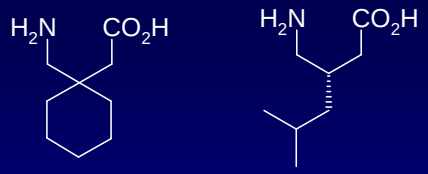
Gabapentin and Pregabalin



140nM 80nM

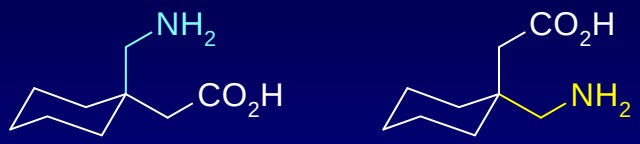
- Gabapentin found to be effective against neuropathic pain (launched for epilepsy).
- Follow-up compound, Pregabalin (Lyrica) approved for neuropathic pain associated with diabetic peripheral neuropathy, post-herpetic neuralgia and fibromyalgia.
- Pregabalin made 4.6 billion dollars in 2013.
- Gabapentin and Pregabalin are $\alpha_2\delta$ ligands.

Aims

- To investigate SAR of Gabapentin at $\alpha_2\delta$ site.
- Identify a more potent and efficacious molecule than Pregabalin against neuropathic pain.

Project was completely driven by synthesis – complexity of molecules meant that novel chemistry was required to synthesise them.

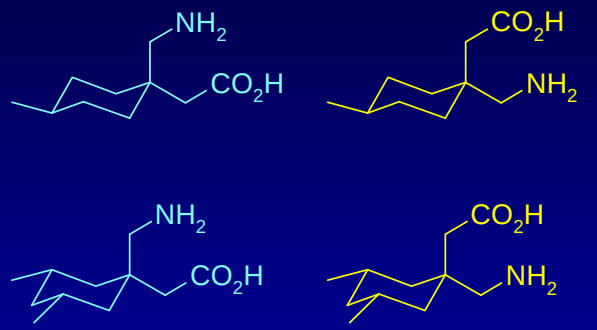
Binding Conformation



Is aminomethyl axial or equatorial in binding pocket?

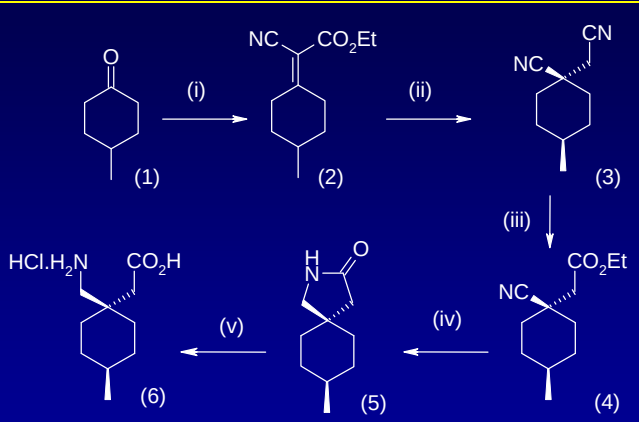
Synthesize conformationally constrained analogues

Conformationally constrained analogues



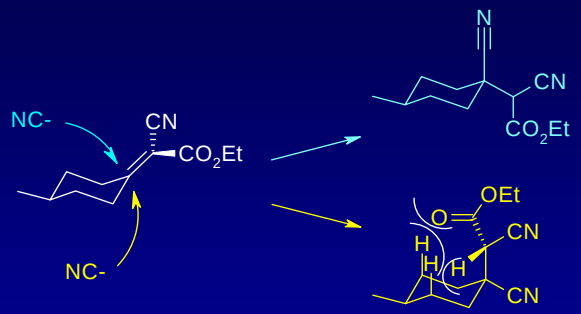
Bryans et al, *Bioorg. Med. Chem. Lett* **1997**, 7, 2481

Conformationally constrained analogues



(i) $NCCH_2CO_2Et$, NH_4OAc, PhMe, azeotrope (82%); (ii) KCN, EtOH, H_2O, reflux (87%);
(iii) EtOH, HCl, 0°C then H_3O^+ (76%); (iv) H_2, Raney Ni, MeOH, 30°C (98%);
(v) 1,4-Dioxane, 6N HCl, reflux (59%).

