N°6 | Scientific Letter

EDITO

ATLANCHIM PHARMA What's new in 2011 ?

Chers lecteurs,

Nous sommes heureux de vous présenter la nouvelle édition de notre Lettre Scientifique, dans laquelle nous avons plaisir à vous faire partager les Recherches de notre Direction Scientifique, ainsi que les évolutions d'AtlanChim Pharma et d'Atlantic Bone Screen.

Pour cette nouvelle année, la première étape notoire est la certification ISO 9001 : 2008 d'AtlanChim Pharma et le renouvellement de celle-ci pour Atlantic Bone Screen (certifications délivrées par l'organisme LRQA). Toujours très attachés à la qualité de notre travail, cette certification vous garantit le respect de nos engagements et surtout, votre entière satisfaction.

Nous en profitons également pour vous annoncer l'agrandissement de l'équipe d'AtlanChim Pharma, avec l'arrivée mi-mai d'un Chef de projet spécialisé dans la chimie des sucres, l'extension de la capacité de production ainsi que l'acquisition de nouveau matériel en propre.

Dans cette première édition de l'année, vous pourrez découvrir le nouvel article scientifique rédigé par le Dr. André GUINGANT, intitulé «Integrin alpha V beta 3 as a target for treatment of osteoporosis: design and synthesis of non peptide antagonists».

Toute l'équipe d'ATLANCHIM PHARMA vous souhaite une agréable lecture.

Ronan LE BOT
Directeur

ATLANCHIM PHARMA What's new in 2011 ?

Dear readers,

We are glad to present you the new edition of our Scientific Letter, in which we are happy to share with you our Scientific Management Researches, as well as the latest evolutions of AtlanChim Pharma and Atlantic Bone Screen Companies.

For this New Year, the first important step is the obtaining of the ISO 9001 : 2008 Certificate of conformity (delivered by LRQA organization) for AtlanChim Pharma Company and the renewal of it for Atlantic Bone Screen Company. Giving a strong emphasis on the quality of our work, this accreditation guarantees you the respect of our commitments and most likely, your full satisfaction.

We also take this opportunity to announce to you the enlargement of AtlanChim Pharma Company team, through the arrival around mid-May of a new Project Leader with a specialization in Carbohydrates Chemistry. We also extend our production capacity and acquire new equipment.

In this first edition of the year, you will discover the new Scientific article written by Dr. André GUINGANT and entitled "Integrin alpha V beta 3 as a target for treatment of osteoporosis : design and synthesis of non peptide antagonists".

The whole Atlanchim Pharma Company team wishes you a nice reading.

Ronan LE BOT
CEO

SOMMAIRE / *SUMMARY*

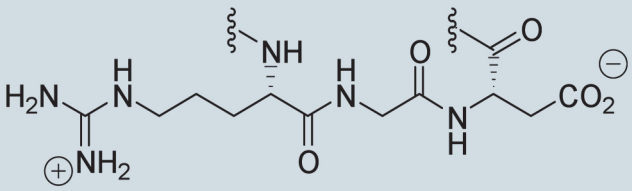
Integrin alpha V beta 3 as a target for treatment of osteoporosis : design and synthesis of non peptide antagonists.

Dr. André GUINGANT
Scientific Director
andre.guingant@atlanchimpharma.com

Osteoporosis is a multifactorial disease resulting from an imbalance between bone resorption and bone formation. Osteoporosis may develop in anyone but is more common in women after menopause because lack of estrogen leads to an increase in bone resorption and a decrease in new bone deposition.The resulting reduction of the bone mineral density (BMD) may be a source of fractures, the hip fracture being the most common one. As life expectancy continues to increase in most countries, the risk of fractures - and associated mortality - due to osteoporosis is expected to increase exponentially. Osteoporosis thus represents an important health issue and, as such, has mobilised many scientists to discover appropriate curative treatments. The most current therapy for the treatment of osteoporosis involved the use of antiresorptive biphosphonate molecules. A small number of other approved therapies with potential osteoblast anabolic function are available but their use may be limited in time due to important associated risks; moreover, their mecanisms of action are not well understood. Continued progress in the biology of osteoblasts (bone forming cells) and osteoclasts (bone resorbing cells) has resulted in the discovery of relevant new targets for osteoporosis treatment. Among these is the transmembrane $\alpha_V\beta_3$ integrin receptor whose binding with bone matrix proteins has been shown to be a key step in bone resorption.

Integrins are heterodimeric transmembrane receptors that play a crucial role for the interaction of cells with the extracellular environment. Widely distributed in the animal reign, the integrins consist of two subunits, an alpha (α) and a beta (β) chain. The pairing of different α and β chains led to different integrins (24 in mammals). For instance, association of integrin subunit α_V with multiple β subunits can form five different receptors ($\alpha_V\beta_1$, $\alpha_V\beta_3$, $\alpha_V\beta_5$, $\alpha_V\beta_6$ and $\alpha_V\beta_8$). As many other integrins, $\alpha_V\beta_3$ integrin binds to several ligands (e.g. vitronectin, osteopontin, fibronectin, thrombospondin, laminin) by recognizing an arginine-glycine-aspartic acid (RGD) tripeptide sequence (Figure 1). These molecular non-covalent associations are crucial in several pathological processes including osteoporosis, various vascular disorders (pathological angiogenesis, arterial restenosis, etc) and cancers. As a result, this area of drug discovery has received considerable attention over the past ten years and a variety of potent and specific $\alpha_V\beta_3$ integrin antagonists have been identified.

Figure 1

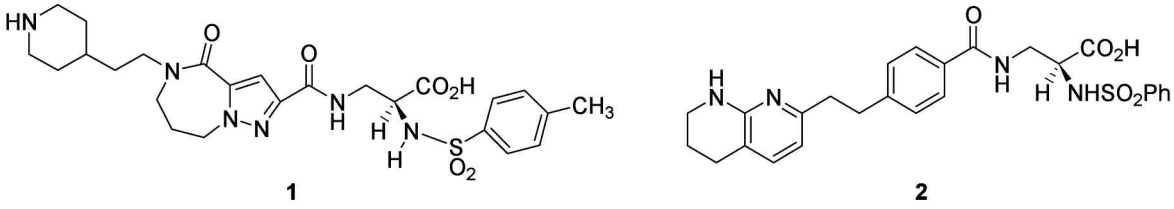


In this short review, we will focus exclusively on non peptide antagonists of $\alpha_V\beta_3$ integrin that were shown to inhibit bone resorption, with special emphasis on their mode of preparation.

Synthesis of $\alpha_v\beta_3$ antagonists

Early work at Merck directed towards the design and preparation of orally-active non-peptide fibrinogen antagonists had shown that $\alpha_{IIb}\beta_3$ selective RGD mimetics could be fashioned by introduction of a rigid central constraint and replacement of the arginine guanidinium functionality by a heterocyclic amine.[1] For example, compound **1** (Figure 2) having a sulfonamide functionality attached to the centrally constrained pyrazolodiazepinone was shown to be a potent platelet aggregation inhibitor. Following this work attempts to modulate receptor specificity and, thereby, to discover high affinity $\alpha_v\beta_3$ ligands, were made by introducing specific alterations in the structure of the potent $\alpha_{IIb}\beta_3$ receptor antagonists. Thus, utilizing compound **1** as a starting point, modifications of both the central moiety and the N-terminus allowed researchers to identify several compounds exhibiting rat osteoclast mediated bone resorption in vitro. Among these, the most interesting derivative was compound **2** (Figure 2) exhibiting a lipophilic 5,6,7,8-tetrahydro[1,8]naphthyridine (THN) moiety at its N-terminus.[2] This compound inhibited rat osteoclast mediated bone resorption with IC_{50} values (3±3 nM) similar to that showed by echistatin, a snake venom-derived peptide used as a reference compound. Furthermore, its ability to inhibit bone-dependent serum calcium increases produced by exogenous parathyroid hormone was evaluated in vivo in the thyroparathyroidectomised (TPTX) rat model and shown to be of the same order (IC_{50} of 200 nM) than that reported for echistatin (IC_{50} of 100 nM).

