j)

Phosphorous tribromide (0.87 ml, 9 mmol) was added dropwise to a cold (-30 °C), stirred
Experimental Part

3-chloro-5-hydroxymethyl-benzoic acid methyl ester 5j (0.60 g, 3 mmol). The reaction mixture was allowed to warm to room temperature and stirred for further 30 min at ambient temperature. The reaction mixture was carefully poured into ice-cold water (20 ml) and extracted with diethyl ether (3 x 15 ml). The combined ether extracts were dried (Na₂SO₄), evaporated under reduced pressure and the residue was purified by column chromatography using system (B) to give 0.47 g (60 %) of 4j as a white powder m.p. 56-58 °C.

TLC of 4j: Rf ~ 0.49 using system (B).

IR (neat) of 4j: v (cm⁻¹) = 1717, 1578, 1448, 1429, 1291.

¹H-NMR (CDCl₃) of 4j: δ (ppm) = 3.96 (s, 3H, CH₃–O–), 4.48 (s, 2H, –CH₂–Br), 7.59 (t, J = 1.83 Hz, 1H, Hₐrom.), 7.96 (d, J = 1.83 Hz, 2H, Hₐrom.).

¹³C-NMR (CDCl₃) of 4j: δ (ppm) = 31.6 (–CH₂–Br), 52.9 (CH₃–O–), 128.7, 129.9, 132.7, 133.7, 135.2, 140.4 (Cₐrom.), 165.7 (carbonyl ester).

3-Azidomethyl-5-chloro-benzoic acid methyl ester (3j)

Sodium azide (1.30 g, 20 mmol) was added to a solution of 3-bromomethyl-5-chloro-benzoic acid methyl ester 4j (0.79 g, 3 mmol) in acetone (15 ml) and water (4 ml). The reaction mixture was refluxed for 18 h. The solvent was removed under reduced pressure, the residue was dissolved in dichloromethane (20 ml) and washed with water (3 x 10 ml). The organic layer was dried (Na₂SO₄) and evaporated under vacuum. The residue was purified by column chromatography using system (B) to afford 0.58 g (86%) of 3j as a pale yellow oil.

TLC of 3j: Rf ~ 0.40 using system (B).

IR (neat) of 3j: v (cm⁻¹) = 2098, 1723, 1582, 1433, 1283.

¹H-NMR (CDCl₃) of 3j: δ (ppm) = 3.96 (s, 3H, CH₃–O–), 4.43 ( s, 2H, –CH₂–N=), 7.53 (t, J = 1.53 Hz, 1H, Hₐrom.), 7.89 (s, 1H, Hₐrom.), 7.99 (t, J = 1.53 Hz, 1H, Hₐrom.).
Experimental Part

$^{13}$C-NMR (CDCl$_3$) of 3$_j$: $\delta$ (ppm) = 52.9 (CH$_3$–O–), 54.1 (–CH$_2$–N=), 127.6, 129.8, 132.6, 132.7, 135.4, 138.3 (C$_{arom}$), 165.8 (carbonyl ester).

3-Azidomethyl-5-chloro-benzoic acid (2$_j$)

![3-Azidomethyl-5-chloro-benzoic acid (2$_j$)](image)

1 N NaOH (2.00 ml, 2 mmol) was added to a solution of 3-azidomethyl–5-chloro-benzoic acid methyl ester 3$_j$ (0.23 g, 1 mmol) in methanol (15 ml). The reaction mixture was refluxed for 2.5 h. The solvent was removed under reduced pressure, the residue was dissolved in saturated sodium bicarbonate solution (10 ml) and extracted with dichloromethane (2 x 5 ml). The aqueous layer was cooled (0-5 °C), acidified with concentrated hydrochloric acid and extracted with dichloromethane (3 x 10 ml). The combined organic extracts were dried (Na$_2$SO$_4$) and evaporated under vacuum to give 0.18 g (83%) of 2$_j$ as a pale yellow powder m.p. 92-94 °C and was used in the next step without further purification.