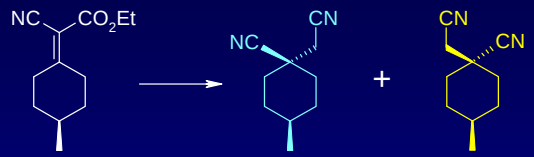
Conformationally constrained analogues



Cyanide attack is axial

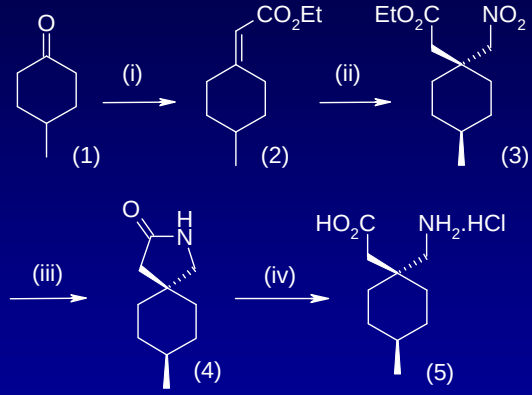
Equatorial attack gives less stable intermediate

Influencing Direction of Cyanide Attack



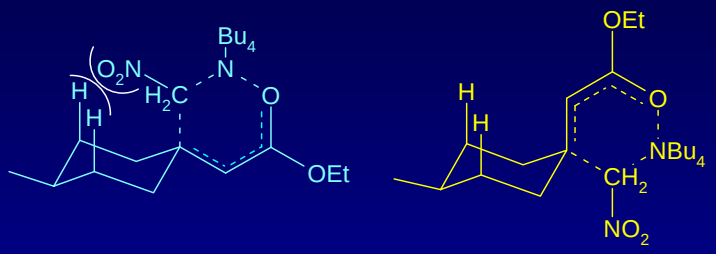
KCN / EtOH/H_2O	12	1
KCN / EtOH	10	1
LiCN / DMF	4	1
Et_2AlCN / PhMe	3	1
KCN / 18-crown-6 / PhH	1	1

Conformationally constrained analogues



(i) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF (88%);
(ii) $MeNO_2$, $Bu_4N^+F^-$, THF, 70°C (56%);
(iii) Raney Ni, H_2, MeOH, 30°C (89%);
(v) 1,4-Dioxane, 6N HCl, reflux (71%).

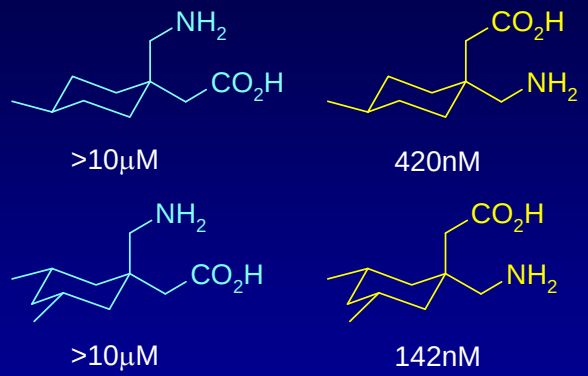
Conformationally constrained analogues



Nitromethane anion and counter-ion form ion pair
6-membered transition-state
Equatorial attack lower energy transition-state

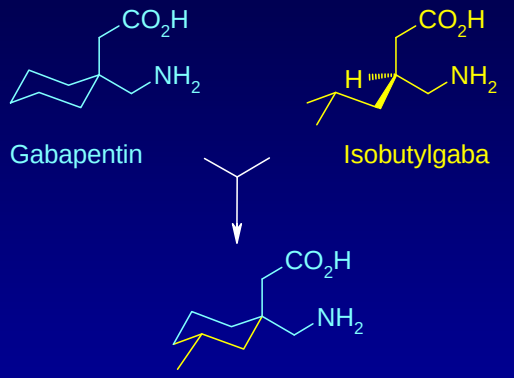
Nasipuri et al *J. Org. Chem.* **1982**, *47*, 2840-2845.