Figure 2

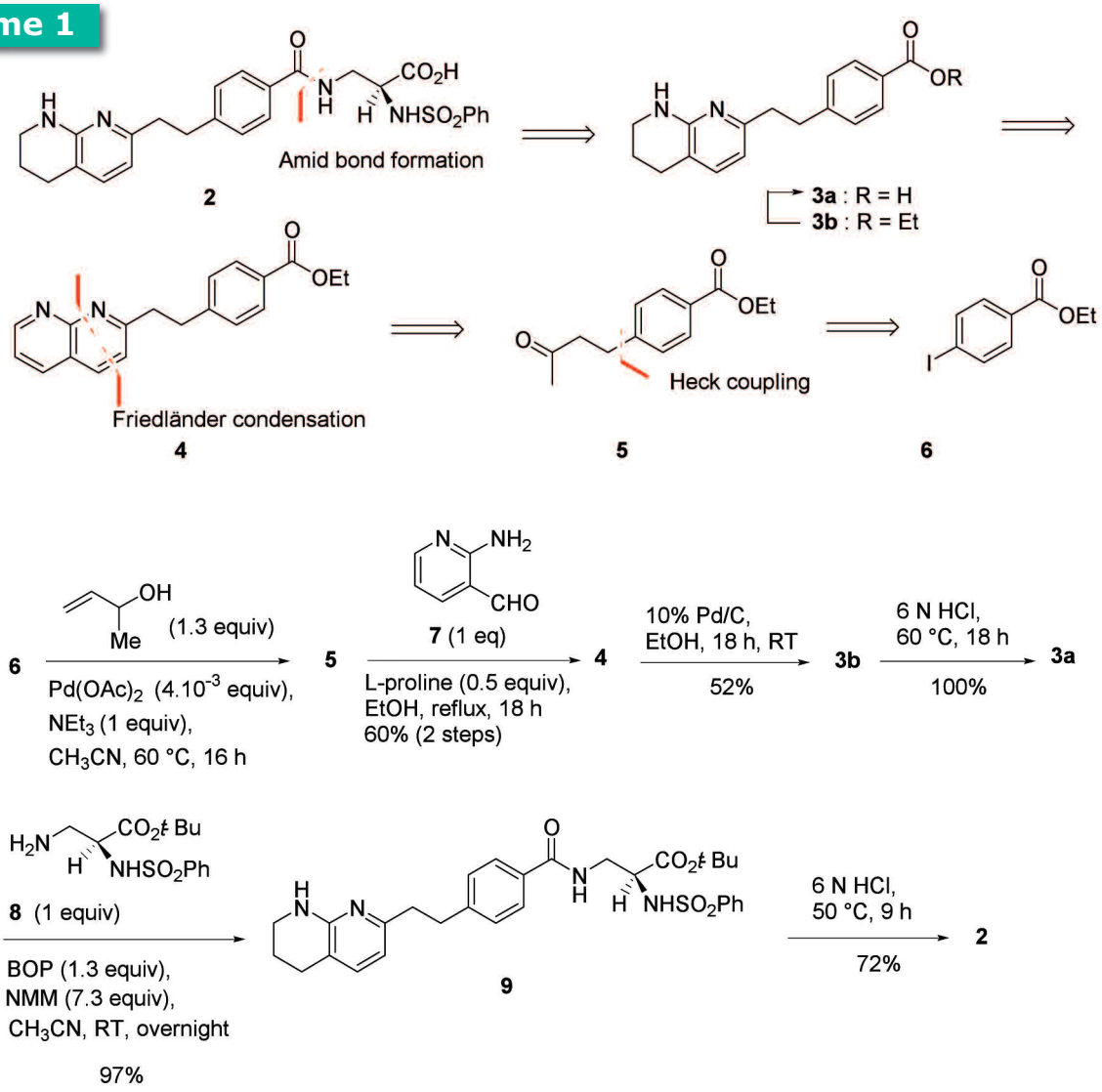


The synthesis of compound **2** is shown in Scheme 1. It commenced with a Heck reaction between ethyl 4-iodobenzoate **6** and 3-buten-2-ol to give ketone **5**, which was next submitted to a proline-catalysed Friedländer annulation with 2-aminopyridine-3-carboxaldehyde **7** to provide the 2-substituted [1,8]naphthyridine **4** in 60% yield. Next, ring selective hydrogenation and ester acidic hydrolysis gave acid **3a**, which was coupled to primary amine **8**, prepared in three steps from L-asparagine, to deliver the 5,6,7,8-tetrahydro[1,8]naphthyridine **9**. Last, the tert-butyl protecting group was removed in acidic conditions to afford compound **2**.

[1] B. C. Askew, R. A. Bednar, B. Bednar, D. A. Claremon, J. J. Cook, C. J. McIntyre, C. A. Hunt, R. J. Gould, R. J. Lynch, J. J. Lynch, Jr., S. L. Gaul, M. T. Stranieri, G. R. Sitko, M. A. Holahan, J. D. Glass, T. Hamill, L. M. Gorham, T. Prueksaritanont, J. J. Baldwin, G. D. Hartman, *J. Med. Chem.* **1997**, *40*, 1779-1788.

[2] M. E. Duggan, L. T. Duong, J. E. Fisher, T. G. Hamill, W. F. Hoffman, J. R. Huff, N. C. Ihle, C-T Leu, R. M. Nagy, J. J. Perkins, S. B. Rodan, G. Wesolowski, D. B. Whitman, A. E. Zartman, G. A. Rodan, G. D. Hartman, *J. Med. Chem.* **2000**, *43*, 3736-3745.

Scheme 1



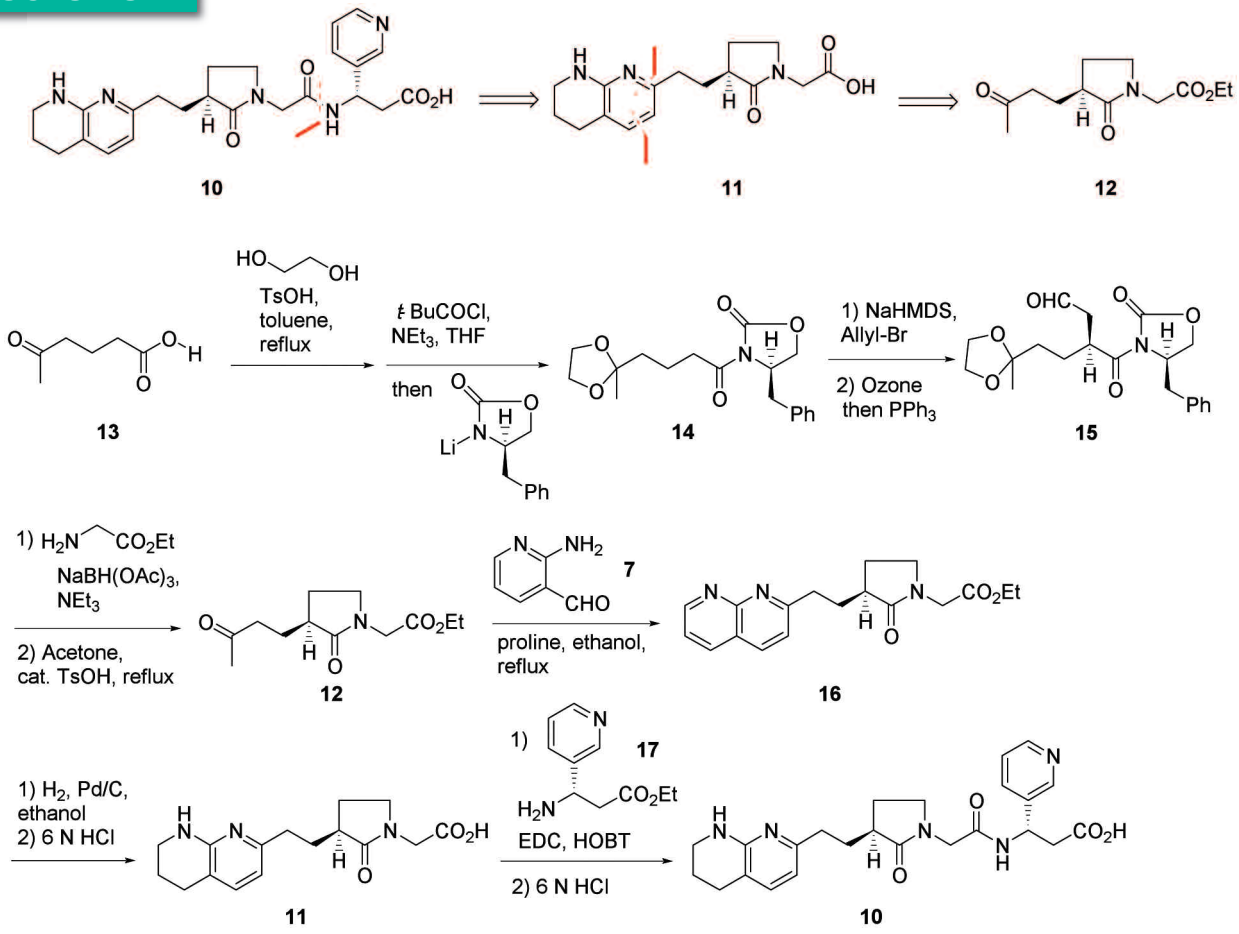
Later on, the Merck group explored a new series of RGD tripeptide mimetics still having a THN group as an N-terminus but featuring different C-terminal 3-substituents and constraining elements of the glycyl amide bond. The SPAV3 and PLAGGIN tests[3] were utilised to evaluate the binding affinity of the new compounds to $\alpha_V\beta_3$and their selectivity to $\alpha_V\beta_3$ over $\alpha_{IIb}\beta_3$, respectively. The *(S)*-pyrrolidinone **10** having a 3-pyridyl substituent at C-3 of the propionic moiety emerged as the most interesting compound in these series.[4] Indeed, it displayed IC_{50} value in the SPAV3 assay of 0.35 nM whereas the PLAGGIN IC_{50} was greater than 10 µM. Moreover, it also inhibited the formation of mouse osteoclasts in vitro (OCFORM assay) with an IC_{50} of 62 nM. Interestingly, the *(R)*-pyrrolidinone enantiomer was only three-fold less potent than **10** whereas elimination of the pyrrolidinone stereogenic center in **10** by conversion of the pyrrolidinone ring to an imidazolidinone ring resulted in a substantial loss of binding-affinity (SPAV3) and cell-based potency (OCFORM). The synthesis of compound **10** is pictured in Scheme 2. The key *(S)*-pyrrolidone **12** was elaborated in a few steps from 2-oxo-hexanoic acid **13**. Thus, keto acid **13** was first transformed to the *(R)*-oxazolidinone **14** following a sequence of carbonyl protection, acid activation and amid bond formation under the action of the lithium salt of the *(4R)*-benzyl-oxazolidin-2-one. Diastereoselective allylation of **14** followed by ozonolysis of the allylic double bond afforded