C$_8$H$_6$ClN$_3$O$_2$ (211.61) of 2$_j$: calcd. C 45.41, H 2.86, N 19.86; found C 45.33, H 2.95, N 19.46.

IR (neat) of 2$_j$: $\nu$ (cm$^{-1}$) = 3093-2406, 2097, 1687, 1603, 1583, 1410, 1295.

$^1$H-NMR (CDCl$_3$) of 2$_j$: $\delta$ (ppm) = 4.47 (s, 2H, –CH$_2$–N=), 7.59 (t, J = 1.83 Hz, 1H, H$_{arom}$), 7.96 (s, 1H, H$_{arom}$), 8.06 (t, J = 1.83 Hz, 1H, H$_{arom}$), 11.8 (br.s, 1H, COOH).

$^{13}$C-NMR (CDCl$_3$) of 2$_j$: $\delta$ (ppm) = 54.0 (–CH$_2$–N=), 128.1, 130.4, 131.8, 133.6, 135.6, 138.5 (C$_{arom}$), 171.2 (carbonyl acid).

3-Aminomethyl-5-chloro-benzoic acid hydrochloride (1$_j$)

![3-Aminomethyl-5-chloro-benzoic acid hydrochloride (1$_j$)](image)
To a solution of 3-azidomethyl-5-chloro-benzoic acid 2j (0.21 g, 1 mmol) in 95% 2-propanol (10 ml) and concentrated hydrochloric acid (0.50 ml) was added PtO₂ (0.04 g). The mixture was hydrogenated on a Parr shaker apparatus under 4 bar of H₂ for 18 h at ambient temperature. The catalyst was removed by filtration and the solvent was evaporated under vacuum. The residue was dissolved in water (10 ml) and extracted with dichloromethane (2 x 25 ml). The aqueous layer was evaporated under reduced pressure and the residue was recrystallized (2-propanol /diethyl ether) to afford 0.18 g (82%) of 1j as a white hygroscopic powder m.p. 260-263 °C.

C₈H₉Cl₂NO₂ (222.07) of 1j: calcd. C 43.27, H 4.09, N 6.31; found C 43.49, H 4.23, N 6.28.

IR (neat) of 1j: ν (cm⁻¹) = 3137-2887, 1709, 1607, 1581, 1395, 1207.

¹H-NMR (D₂O) of 1j: δ (ppm) = 4.13 (s, 2H, –CH₂–NH₂), 7.59 (t, J = 1.83 Hz, 1H, Hₐrom.), 7.81 (t, J = 1.83 Hz, 2H, Hₐrom.).

¹³C-NMR (D₂O) of 1j: δ (ppm) = 42.6 (–CH₂–NH₂), 128.6, 130.5, 132.9, 133.9, 135.0, 135.2 (Cₐrom.), 168.9 (carbonyl acid).

MS (Cl), m/z (%) of 1j: 203 [(100), M⁺ + 18].

(4-Iodo-phenyl)-methanol (9k)(153)

A mixture of 4-iodo-benzoic acid 11k (4.22 g, 17 mmol) and thionyl chloride (3.09 g, 1.9 ml, 26 mmol) in tetrahydrofuran (20 ml) was refluxed for one hour. The solvent was removed under reduced pressure to give 4.53 g (100%) of 4-iodo-benzoyl chloride 10k as a pale yellow solid m.p. 61-63 °C (lit.(198) 65-66 °C). The crude 10k (4.53 g, 17 mmol) was dissolved in dioxane (15 ml) and was added dropwise to a cold (0 °C) stirred suspension of sodium borohydride (0.99 g, 26 mmol) in dioxane (15 ml) over 30 min. The reaction mixture was heated at 100 °C for 90 min, cooled (0-5 °C) and water (15 ml) was
cautiously added. The resulting reaction mixture was extracted with dichloromethane (3 x 20 ml). The combined organic extracts were washed with 1 N hydrochloric acid (2 x 15 ml), water (2 x 15 ml), dried (Na₂SO₄) and evaporated under reduced pressure. The residue was purified by column chromatography using system (C) to give 2.91 g (73%) of 9k as a white powder m.p. 70-72 °C (lit.¹⁵³ 61-66.5 °C).