Conformationally constrained analogues



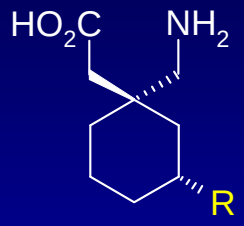
c.f. - Gabapentin 140nM
Suggests aminomethyl must be equatorial

Gabapentin and (S)-isobutylGABA



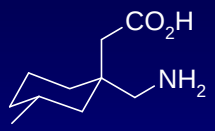
Modelling indicates pro-(R)-3-position

3-Substituted Gabapentin Analogues

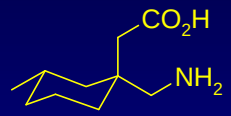


R	IC_{50} (nM)
H	140
Me	45
CF_3	580
Et	277
iPr	6300
nPr	3300
Ph	>10000

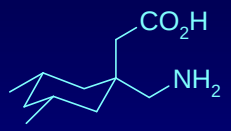
3 and 5-Substituted Gabapentin analogues



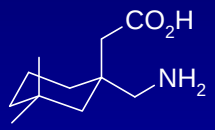
45nM



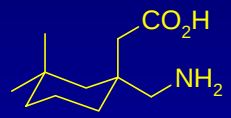
70nM



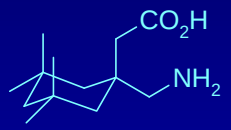
142nM



570nM

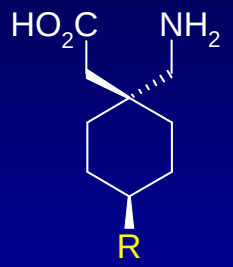


980nM



>10,000nM

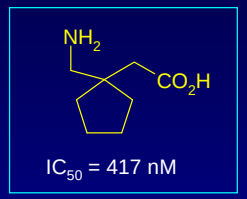
4-Substituted Gabapentin analogues



R	IC_{50} (nM)
H	140
Me	440
Et	700
iPr	7020
Ph	>10000

Also 4,4-Dimethyl 1,300nM

Extension of gabapentin SAR - the gababutins

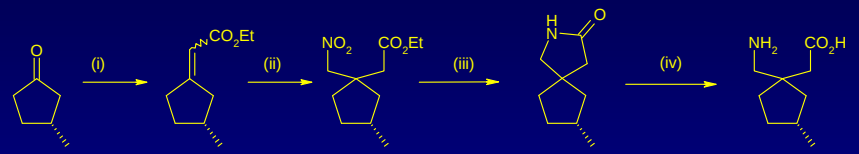


IC_{50} = 417 nM

R = Me, Et or n-Pr significantly improves binding affinity
R larger or branched reduces binding affinity

- Binding pocket not optimally filled in parent gababutin - SAR explored by appending substituents.
- 3-substituted Gababutins investigated first due to ready availability of 3-methyl cyclopentanone.
- Aim: to find a compound that is more efficacious and potent than Pregabalin in neuropathic pain models.

Standard route to 3-alkyl gababutins and synthetic issues

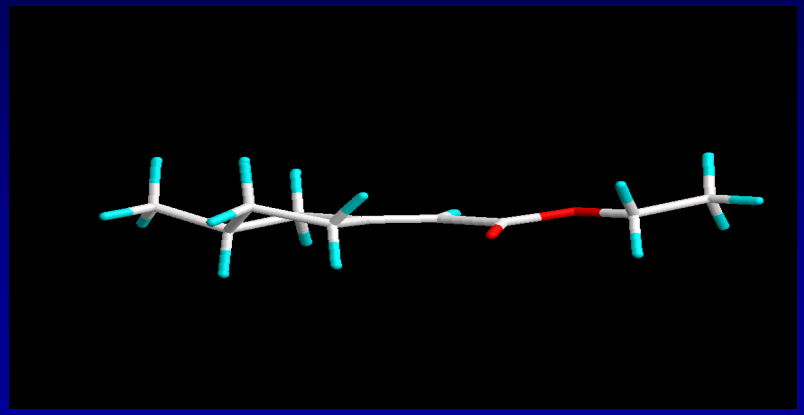


55:45 mixture of diastereoisomers

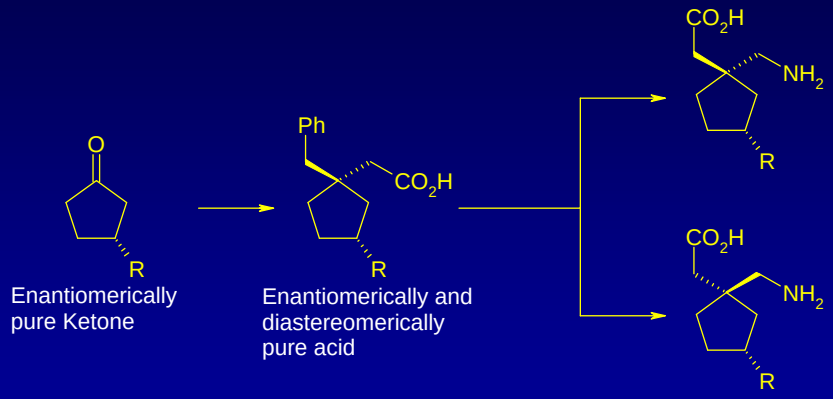
(i) Triethylphosphonoacetate, NaH, THF, 0 °C to RT (95%); (ii) $MeNO_2$, TBAF, THF, Reflux (65%); (iii) H_2, Ni, MeOH; (iv) 6N HCl, 1,4-dioxane, Reflux (69% from nitroester).