[3] The SPAV3 test measures the ability to displace a non-peptide radioligand from human recombinant $\alpha_V\beta_3$, whereas the PLAGGIN test evaluates the ability to inhibit the rate of ADP-stimulated aggregation of gel-filtered human platelets mediated through the integrin $\alpha_{IIb}\beta_3$.

[4] R. S. Meissner, J. J. Perkins, L. T. Duong, G. D. Hartman, W. F. Hoffman, J. R. Huff, N. C. Ihle, C-T. Leu, R. M. Nagy, A. Naylor-Olsen, G. A. Rodan, S. B. Rodan, D. B. Whitman, G. A. Wesolowski, M. E. Duggan, *Bioorg. Med. Chem. Lett.* **2002**, *12,* 25-29.

aldehyde **15**, which was next transformed to the key *(R)*-pyrrolidinone **12** through a sequence of reductive amination and intramolecular oxazolidinone displacement. Compound **12** was then reacted with 2-aminopyridine-3-carboxaldehyde **7** under the conditions of the Friedländer annulation to give the naphthyridine **16**, which, after selective ring hydrogenation and acidic acid hydrolysis, afforded acid **11**. Finally, the synthesis of **10** was completed upon coupling of acid **11** with the *(S)*-amino ester **17** prepared following the Davies protocol (see the synthesis of **22**, Scheme 5 below).

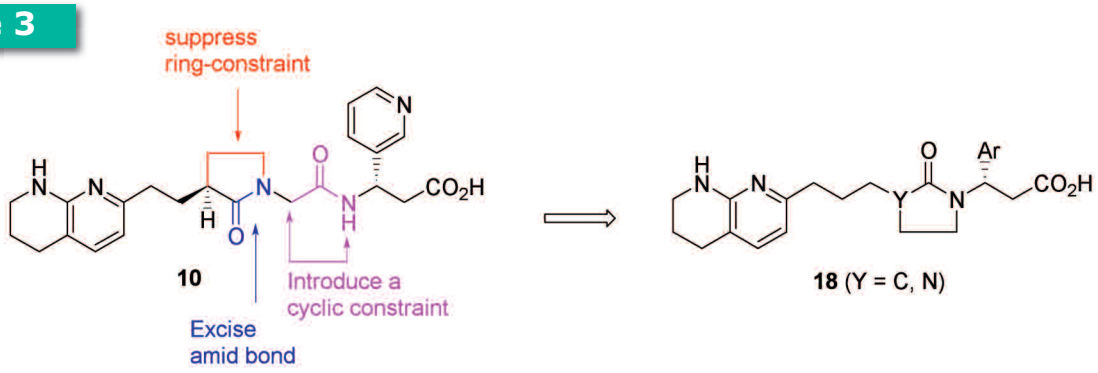
Scheme 2



The utility of compound **10**, in spite of its excellent binding affinity, was hampered by its poor oral pharmacokinetics. The search for new compounds with a better pharmacological profile was thus next undertaken. Utilising compound **10** as a starting point and keeping the THN moiety, work was first centered on the replacement of the 3-pyridinyl motif by more lipophilic aryl moieties.[5] Although this approach allowed for the identification of several compounds with pharmacokinetic parameters better than **10**, their bioavailability in dogs remained poor so that this route was not pursued further. Efforts were instead undertaken towards the development of new chain-shortened (2C) antagonists still having a cyclic constraint. In this direction, structural modifications of compound **10** led to new pyrrolidinone and imidazolidinone structures of general formula **18** (Scheme 3).

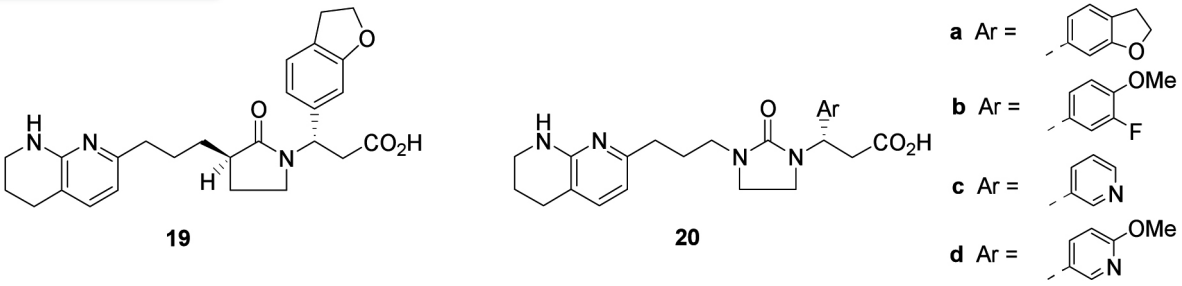
[5] P. J. Coleman, K. M. Brashear, C. A. Hunt, W. F. Hoffman, J. H. Hutchinson, M. J. Breslin, C. A. McVean, B. C. Askew, G. D. Hartman, S. B. Rodan, G. A. Rodan, C-T. Leu, T. Prueksaritanont, C. Fernandez-Metzler, B. Ma, L. A. Libby, K. M. Merkle, G. L. Stump, A. A. Wallace, J. J. Lynch, R. Lynch, M. E. Duggan, *Bioorg. Med. Chem. Lett.* **2002**,*12,* 31-34.

Scheme 3



Among this series of new compounds, pyrrolidinone *(R)*-**19** (Figure 3) displayed interesting properties.[6] This compound had an SPAV3 IC_{50} of 0.39 nM and showed an oral bioavailibility in dogs of 52%, a clearance of 2.1 mL/min/kg and a plasma half-life of 8 h. By comparaison with its more polar *(S)*-enantiomer, the *(R)*-pyrrolidone **19** displayed a much better pharmacokinetic profile. Because of its attractive profile, *(R)*-pyrrolidinone **19** was evaluated in an in vivo model of osteoporosis. After 10 consecutive days of a twice-daily mpk oral dose to young, rapidly growing rats, it produced a 5.6% increase in bone mineral density as compared to dosing vehicle.