TLC of 9k: R$_f$~ 0.22 using system (C).

IR (neat) of 9k: ν (cm$^-1$) = 3260-3055, 1584, 1482, 1399, 997.

$^1$H-NMR (CDCl$_3$) of 9k: δ (ppm) = 2.40 (s, 1H, –OH), 4.60 (s, 2H, –CH$_2$–C$_6$H$_4$), 7.09 (d, $J_{AB}$ = 8.23 Hz, 2H, H$_{arom.}$), 7.69 (d, $J_{AB}$ = 8.53 Hz, 2H, H$_{arom.}$).

$^{13}$C-NMR (CDCl$_3$) of 9k: δ (ppm) = 64.9 (–CH$_2$–C$_6$H$_4$), 93.4, 129.2, 137.9, 140.8 (C$_{arom.}$).

4-Iodo-benzaldehyde (8k)¹⁵³

\[
\begin{align*}
\text{H} & \quad \text{I} \\
\text{O} & \quad \text{C} \\
\text{H} & \quad \text{H}
\end{align*}
\]

A solution of (4-iodo-phenyl)-methanol 9k (3.51 g, 15 mmol) in dichloromethane (25 ml) was added to a suspension of pyridinium dichromate (8.66 g, 23 mmol) in dichloromethane (50 ml). The reaction mixture was stirred vigorously for 2.5 h (TLC monitoring). The reaction mixture was diluted with diethyl ether (60 ml) and filtered through a pad of celite and a pad of silica gel. The filtrate was evaporated under vacuum and the residue was purified by column chromatography using system (A) to afford 2.9 g (83%) of 8k as a white powder m.p. 75-76 °C (lit.¹⁵³ 71-73.5 °C).

TLC of 8k: R$_f$~ 0.58 using system (C).

IR (neat) of 8k: ν (cm$^-1$) = 1684, 1654, 1580, 1377, 802.

$^1$H-NMR (CDCl$_3$) of 8k: δ (ppm) = 7.61 (d, $J_{AB}$ = 8.23 Hz, 2H, H$_{arom.}$), 7.93 (d, $J_{AB}$ = 8.23 Hz, 2H, H$_{arom.}$), 9.98 (s, 1H, O=C–H).

$^{13}$C-NMR (CDCl$_3$) of 8k: δ (ppm) = 103.2, 131.2, 135.9, 138.8 (C$_{arom.}$), 191.8 (carbonyl aldehyde).
(E)-3-(4′-Iodo-phenyl)-acrylic acid methyl ester (7k)\(^{(155)}\)

To a solution of (methoxycarbonylmethylene)-triphenylphosphorane 105 (3.35 g, 10 mmol) in dry dichloromethane (10 ml) was added dropwise a solution of 4-iodo-benzaldehyde 8k (2.32 g, 10 mmol) in dichloromethane (5 ml). The resulting pale yellow solution was stirred at room temperature for one hour (NMR monitoring). The reaction mixture was evaporated under vacuum and the residue was purified by column chromatography using system (A) to yield 2.68 g (93%) of 7k as a pale yellow powder m.p. 128-130 °C.

TLC of 7k: \( R_f \approx 0.56 \) using system (C).

C\(_{10}\)H\(_9\)IO\(_2\) (288.08) of 7k: calcd. C 41.69, H 3.15; found C 41.29, H 3.15.

IR (neat) of 7k: \( \nu \) (cm\(^{-1}\)) = 1711, 1636, 1437, 1310, 1189.