- No facial selectivity in conjugate addition of nitromethane anion to unsaturated ester.
- Of 3-Alkyl cyclopentanones, only 3-(R)-Methyl cyclopentanone readily available.
- Separation of diastereoisomers along route is non-trivial.

Minimized energy conformation of unsaturated ester



New Synthetic Approach to 3-Alkyl Gababutins

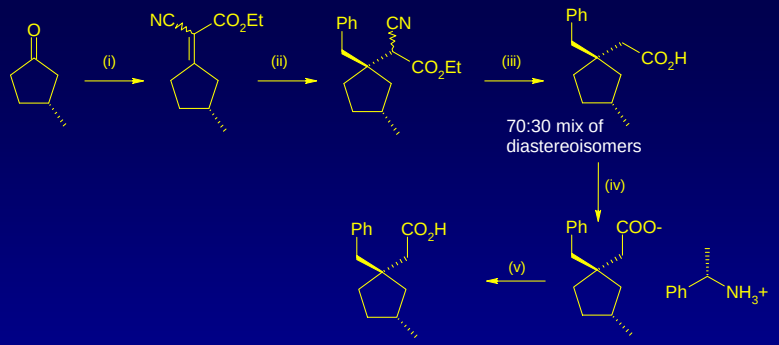


Enantiomerically pure Ketone

Enantiomerically and diastereomerically pure acid

- Generation of diastereomerically pure benzyl acid allows access to either diastereomerically pure Gababutin.
- Synthesis requires access to enantiomerically pure cyclopentanone.

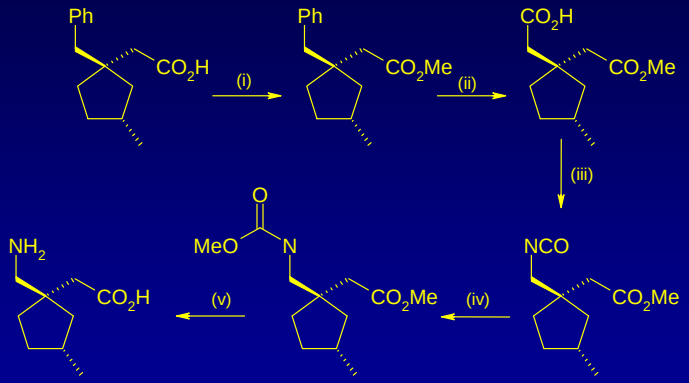
New Approach to 3-Methyl Gababutin



(i) $NCCH_2CO_2Et$, NH_4OAc, AcOH, Toluene, Reflux (97%); (ii) BnMgCl, THF, -78 °C (94%); (iii) KOH, Ethylene glycol, 160 °C (77%); (iv) (s)-(-)-α-Methylbenzylamine, EtOAc, 0 °C; 2 x Recrystallisation (40%); (v) Dilute HCl (91%).

- Benzyl acid diastereoisomers can be separated by recrystallisation of salt.
- This procedure has allowed 100 g of diastereomerically pure acid to be obtained.

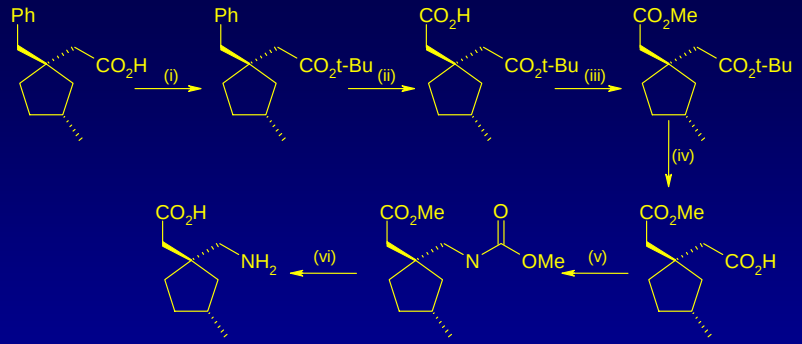
New Synthetic Route to 3-Methyl Gababutins



(i) $TMSCHN_2$, Toluene (99%); (ii) $RuCl_3$, $NaIO_4$, CCl_4, MeCN, H_2O (82%); (iii) DPPA, NEt_3, Toluene, Reflux; (iv) MeOH, Toluene, Reflux (47% from acid); (v) 6N HCl, 1,4-dioxane, Reflux (85%).