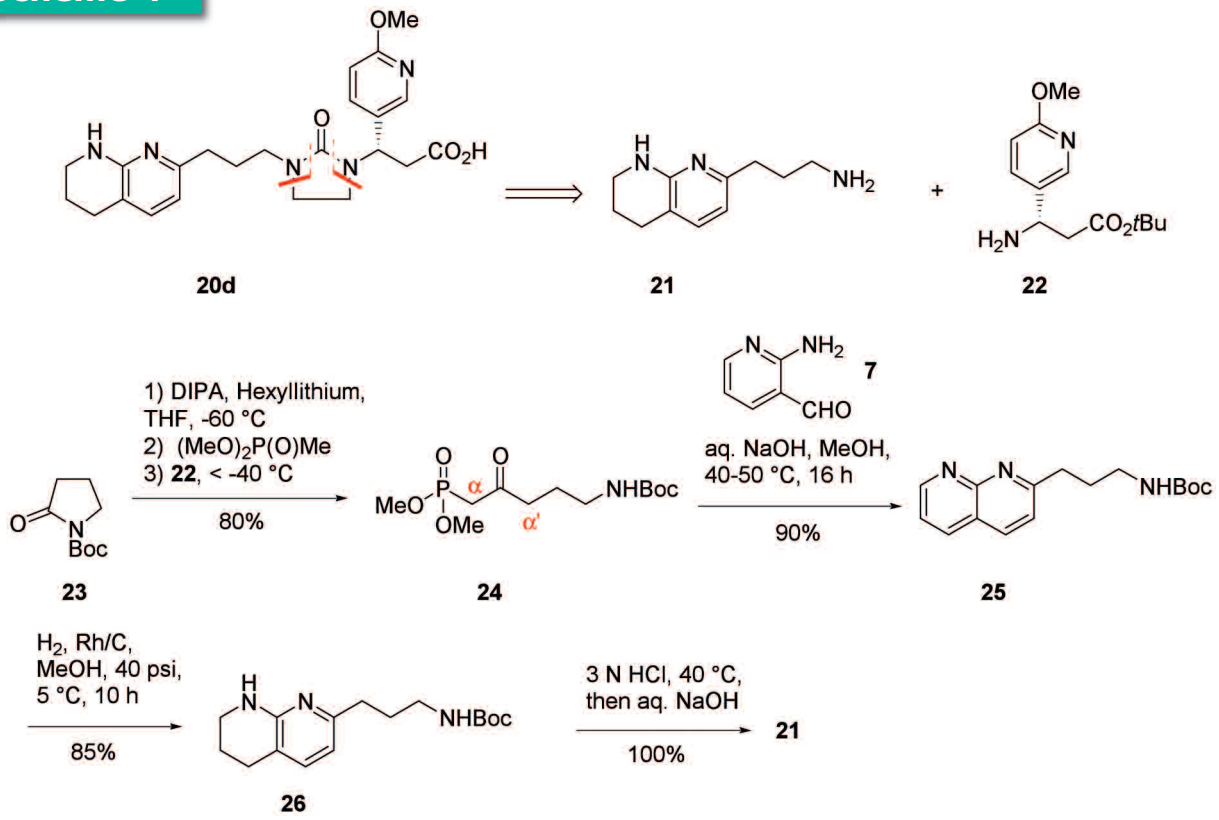
Figure 3



Imidazolidinones **20a** and **20b** (Figure 3) displayed properties not markedly different from those of pyrrolidinone **19**. They had good pharmacokinetic profiles but demonstrated extensive binding to human plasma proteins and were thus unsuitable for clinical development.[7] Compound **20c** having a 3-pyridyl substituent was considerably more polar than **20a** and **20b** and, in conjunction with this, its human plasma proteins value was significantly improved (66% instead of 98% for **20a** and 95% for **20b**). However, its pharmacokinetic parameters, in the dog, were unacceptable. [7] Finally, compound **20d** having a polarity intermediate between that of **20a**,**b** and **20c** proved to be optimal.[8] It was the most potent of the series with an IC_{50} = 0.08 nM in the SPAV3 assay while its binding value to human plasma proteins (88%) remained acceptable. On the basis of this interesting profile, compound **20d** was tested as a potential inhibitor of bone resorption in ovariectomised (OVX) female rats (**20d** was dosed orally at 30 mpk for 28 days) and in young, rapidly growing male rats (constant infusion to give a steady state concentration of 800nM for a period of 10 days). In both animal models a significant increase in bone mineral density was observed among the treated rats (12.8% and 20.5%, respectively). Compound **20d** was also tested in an OVX rhesus monkey to monitor its effect on urinary markers of bone degradation. When it was orally administered at mpk per day for 2 weeks, there was a 39% reduction in the level of n-telopeptides (normalised for creatinine) excreted in the urine compared to placebo controls. On the basis of the efficacy shown in the three animal models, compound **20d** was selected as a potential clinical candidate. Several synthetic approaches to this compound were thus attempted. [9] The most practical achievement is pictured in Schemes 4-6.

[6] J. J. Perkins, L. T. Duong, C. Fernandez-Metzler, G. D. Hartman, D. B. Kimmel, C-T. Leu, J. J. Lynch, T. Prueksaritanont, G. A. Rodan, S. B. Rodan, M. E. Duggan, R. S. Meissner *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4285-4288.
[7] J. H. Hutchinson, W. Halczenko, K. M. Brashear, M. J. Breslin, P. J. Coleman, L. T. Duong, C. Fernandez-Metzler, M. A. Gentile, J. E. Fisher, G. D. Hartman, J. R. Huff, D. B. Kimmel, C-T. Leu, R. S. Meissner, K. Merkle, R. Nagy, B. Pennypacker, J. J. Perkins, T. Prueksaritanont, G. A. Rodan, S. L. Varga, G. A. Wesolowski, A. E. Zartman, S. B. Rodan, M. E. Duggan, *J. Med. Chem.* **2003**, *46*, 4790-4798.
[8] D. Cui, R. Subramanian, M. Shou, Y. Xiao, M. A. Wallace, M. P. Braun, B. H. Arison, J. A. Yergey, T. Prueksaritanont, *Drug Metab. Dispos.* **2004**, *32*, 848-861.
[9] N. Yasuda, Y. Hsiao, M. S. Jensen, N. R. Rivera, C. Yang, K. M. Wells, J. Yau, M. Palucki, L. Tan, P. G. Dormer, R. P. Volante, D. L. Hughes, P. J. Reider, *J. Org. Chem.* **2004**, *69*, 1959-1966.

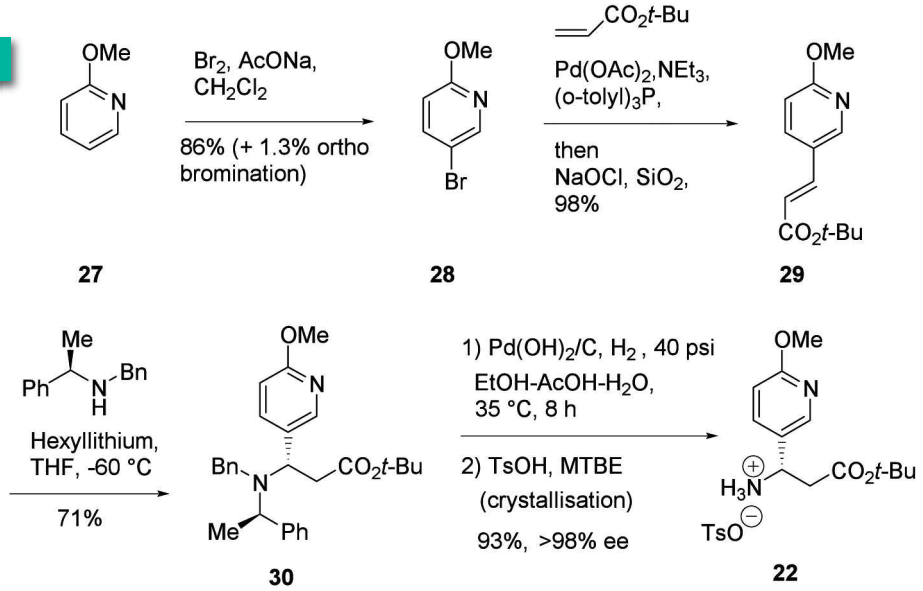
Scheme 4



The initial operations of the retrosynthetic analysis of **20d** logically excised the imidazolidinone ring at the indicating bonds, leading to two primary amine precursors, **21** and **22** (Scheme 4). In the forwards direction, synthesis of amine **21** (Scheme 4) commenced with opening of the *N*-Boc-2-pyrrolidinone **23** by the anion derived from dimethyl methylphosphonate. In the best experimental conditions [$(MeO)_2P(O)Me$ added to a solution of LDA, **23** next added at -60 °C and temperature maintained between -60 °C and -40 °C], the β-keto phosphonate **24** was isolated in 80-85% yield. The advantage of using β-keto phosphonate **24** in place of a simple methylketone is that it will allowed, due to the increase in the acidity of the α vs α′ protons (see **24** in Scheme 4), the perfect control of the regioselectivity of the Friedländer reaction. Indeed, reaction of **24** with 2-amino-3-pyridinecarboxaldehyde **7** proceeded smoothly and selectively to provide naphthyridine **25** in excellent yield. The sequence to amine **21** was then completed in two additional steps. Ring-selective reduction of **25** was best accomplished using Rh/C as catalyst. After crystallisation of the crude material, tetrahydronaphthyridine **26** was isolated in 85% yield and 99.8 wt % purity. Acidic *N*-Boc deprotection then completed the sequence, providing the key amine intermediate **21** in good overall yield.