\(^1\)H-NMR (CDCl\(_3\)) of 7k: \( \delta \) (ppm) = 3.83 (s, 3H, –O–CH\(_3\)), 6.74 (d, \( J = 16.18 \) Hz, 1H, 2-H), 7.27 (d, \( J_{AB} = 8.53 \) Hz, 2H, H\(_{arom.}\)), 7.63 (d, \( J = 16.15 \) Hz, 1H, 3-H), 7.75 (d, \( J_{AB} = 8.55 \) Hz, 2 H, H\(_{arom.}\)).

\(^{13}\)C-NMR (CDCl\(_3\)) of 7k: \( \delta \) (ppm) = 52.2 (–O–CH\(_3\)), 96.9 (C\(_{arom.}\)), 118.9 (C-2), 129.9, 134.3, 138.5 (C\(_{arom.}\)), 144.0 (C-3), 167.5 (C-1).
To a stirred solution of \((E)-3-(4^-\text{ido-phenyl})-\text{acrylic acid methyl ester} \ 7k\) (2 g, 7 mmol) in nitromethane (14 ml) was added Triton B (0.6 ml). The resulting pale yellow solution was heated at 85 °C for two hours. The reaction mixture was cooled (0-5 °C), acidified using 1 N hydrochloric acid and evaporated under reduced pressure. The residue was dissolved in diethyl ether (20 ml) and washed with water (2 x 10 ml). The organic layer was dried (Na₂SO₄), evaporated under vacuum and the residue was purified by column chromatography using system (C) to give 2.18 g (89%) of \(6k\) as a white powder m.p. 58-60 °C.

TLC of \(6k\): \(R_f \approx 0.33\) using system (C).

\(C_{11}H_{12}INO_4\) (349.12) of \(6k\): calcd. C 37.84, H 3.46, N 4.01; found C 37.80, H 3.38, N 3.71.

IR (neat) of \(6k\): \(\nu\) (cm\(^{-1}\)) = 1730, 1721, 1561, 1536, 1374.

\(^1\)H-NMR (CDCl₃) of \(6k\): \(\delta\) (ppm) = 2.77 (2 x dd, 2H, 2-H), 3.66 (s, 3H, –O–CH₃), 3.96 (m, 1H, 3-H), 4.63 (dd, \(J_{4a-3} = 8.25\) Hz, \(J_{\text{gem}} = 12.83\) Hz, 1H, 4-Hₐ), 4.74 (dd, \(J_{4b-3} = 6.7\) Hz, \(J_{\text{gem}} = 12.83\) Hz, 1H, 4-Hₐ), 7.01 (d, \(J_{\text{AB}} = 8.25\) Hz, 2H, \(H_{\text{arom.}}\)), 7.69 (d, \(J_{\text{AB}} = 8.53\) Hz, 2H, \(H_{\text{arom.}}\)).

\(^{13}\)C-NMR (CDCl₃) of \(6k\): \(\delta\) (ppm) = 37.7 (C-2), 40.1 (C-3), 52.5 (–O–CH₃), 79.4 (C-4), 94.1, 129.7, 138.4, 138.6 (\(C_{\text{arom.}}\)), 171.2 (C-1).
**Experimental Part**

**RS**-4-(4'-Iodo-phenyl)-2-pyrrolidinone (5k)

To a cold (0 °C) mixture of concentrated hydrochloric acid (1.5 ml) and methanol (1.5 ml) was added simultaneously in portions both zinc dust (0.46 g, 7 mmol) and a solution of **RS**-3-(4'-iodo-phenyl)-4-nitro-butanoic acid methyl ester 6k (0.25 g, 0.7 mmol) in methanol (3 ml) through 30 min. The reaction mixture was further stirred for 30 min at 0 °C. The reaction mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was dissolved in water (10 ml), extracted with diethyl ether (3 x 5 ml). The aqueous layer was basified with 6 N sodium hydroxide solution (15 ml), extracted with diethyl ether (3 x 20 ml). The combined organic extracts were dried (Na₂SO₄) and evaporated under vacuum to afford 0.13 g (65%) of 5k as a white powder m.p. 144-145 °C (lit.(158) 143-145 °C) which was pure enough to be used in the next step without further purification.