- Aminomethyl is *trans* to methyl group in final product.

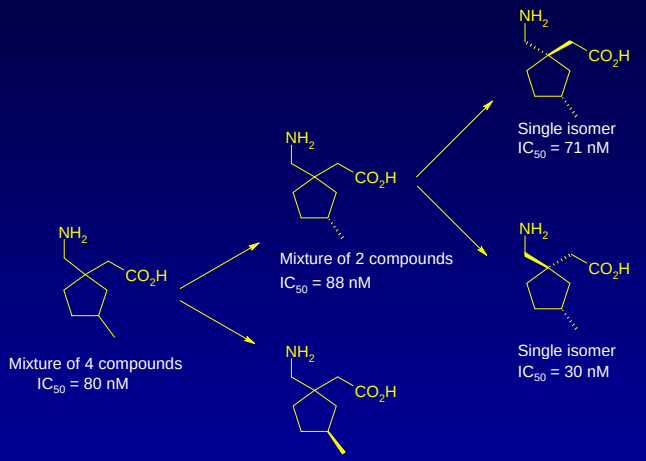
New Synthetic Route to 3-Methyl Gababutins



(i) Oxalyl Chloride, DMF, DCM; t-BuOH, DIPEA, DCM (88%); (ii) $RuCl_3$, $NaIO_4$, CCl_4, MeCN, H_2O (73%); (iii) $TMSCHN_2$, Toluene (92%); (iv) TFA, DCM (98%); (v) DPPA, NEt_3, Toluene, Reflux; MeOH, Toluene, Reflux (63%); (vi) 6N HCl, 1,4-dioxane, Reflux (60%).

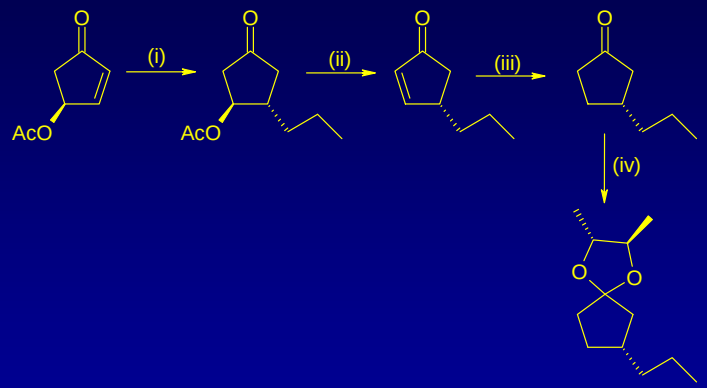
- Sharpless oxidation of phenyl in presence of t-butyl ester protecting group goes in good yield.
- Aminomethyl is *cis* to methyl group in final product.

SAR of 3-methyl gababutins



- Two diastereoisomers identified with better potency than gabapentin.
- Similar potencies found for ethyl and propyl derivatives