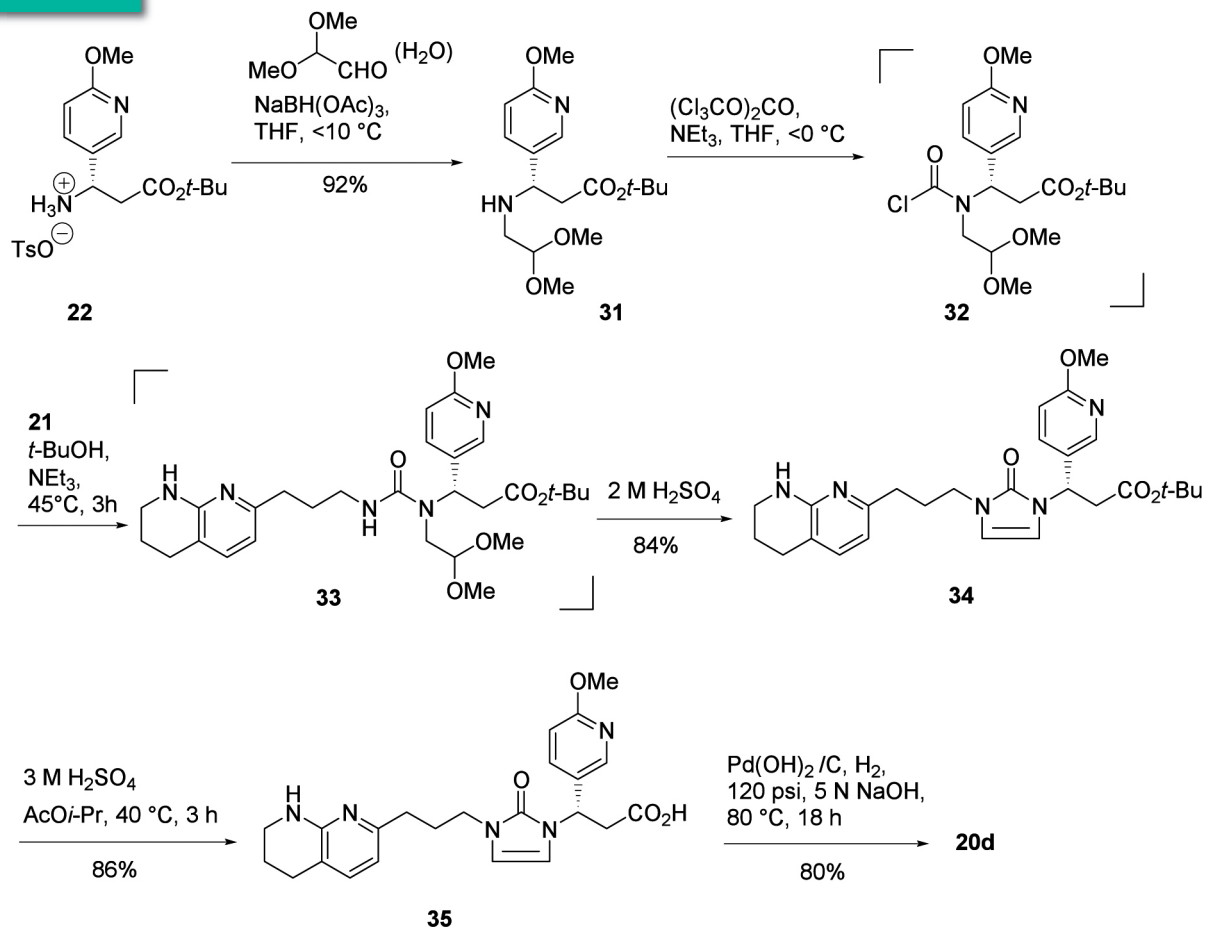
The way for the preparation of the second amine fragment **22** is shown in Scheme 5. The key step is the Michael addition of the lithium salt of *(R)*-(+)-α-methylbenzylamine to α,β-unsaturated *tert*-butyl ester **29** using the Davies protocol. Ester **29** was prepared from 2-methoxypyridine **27** in two steps featuring a para selective bromination (→ **28**) followed by a Heck coupling reaction. The treatment of the latter reaction involved an oxidative step to eliminate tri-o-tolylphosphine, which was found to be a poison of the subsequent hydrogenation step. To obtain a good diastereoselectivity, Davies reaction was best accomplished at low temperature to give the addition product **30** in 71% isolated yield. After removal of both the benzyl and methyl benzyl groups, amine **22** was isolated in 97% yield and 95-96% ee. Finally, tosylate salt formation proceeded in high yield (97%) while upgrading the ee of **22** to >98%.

Scheme 5



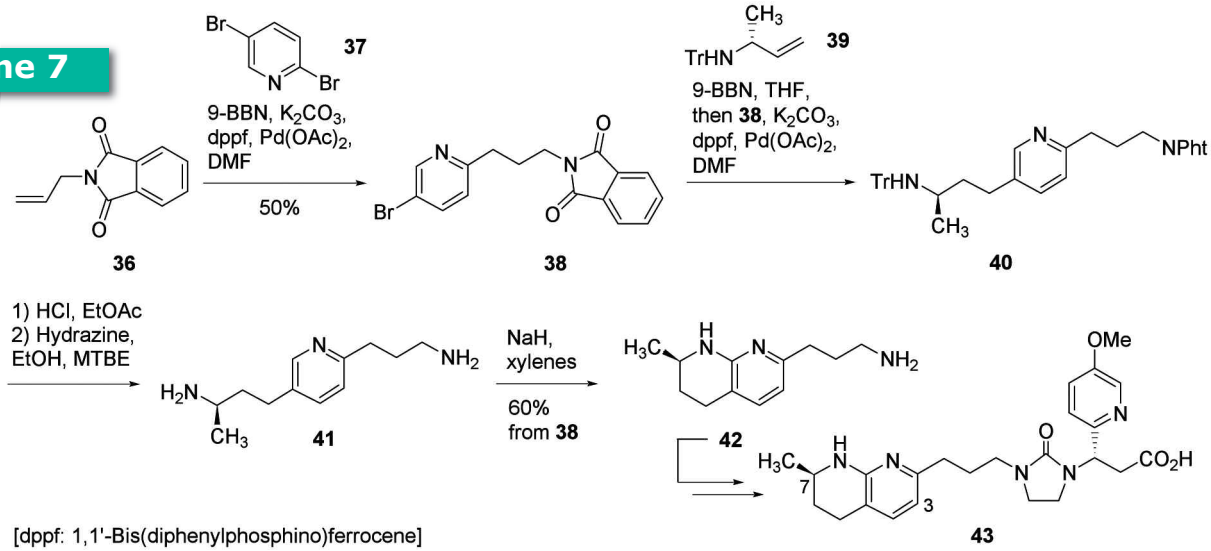
With both fragments **21** and **22** in hand, the final construction of the imidazolidinone ring could now be envisaged. The most successful route among those attempted to reach this goal is shown in Scheme 6. Condensation of dimethoxyacetaldehyde with amine **22** under reductive conditions afforded secondary amine **31** in high yield. The latter was then submitted to the action of triphosgene (0.41 equiv) to give the chlorocarbamate **32**, which, without isolation, was reacted with amine **21**. The resulting urea **33** was not isolated but directly treated with dilute sulphuric acid to provide imidazoline-2-one **34** in good overall yield. Subsequent acidic hydrolysis of the tert-butyl ester function of **34** afforded acid **35**, the enantiopurity of which could be easily upgraded to >99.8% ee by crystallisation. Finally, hydrogenation of the imidazoline-2-one ring completed the synthesis of drug candidate **20d**.