**TLC** of 5k: Rf ~ 0.33 using system (D).

**IR** (neat) of 5k: ν (cm⁻¹) = 3181, 1703, 1477, 1285, 1267.

H-NMR (CDCl₃) of 5k: δ (ppm) = 2.28 (dd, J₃a-4 = 8.55 Hz, J_gem = 16.78 Hz, 1H, 3-Ha), 2.77 (dd, J₃b-4 = 8.85 Hz, J_gem = 16.78 Hz, 1H, 3-Hb), 3.41 (dd, J₅a-4 = 7 Hz, J_gem = 9.15 Hz, 1H, 5-H), 3.66 (quin, J₄-3a = J₄-3b = J₄-5a = J₄-5b = 8.23 Hz, 1H, 4-H), 3.82 (m, 1H, 5-Hb), 7.03 (d, J_AB = 8.23 Hz, 2H, H_arom.), 7.09 (br.s, 1H, N–H), 7.68 (d, J_AB = 8.23 Hz, 2H, H_arom.).

13C-NMR (CDCl₃) of 5k: δ (ppm) = 38.3 (C-3), 40.2 (C-4), 49.9 (C-5), 92.7, 129.2, 138.3, 142.2 (C_arom.), 178.1 (lactam).
(RS)-1-tert-Butyloxycarbonyl-4-(4′-iodo-phenyl)-2-pyrrolidinone (4k)

To a stirred solution of (RS)-4-(4′-iodo-phenyl)-2-pyrrolidinone 5k (0.23 g, 0.8 mmol), di-tert-butyl dicarbonate (0.35 g, 1.6 mmol) and 4-(dimethylamino)-pyridine (0.1 g, 0.8 mmol) in dichloromethane (15ml) was added triethylamine (0.08 g, 0.11 ml, 0.8 mmol) under nitrogen atmosphere. The resulting solution was stirred for 30 h at ambient temperature. The volatiles were removed under reduced pressure, the residue was dissolved in diethyl ether (15 ml), washed with 1 N hydrochloric acid (2 x 5 ml), washed with water (2 x 5 ml), dried (Na₂SO₄) and evaporated under vacuum. The residue was purified by column chromatography using system (C) to yield 0.26 g (84%) of 4k as a white powder m.p. 100-102 °C.

TLC of 4k: Rf ~ 0.17 using system (C).

C₁₅H₁₈INO₃ (387.21) of 4k: calcd. C 46.53, H 4.69, N 3.62; found C 46.72, H 4.69 N 3.24

IR (neat) of 4k: ν (cm⁻¹) = 1774, 1696, 1369, 1343, 1288.

¹H-NMR (CDCl₃) of 4k: δ (ppm) = 1.31 (s, 9H, t-Bu.), 2.43 (dd, J₃a-4 = 9.45 Hz, J_gem = 17.08 Hz, 1H, 3-Ha), 2.67 (dd, J₃b-4 = 8.25 Hz, J_gem = 17.08 Hz, 1H, 3-Hb), 3.27 (quin, J₄-3a = J₄-3b = J₄-5a = J₄-5b = 8.55 Hz, 1H, 4-H), 3.43 (dd, J₅a-4 = 8.23 Hz, J_gem = 10.68 Hz, 1H, 5-Ha), 3.93 (dd, J₅b-4 = 7.95 Hz, J_gem = 10.68 Hz, 1H, 5-Hb), 6.78 (d, J_AB = 8.25 Hz, 2H, H_arom.), 7.46 (d, J_AB = 8.25 Hz, 2H, H_arom.).