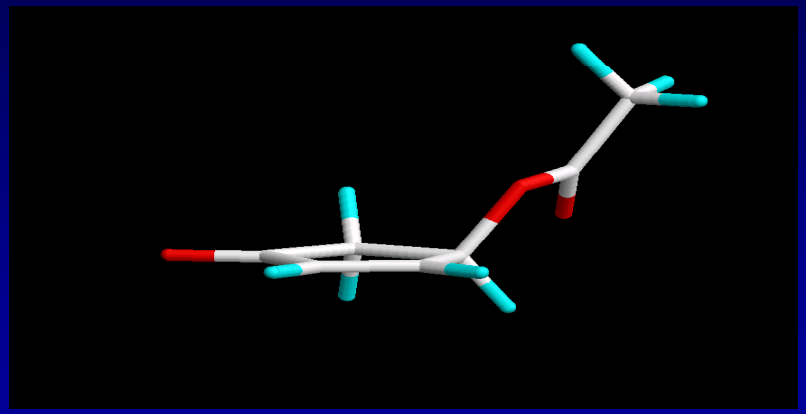
Synthesis of 3-Propyl Cyclopentanone



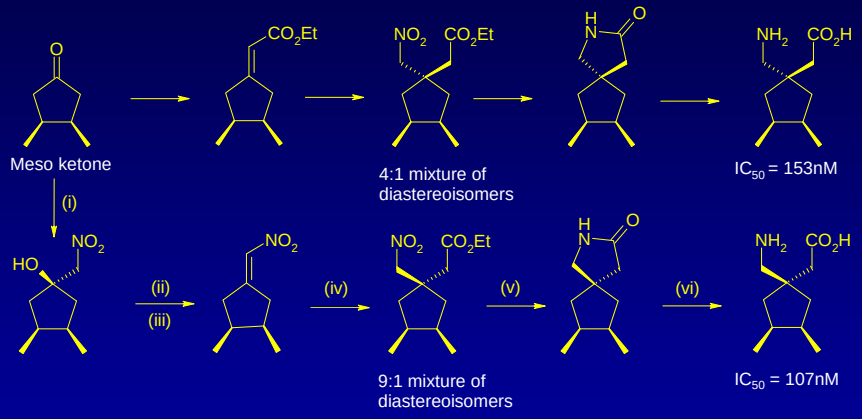
Single diastereoisomer by ^{13}C NMR

(i) n-PrMgCl, Me_2Zn, THF, -78 °C; (ii) DBU, DCM, -30 °C (68% from 4-acetoxycyclopentenone); (iii) H_2, Pd/C (88%); (iv) (2R,3R)-(-)-2,3-Butanediol, p-TSA, Benzene (64%).

Minimized energy conformation of 4-Acetoxy Cyclopentenone



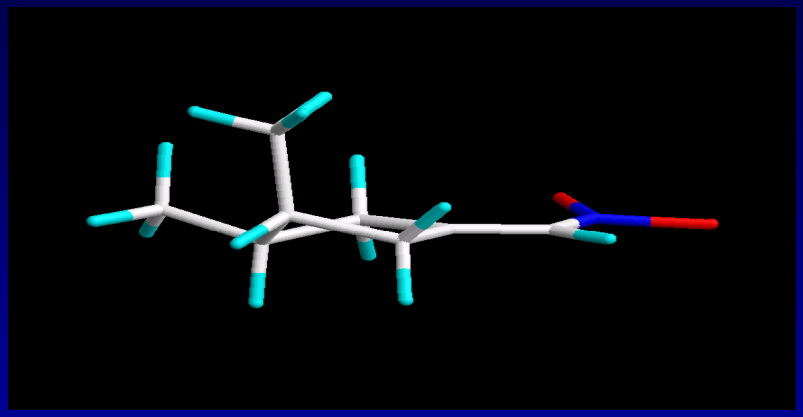
Synthesis of both Diastereoisomers of *cis*-3,4-DimethylGababutin



(i) $MeNO_2$, BuLi (2 equiv), THF/HMPA, -78 °C (40%); (ii) Ac_2O, conc H_2SO_4, reflux (98%); (iii) KOMe, MeOH, 0 °C (53%); (iv) $MeCO_2Et$, LHMDS, THF, -78 °C (45%); (v) H_2, Ni, MeOH; (vi) 6N HCl, 1,4-dioxane, Reflux (45% from nitro-ester).

- Low temperature addition of ester enolate gives better diastereoselectivity than nitromethane anion addition.

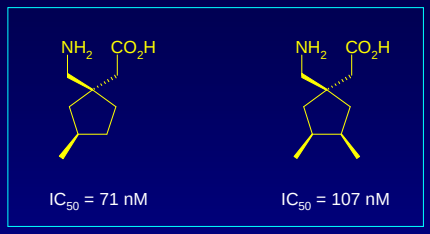
Minimized energy conformation of nitroalkene



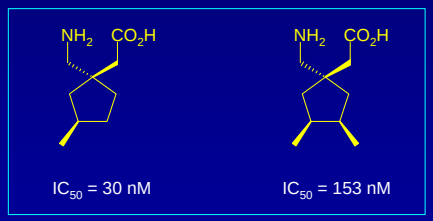
- *cis*-Dimethyl groups arrange themselves such that one methyl group is in plane of ring and one is blocking top face.
- Enolate attack is favoured from lower face of double bond under kinetic conditions.