Scheme 6



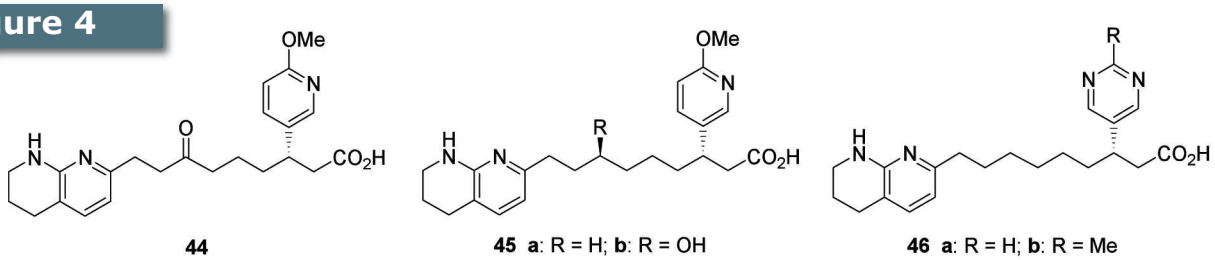
Further studies concentrated on the synthesis of close derivatives of **20d** having a methyl group at C3 or C7 of the THN moiety. Among the small series of compounds prepared, compound **43** (Scheme 7) appeared as the most interesting one.[10] Compared to **20d**, this compound exhibited reduced clearance and improved bioavailability both in dog and monkey. When administered to young, rapidly growing rats by minipump infusion over ten days, it proved to be as efficacious as compound **20d** for inhibiting bone resorption. Its synthesis followed the synthetic plan previously validated for compound **20d**. The preparation of the chiral key amine intermediate **42** is detailed in Scheme 7. Suzuki coupling of phtalimido-protected allylamine **36** with 2,5-dibromopyridine **37** afforded **38**, which was subsequently coupled with alkene **39** (prepared in four steps from D-alanine methyl ester, 93% yield) to deliver the bis-protected diamine **40**. Bis-deprotection of the latter (→ **41**) followed by an intramolecular Chichibabin reaction achieved the preparation of the key amine **42**.

Scheme 7



In parallel with the work reported above, and with the aim of finding a compound that could be effective in humans following once-a-day administration, studies also focused on the preparation of RGD chain-shortened mimetics without inclusion of a cyclic constraint. Exploration in this direction led to the identification of several $\alpha_V\beta_3$ antagonists with good to excellent pharmacokinetic profiles.[11-15] The structures of the most promising compounds are shown in Figure 4.

Figure 4

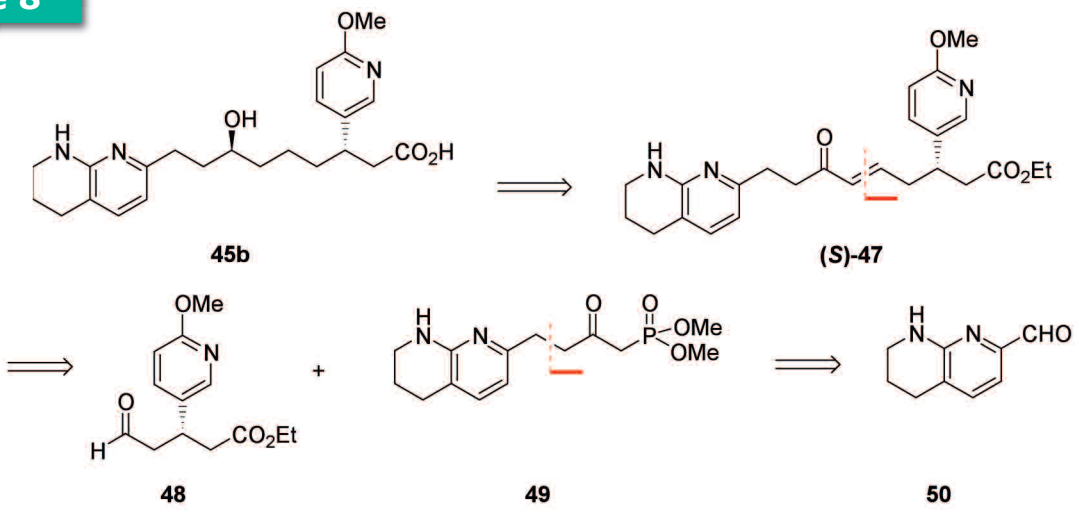


[10] M. J. Breslin, M. E. Duggan, W. Halczenko, G. D Hartman, L. T Duong, C. Fernandez-Metzler, M. A Gentile, D. B. Kimmel, C-T. Leu, K. Merkle, T. Prueksaritanont, G. A. Rodan, S. B. Rodan, J. H. Hutchinson, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4515-4518.
[11] P. J. Coleman, B. C. Askew, J. H. Hutchinson, D. B. Whitman, J. J. Perkins, G. D. Hartman, G. A. Rodan, C-T. Leu, T. Prueksaritanont, C. Fernandez-Metzler, K. M. Merkle, R. Lynch, J. J. Lynch, S. B. Rodan, M. E. Duggan, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2463-2465.
[12] K. M. Brashear, C. A. Hunt, B. T. Kucer, M. E. Duggan, G. D. Hartman, G. A. Rodan, S. B. Rodan, C-T. Leu, T. Prueksaritanont, C. Fernandez-Metzler, A. Barrish, C. F. Homnick, J. H. Hutchinson, P. J. Coleman, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3483-3485.
[13] J. Wang, M. J. Breslin, P. J. Coleman, M. E. Duggan, C. A. Hunt, J. H. Hutchinson, C-T. Leu, S. B. Rodan, G. A. Rodan, L. T. Duong, G. D. Hartman, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1049-1052.
[14] A. E. Zartman, L. T. Duong, C. Fernandez-Metzler, G. D. Hartman, C-T. Leu, T. Prueksaritanont, G. A. Rodan, S. B. Rodan, M. E. Duggan, R. S. Meissner, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1647-1650.
[15] P. J. Coleman, K. M. Brashear, B. C. Askew, J. H. Hutchinson, C. A. McVean, L. T. Duong, B. P. Feuston, C. Fernandez-Metzler, M. A. Gentile, G. D. Hartman, D. B. Kimmel, C-T. Leu, L. Lipfert, K. Merkle, B. Pennypacker, T. Prueksaritanont, G. A. Rodan, G. A. Wesolowski, S. B. Rodan, M. E. Duggan, *J. Med. Chem.* **2004**, *47*, 4829-4835.