¹³C-NMR (CDCl₃) of 4k: δ (ppm) = 28.4 (OC(CH₃)₃), 36.4 (C-4), 40.5 (C-3), 53.2 (C-5), 83.6 (OC(CH₃)₃), 93.1, 129.2, 138.4, 140.8 (C_arom.), 150.2 (urethane), 172.9 (lactam).
(RS)-1-\textit{tert}-Butyloxycarbonyl-4-(4′-trimethylsilanylethynyl-phenyl)-2-pyrrolidinone (3_k)

To a stirred suspension of (RS)-1-\textit{tert}-butyloxycarbonyl-4-(4′-ido-phenyl)-2-pyrrolidinone 4_k (0.78 g, 2 mmol), bis (triphenylphosphine)-palladium dichloride (0.014 g, 0.02 mmol) and cuprous iodide (0.008 g, 0.04 mmol) in triethylamine (20 ml) was added trimethylsilylacetylene (0.24 g, 0.34 ml, 2.4 mmol) under nitrogen atmosphere. The yellow reaction mixture was stirred at ambient temperature for 12 h. The black reaction mixture was filtered and washed with diethyl ether (10 ml). The filtrate was evaporated under reduced pressure, the residue was dissolved in diethyl ether (20 ml), washed with water (3 x 10 ml), dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by column chromatography using system (C) to afford 0.64 (90%) of 3_k as a pale yellow powder m.p. 118-120 °C.

TLC of 3_k: R_f ~ 0.20 using system (C).

C_{20}H_{27}SINO_3 (357.52) of 3_k: calcd. C 67.19, H 7.61, N 3.92; found C 67.48, H 7.69 N 3.77.

IR (neat) of 3_k: ν (cm\textsuperscript{-1}) = 1790, 1690, 1352, 1293, 1156.

\textsuperscript{1}H-NMR (CDCl_3) of 3_k: δ (ppm) = 0.01 (s, 9H, SI(CH_3)_3), 1.30 (s, 9H, \textit{t}-Bu.), 2.44 (dd, J_{3a-4} = 9.45 Hz, J_{gem} = 17.38 Hz, 1H, 3-Ha), 2.66 (dd, J_{3b-4} = 8.25 Hz, J_{gem} = 17.38 Hz, 1H, 3-Hb), 3.29 (quin, J_{4-3a} = J_{4-3b} = J_{4-5a} = J_{4-5b} = 8.53 Hz, 1H, 4-H), 3.43 (dd, J_{5a-4} = 8.55 Hz, J_{gem} = 10.68 Hz, 1H, 5-Ha), 3.92 (dd, J_{5b-4} = 7.93 Hz, J_{gem} = 10.68 Hz, 1H, 5-Hb), 6.94 (d, J_{AB} = 8.23 Hz, 2H, H_{arom.}), 7.22 (d, J_{AB} = 8.25 Hz, 2H, H_{arom.}).

\textsuperscript{13}C-NMR (CDCl_3) of 3_k: δ (ppm) = 0.00 (SI(CH_3)_3), 28.1 (OC(CH_3)_3), 36.3 (C-4), 40.1 (C-3), 52.9 (C-5), 83.2 (OC(CH_3)_3), 94.8, 104.5 (C=\textit{C}), 122.4, 126.7, 132.6, 141.0 (C_{arom.}), 149.9 (urethane), 172.7 (lactam).
**Experimental Part**