Key initial compounds and their *in vivo* effects

Active orally in CITH model of pain:

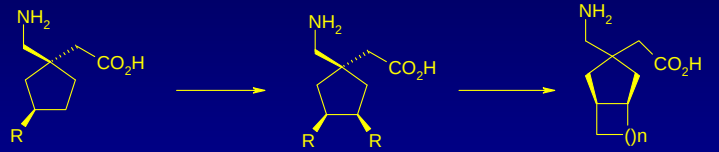


Inactive orally (active intrathecally) in CITH model of pain:



- Different diastereoisomers behaved differently *in vivo*
- Binding affinity alone not a good guide to *in vivo* activity.

Space in binding pocket key - move to bicyclic analogues

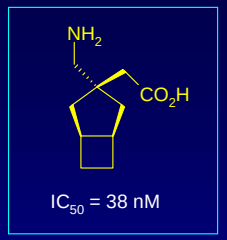


R = Me,Et,Pr - OK
R = Bu, less potent

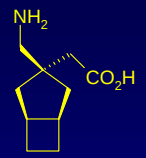
R = Me - OK
R = Et - less potent

n = 1 - optimum fit?
n =2 or 3 - too big?

SAR of bicyclic gababutins



IC_{50} = 38 nM



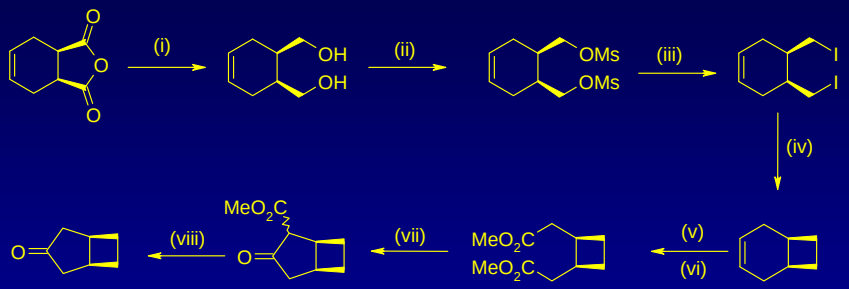
IC_{50} = 332 nM

n=1 or 2

IC_{50} > 720nM
for all diastereoisomers

- Space in binding pocket again shown to be tight.
- One stand-out compound with superior potency to gabapentin and pregabalin.

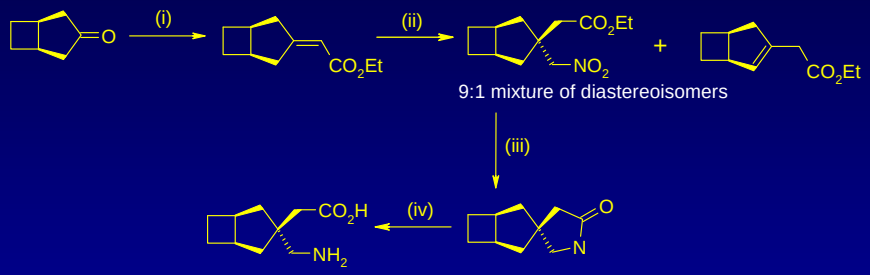
Synthesis of cyclobutyl gababutin (i)



(i) $LiAlH_4$, THF, Reflux (80%); (ii) MsCl, NEt_3, DCM, -40 °C to RT (80%); (iii) NaI, Acetone, Reflux (70%); (iv) t-BuLi, Pentane-ether (3:2), -25 °C; (v) $NaIO_4$, $RuCl_3.H_2O$, MeCN, EtOAc, H_2O; (vi) MeOH, Conc H_2SO_4 (85% from di-iodide); (vii) KOt-Bu, THF, Reflux (95%); (viii) DMSO, H_2O, 155 °C (97%).

- Starting *cis*-tetrahydrophthalic anhydride is readily available and cheap.
- Key step is closure of 4-membered ring.