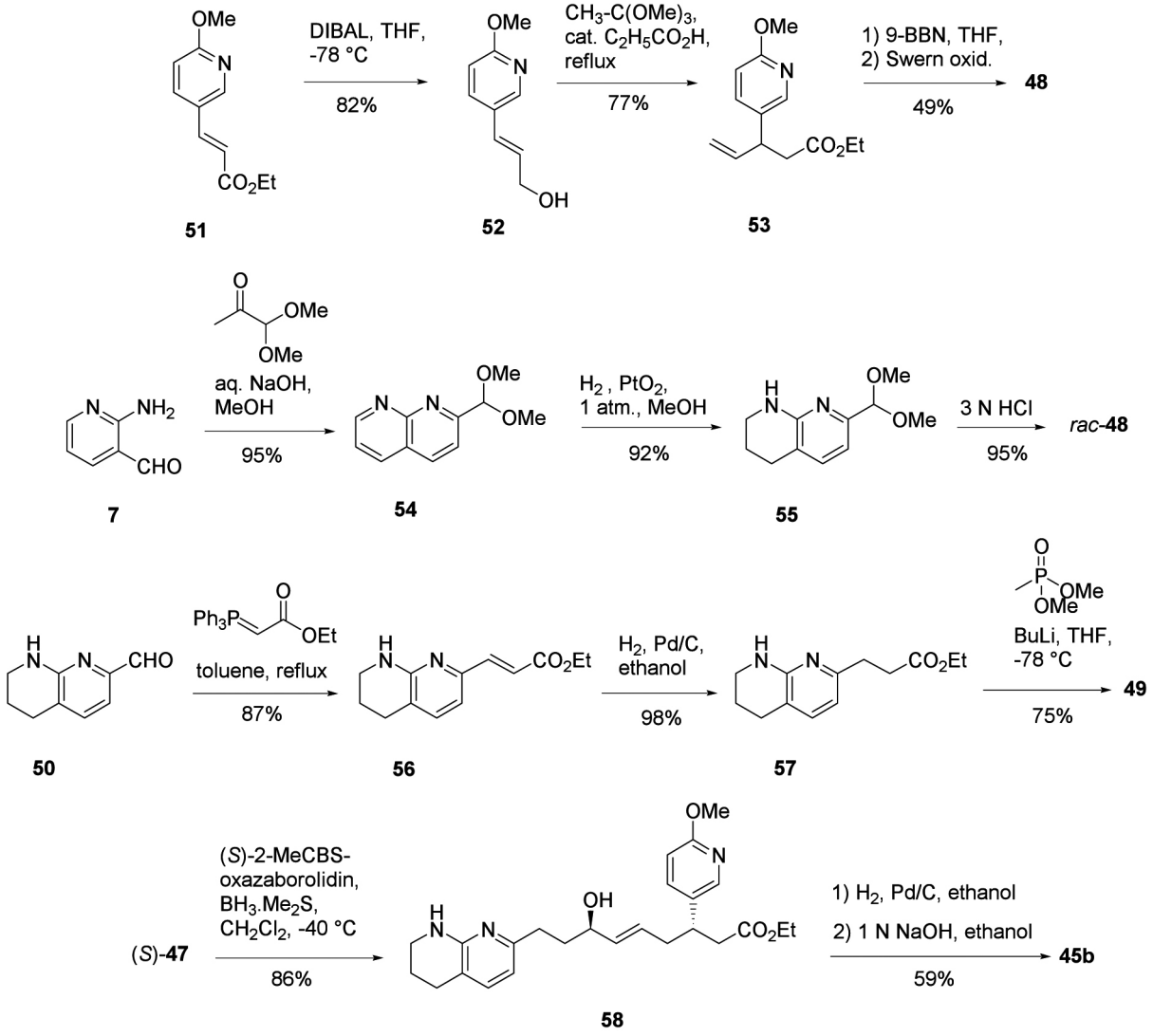
Compound **44**[14] was highly selective for the $\alpha_V\beta_3$ integrin (SPAV3 IC_{50} 0.65 nM)) and was potent in the OCFORM assay (IC_{50} 39 nM). In the dog it displayed good oral availability, low clearance, and excellent plasma half-time (9 h); however, it was highly bound by human proteins with a free fraction of less than 1%. Compound **45a**[15] demonstrated increased potency in both the SPAV3 and OCFORM assays (IC_{50} 0.16 and 12 nM, respectively) and showed a decreased in plasma protein binding. The more polar **45b**[14], while maintaining an excellent pharmacokinetic profile, had a 7% plasma free fraction and thus appeared more potent than the other two analogues.
The binding affinities of **46a** and **46b**[15] to $\alpha_V\beta_3$ were excellent (IC_{50} = 0.07 and 0.08 nM, respectively, in SPAV3 assays). They had a plasma free fraction of 6 and 4%, respectively. When dosed for dogs, both compounds provided excellent bioavailability, low clearance and a good half time. **46b** had an exceptional pharmacokinetic profile in the rat. Remarkably, the methyl group on the pyrimidine of **46b** reduced plasma clearance in the rat by 8-fold and in the monkey by 4-fold. When evaluated in an OVX assay, both compounds had significant effects on bone mineral density determined at the distal femoral metaphysic (DFM BMD). In particular, **46b** was fully efficacious in BMD when administered orally at 3 mg/kg/day in the rat OVX model (DFM BMD 173 mg/cm2; +10.6% from OVX + vehicle). Assays also demonstrated that both **46a** and **46b** are potent inhibitors of bone resorption in vivo. Moreover, metabolism studies on compound **46a** and **46b** indicated that only once-daily oral administration in man would be necessary to provide sufficient plasma coverage for the treatment of osteoporosis. Because of their favourable profiles, these compounds were selected as development candidates for the treatment of osteoporosis. Synthesis of compounds **45b** and **46a**,**b** are reported in Schemes 8-9 and Scheme 10, respectively.

Synthesis of alcohol **45b** was envisaged from enone precursor *(S)*-**47**, the disconnection of which led to the two simplified fragments **48** and **49**, the latter would be derived from aldehyde **50** (Scheme 8).[14] The synthesis of aldehyde *rac*-**48** (Scheme 9) was readily accomplished from unsaturated ester **51** following a four-step sequence of reactions. β-Keto phosphonate **49** was prepared from aldehyde **50**, itself efficiently prepared from 2-aminopyridine-3-carboxaldehyde **7** through a sequence of three reactions featuring a Friedländer cyclisation, a ring-selective reduction and a final acetal hydrolysis (Scheme 9). Aldehyde **50** was submitted to the action of ethyl (triphenylphosphoranylidene)acetate to give the unsaturated ester **56**. Hydrogenation of **56** to give **57** followed by addition of the lithium anion of dimethyl methyl phosphonate afforded the desired intermediate **49**. Key fragments **48** and **49** were then condensed (K_2CO_3, DMF, 64%) to give enone **47**. The enantiomers of **47** were separated on a Chiralcel® AS column and enantiomer *(S)*-**47** subsequently reduced to the corresponding alcohol 58 (de >90%) with (S)-2-methyl-CBS-oxazaborolidine. Finally, a reduction/saponification sequence afforded the target alcohol **45b**.

Scheme 8

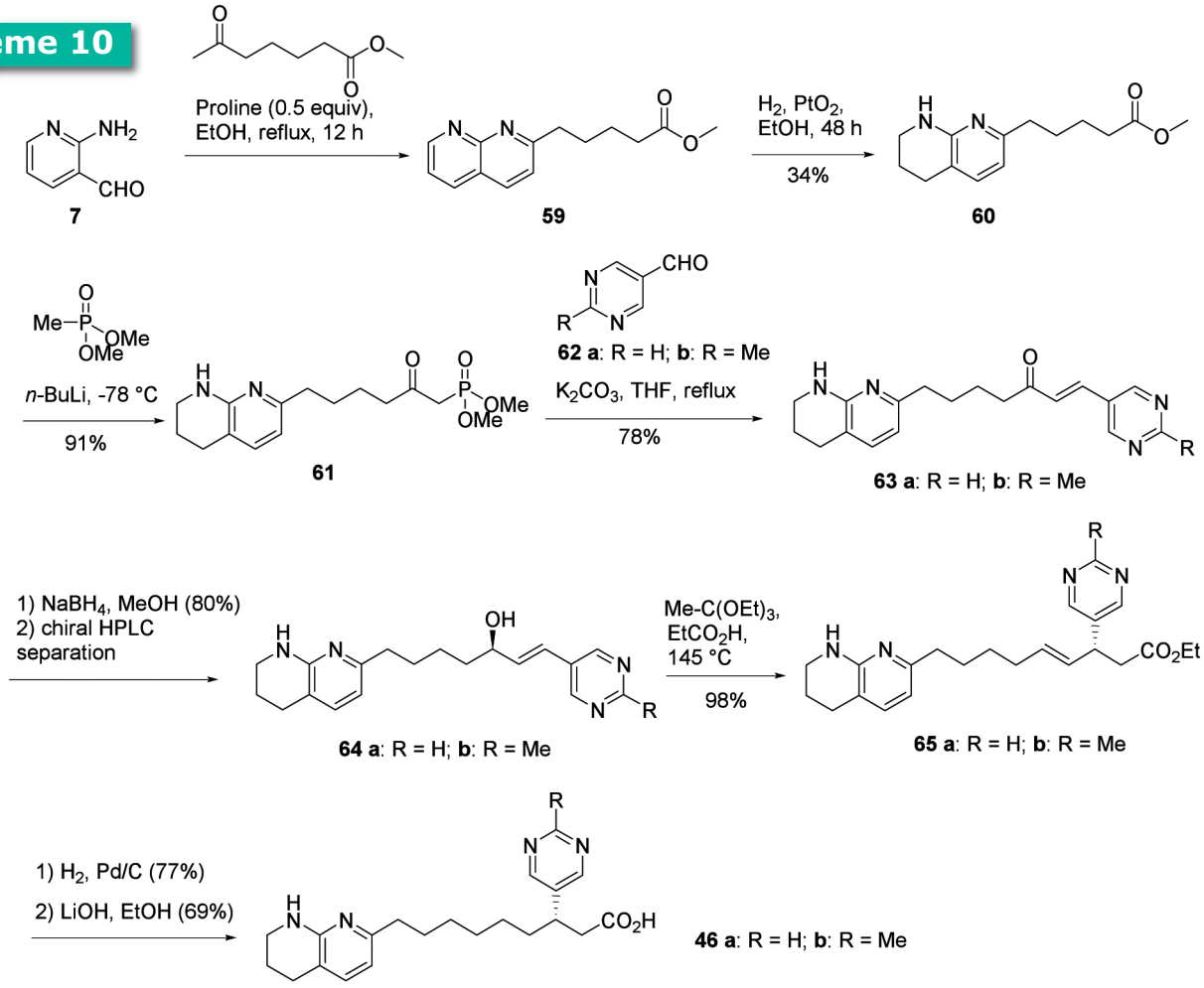


Scheme 9



For the synthesis[15] of compounds **46a** and **46b**, β-keto phosphonate **61**, prepared from 2-aminopyridine-3-carboxaldehyde **7** in three steps was first reacted with aldehydes **62a** and **62b** to provide enones **63a** and **63b**, respectively. Reduction of these enones with $NaBH_4$ led to the corresponding allylic alcohols which were resolved by chiral HPLC on a Chiralpak AD column. Next, chiral alcohols **64a** and **64b** were each submitted to a Claisen-Johnson rearrangement to provide unsaturated esters **65a** and **65b**. Target compounds **46a** and **46b** were finally generated by olefin hydrogenation and ester saponification (Scheme 10).