**(RS)-4-Amino-3-(4′-ethynyl-phenyl)-butanoic acid hydrochloride (1k)**

To a stirred solution of (RS)-1-tert-butyloxycarbonyl-4-(4′-trimethylsilanylethynyl-phenyl)-2-pyrrolidinone 3k (0.25 g, 0.7 mmol) in tetrahydrofuran (9 ml) was added 1 M lithium hydroxide (3 ml). The resulting reaction mixture was stirred at room temperature for 18 h. The solvent was evaporated under reduced pressure, the residue was diluted with water (10 ml) and extracted with diethyl ether (3 x 5 ml). The aqueous layer was acidified using 0.5 M potassium hydrogen sulfate solution and extracted with diethyl ether (3 x 15 ml). The organic extracts were dried (Na₂SO₄) and evaporated under vacuum to give 0.19 g (90%) of (RS)-4-tert-butyloxycarbonylamino-3-(4′-ethynyl-phenyl)-butanoic acid 2k as a yellow powder m.p. 126-128 °C. The crude 2k was dissolved in ~2.5 M dry hydrogen chloride/ethyl acetate solution (1.6 ml) and the reaction mixture was stirred at room temperature for one hour. The reaction mixture was filtered and washed with diethyl ether (5 ml). The precipitated solid was recrystallized from 2-propanol/diethyl ether to yield 0.13 g (87%) of 1k as a white powder m.p. 209-211 °C.

C₁₂H₁₄ClNO₂ (239.70) of 1k: calcd. C 60.13, H 5.89, N 5.84; found C 59.78, H 5.90 N 5.55.

IR (neat) of 1k: v (cm⁻¹) = 3244-2654, 1724, 1520, 1185, 823.

¹H-NMR (D₂O) of 1k: δ (ppm) = 2.66 (dd, J₂a-3 = 8.53 Hz, J₆₇ = 16.18 Hz, 1H, 2-Hₚ), 2.79 (dd, J₂b-3 = 5.80 Hz, J₆₇ = 16.18 Hz, 1H, 2-Hₚ), 3.12-3.41 (m, 3H, 3-H and 4-H), 3.45 (s, 1H, C≡CH), 7.28 (d, J AB = 8.23 Hz, 2H, Hₕₐᵢₙ.), 7.48 (d, J AB = 8.25 Hz, 2H, Hₕₐᵢₙ.).

¹³C-NMR (D₂O) of 1k: δ (ppm) = 28.8 (C-2), 40.2 (C-3), 43.9 (C-4), 79.1 (C≡CH), 83.8 (C=CH), 121.7, 128.5, 133.3, 139.9 (Cₕₐᵢₙ.), 175.7 (C-1).

MS (Cl), m/z (%) of 1k: 203.1 [(100), M⁺].
Experimental Part

**(RS)-4-Amino-3-(4'-iodo-phenyl)-butanoic acid hydrochloride (1)**

![Chemical Structure](image)

To a stirred solution of (RS)-1-tert-butyloxycarbonyl-4-(4'-iodo-phenyl)-2-pyrrolidinone 4k (0.25 g, 0.7 mmol) in tetrahydrofuran (6 ml) was added 1 M lithium hydroxide (2 ml). The resulting reaction mixture was stirred at ambient temperature for two hours. The solvent was evaporated under reduced pressure, the residue was diluted with water (10 ml) and extracted with diethyl ether (3 x 5 ml). The aqueous layer was acidified using 0.5 M potassium hydrogen sulfate solution, extracted with diethyl ether (3 x 15 ml). The organic extracts were dried (Na₂SO₄), and evaporated under vacuum to yield 0.23 g (81%) of (RS)-4-tert-butyloxycarbonylamino-3-(4'-ido-phenyl)-butanoic acid 2 as a pale yellow powder m.p. 146-148 °C. The crude 2 was dissolved in ~2.5 M dry hydrogen chloride/ethyl acetate solution (1.5 ml) and the reaction mixture was stirred at ambient temperature for one hour. The reaction mixture was filtered, washed with diethyl ether (5 ml). The precipitated solid was recrystallized from 2-propanol/diethyl ether to give 0.16 g, (83%) of 1 as a white powder m.p. 208-210 °C (lit. 190-195 °C).