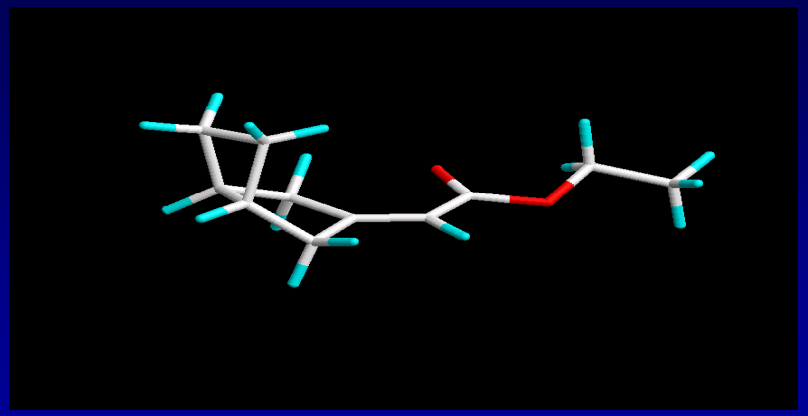
Completing synthesis of cyclobutyl gababutin



(1) Triethylphosphonoacetate, NaH, THF, 0 °C to RT (68%); (ii) $MeNO_2$, TBAF, THF, Reflux (25%); (iii) H_2, Ni, MeOH; (iv) 6N HCl, 1,4-Dioxane, Reflux (84% from nitroester)

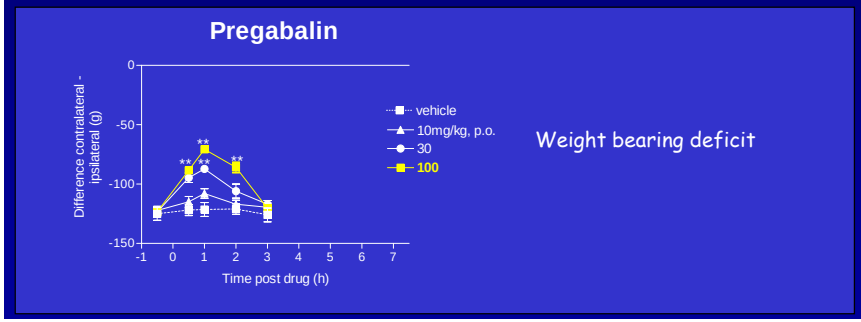
- Nitromethane addition to unsaturated ester gives 9:1 mixtures of diastereoisomers.
- Major side-product of nitromethane reaction is from double bond migration.
- Aminomethyl group on *exo*-face of bicyclic system.

Minimized energy conformation of unsaturated ester

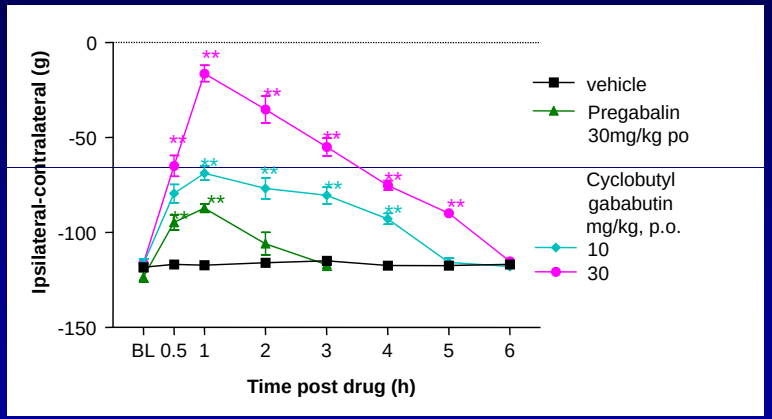


Behavioural assessment of neuropathic pain

- Aiming for compound with greater efficacy than Gabapentin or Pregabalin.
- However, both show full efficacy in most neuropathic pain models.
- Gabapentin and Pregabalin show only a partial effect in weight bearing model of pain.
- This model might be a way to identify compound with increased efficacy compared to Gabapentin.



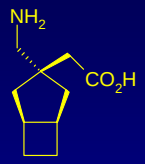
Cyclobutyl gababutin in the CCI Weight-bearing model of Neuropathic Pain



- Cyclobutyl analogue is significantly more efficacious than pregabalin in this model.

Cyclobutyl gababutin
Summary of Key Data

Binding affinity:	38nM
Bioavailability:	84% (oral, rat).
Half-life:	2.4hrs (p.o. rat).
Clearance:	4.3ml/min/kg (p.o. rat).



- Superior efficacy to gabapentin and pregabalin in the CCI weight-bearing model of neuropathic pain.
- Highly polar, low mol weight zwitterion (logD = -1).
- High bioavailability driven by paracellular absorption/active transport and negligible first pass metabolism.
- Renally cleared at around glomerular filtration rate.
- Compound progressed to clinical trials.

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