**C₁₀H₁₃ClINO₂ (341.57) of 1:** calcd. C 35.16, H 3.84, N 4.10; found C 35.52, H 3.79 N 4.00.

**IR (neat) of 1:** ν (cm⁻¹) = 3154-2720, 1719, 1587, 1520, 1414, 1194.

**¹H-NMR (D₂O) of 1:** δ (ppm) = 2.63 (dd, J₂a-₃ = 8.55 Hz, J gem = 16.18 Hz, 1H, 2-Ha), 2.76 (dd, J₂b-₃ = 5.78 Hz, J gem = 16.18 Hz, 1H, 2-Hb), 3.08-3.18 (m, 1H, 4-Ha), 3.24-3.36 (m, 2H, 3-H and 4-Hb), 7.04 (d, J_AB = 8.55 Hz, 2H, H arom.), 7.68 (d, J_AB = 8.23 Hz, 2H, H arom.).

**¹³C-NMR (D₂O) of 1:** δ (ppm) = 38.4 (C-2), 39.9 (C-3), 43.9 (C-4), 93.7, 130.3, 138.5, 138.7 (C arom.), 175.4 (C-1).

**MS (Cl), m/z (%) of 1:** 322.1 [(100), M⁺ + 17].
9 References


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163- H. Keberle, J. W. Faigle and M. Wilhelm, Swiss Pat., 449046, **1963**.
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179- Tripos Assoc. St. Louis, MO, USA.
180- SUSE Linux AG, Deutschherrnstr. 15-19, D-90429, Nürnberg, Germany.
188- OriginLab Corporation, One Roundhouse Plaza Northampton, MA 01060, USA.
189- Accelrys Ltd., 334 Cambridge Science, Cambridge, CB4 0WN.


10 Abbreviations

arom    aromatic
BE      binding energy
Boc     tert. butyloxycarbonyl
b.p.    boiling point
br      broad
calcd   calculated
d       doublet
DAVA    δ-amino valeric acid
DBB     dibenzoyl peroxide
DISCO   DIStance COmparisons
DMAP    4-(dimethyl-amino)-pyridine
GABA    γ-amino-butryic acid
GABA_{B}R GABA_{B} receptor
gem     geminal
Glu     glutamic acid
h       hour
HWE     Horner-Wadsworth-Emmons
m       multiplet
min     minute
m.p.    melting point
NBS     N-bromosuccinimide
q       quartet
quin    quintet
RMS     root mean square
r.t.    room temperature
s       singlet, second
S.E.M   standarad error of the mean
t       triplet
TEAC    tetraethylammonium cyanide
THF     tetrahydrofuran
TLC     thin layer chromatography
TMSA    trimethylsilylacetylene
VFTM    venus flytrap module
### CURRICULUM VITAE

**Mohamad Ibrahim Attia**

<table>
<thead>
<tr>
<th>Date</th>
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<tbody>
<tr>
<td>03.09.1968</td>
<td>Birth in El-Minia, Egypt</td>
</tr>
<tr>
<td>September 73- Jun 79</td>
<td>Primary school</td>
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<tr>
<td>September 79- Jun 82</td>
<td>Preparatory school</td>
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<td>September 82 - Jun 85</td>
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<td>September 85- May 90</td>
<td>Faculty of Pharmacy, Tanta University, Tanta, Egypt</td>
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<tr>
<td>Jun 91- September 92</td>
<td>Military service</td>
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<tr>
<td>September 92- December 96</td>
<td>Making Master of Sciences, Pharmaceutical Chemistry, Pharmaceutical Sciences Dep., National Research Centre (NRC), Cairo, Egypt.</td>
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<tr>
<td>December 96</td>
<td>Master degree entitled “Synthesis of certain substituted pyrrolidines of biological activity” Faculty of Pharmacy, Cairo University.</td>
</tr>
<tr>
<td>January 97- December 99</td>
<td>Assistant researcher, Pharmaceutical Sciences Dep., National Research Centre (NRC), Cairo, Egypt.</td>
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**Publications**


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<tr>
<td>May 2000</td>
<td>Starting Ph. D. work under supervision of Prof. Dr. C. Herdeis, Institute of Pharmacy and Food Chemistry, Würzburg University.</td>
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<tr>
<td>Since May 2000</td>
<td>Assistant researcher, Institute of Pharmacy and Food Chemistry, Würzburg University.</td>
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