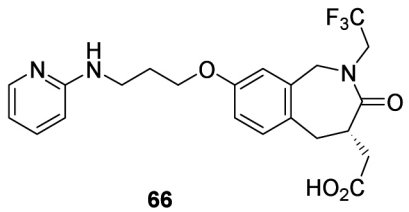
Scheme 10



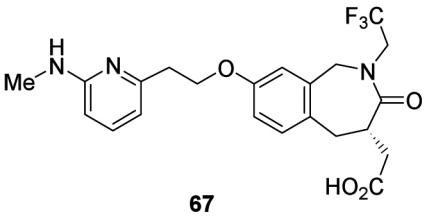
On the basis of the published crystal of the extracellular domain structure of integrin $\alpha_V\beta_3$, docking analyses of several non peptide antagonists of integrin $\alpha_V\beta_3$ allowed the Merck group to develop a binding model.[16] Results of docking analyses of antagonist **46a** to $\alpha_V\beta_3$ were in agreement with this model.[15] In particular, they revealed that the basic naphthyridine interacts with the carboxylic acid side chain of Asp150 in α_V, whereas the C-terminal carboxylic acid is engaged in a salt bridge with Arg214 in β_3. In addition, an energetically favourable Π-Π stacking interaction is found between the Tyr178 side chain of α_V and the 3-pyrimidinyl substituent. This interaction is made possible by the flexible extended conformation adopted by compound **46a**, which also provides sufficient length to allow for the key electrostatic interactions of the C- and N-termini with the protein.
NMR conformational analyses, mainly of cyclic peptides, led researchers at SmithKline Beecham Pharmaceuticals to think that a conformation of the RGD sequence containing turns about both Gly and Asp could be favourable for selective binding to $\alpha_V\beta_3$. Several compounds embodying the 1,4-benzodiazepine ring, hypothesised to act as a Gly-Asp mimic, were thus synthesised and tested.[17-19] Starting from selected first-generation compounds, subsequent SAR and biological studies finally led to identification of two highly selective $\alpha_V\beta_3$ antagonists, i.e **66** and **67** (Figure 5).[20-21]

[16] B. P. Feuston, J. C. Culberson, M. E. Duggan, G. D. Hartman, C-T. Leu, S. B. Rodan, *J. Med. Chem.* **2002**, *45*, 5640-5648.
[17] R. M. Keenan, W. H. Miller, C. Kwon, F. E. Ali, J. F. Callahan, R. R. Calvo, S-M. Hwang, K. D. Kopple, C. E. Peishoff, J. M. Samanen, A. S. Wong, C-K. Yuan, W. F. Huffman, *J. Med. Chem.* **1997**, *40*, 2289-2292.
[18] W. H. Miller, W. E. Bondinell, R. D. Cousins, K. F. Erhard, D. R. Jakas, R. M. Keenan, T. W. Ku, K. A. Newlander, S. T. Ross, R. C. Haltiwanger, J. Bradbeer, F. H. Drake, M. Gowen, S. J. Hoffman, S-M. Hwang, I. E. James, M. W. Lark, B. Lechowska, D. J. Rieman, G. B. Stroup, J. A. Vasko-Moser, D. L. Zembryki, L. M. Azzarano, P. C. Adams, K. L. Salyers, B. R. Smith, K. W. Ward, K. O. Johanson, W. F. Huffman, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1807-1812.
[19] M. W. Lark, G. B. Stroup, S-M. Hwang, I. E. James, D. J. Rieman, F. H. Drake, J. N. Bradbeer, A. Mathur, K. F. Erhard, K. A. Newlander, S. T. Ross, , K. L. Salyers, B. R. Smith, W. H. Miller, W. F. Huffman, M. Gowen, *J. Pharmacol. Exp. Ther.* **1999**, *291*, 612-617.
[20] W. H. Miller, D. P. Alberts, P. K. Bhatnagar, W. E. Bondinell, J. F. Callahan, R. R. Calvo, R. D. Cousins, K. F. Erhard, D. A. Heerding, R. M. Keenan, C. Kwon, P. J. Manley, K. A. Newlander, S. T. Ross, J. M. Samanen, I. N. Uzinskas, J. W. Venslavsky, C. C-K. Yuan, R. C. Haltiwanger, M. Gowen, S-M. Hwang, I. E. James, M. W. Lark, D. J. Rieman, G. B. Stroup, L. M. Azzarano, K. L. Salyers, B. R. Smith, K. W. Ward, K. O. Johanson, W. F. Huffman, *J. Med. Chem.* **2000**, *43*, 22-26.
[21] M. W. Lark, G. B. Stroup, R. A. Dodds, R. Kapadia, S. J. Hoffman, S-M. Hwang, I. E. James, B. Lechowska, X. Liang, D. J. Rieman, K. L. Salyers, K. Ward, B. R. Smith, W. H. Miller, W. F. Huffman, M. Gowen, *J. Bone Miner. Res.* **2001**, *16*, 319-327.

Figure 5



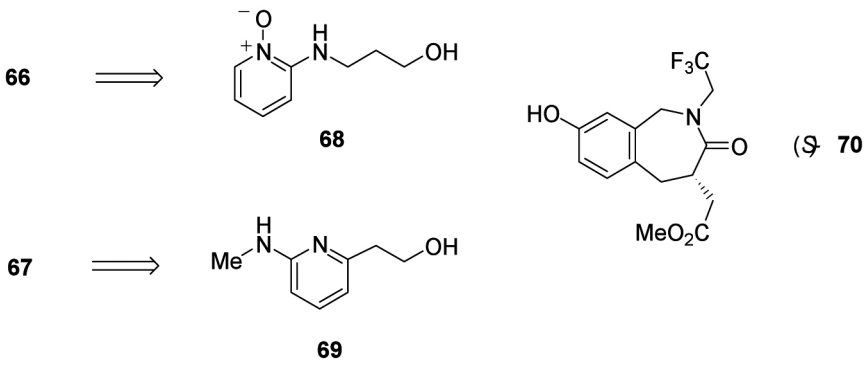
66



67

In an vitro human osteoclast resorption assay, compounds **66** and **67** displayed IC_{50} values of 29 nM and 11 nM, respectively. In an in vivo thyroidectomised-parathyroidectomised rat model of bone resorption, these compounds, on continuous intravenous infusion, had EC_{50} = 35 mM and 20 mM, respectively. On twice-daily oral administration in the ovariectomised rat both compounds inhibited bone loss in a dose-dependent fashion. At the highest dose (60 mg/kg for **66** and 30 mg/kg for **67**), inhibition was greater than 50%. The synthesis of **66** and **67** are reported below in Schemes 11-14. As depicted in Scheme 11, the backbones of these compounds were constructed by assemblage of the pyridine derivatives **68** and **69** with the *(S)*-enantiomer of benzazepine **70**. The preparation of *(S)*-**70** (Scheme 12) commenced with NBS bromation of commercially available 4-bromo-3-methylanisole **71** to afford the dibromo derivative **72**. Benzylic bromide substitution with the sodium salt of N-Boc-protected trifluoroethylamine (→ **73**) followed by a Heck coupling reaction with dimethylitaconate delivered compound **74**, which was transformed to racemic **75** through a sequence of three reactions involving double bond reduction, N-Boc deprotection and lactamisation. The racemic **75** was then resolved into its enantiomers by chiral HPLC on a Daicel Chiralcel OJ column and the *(S)*-enantiomer ultimately O-Me deprotected to afford *(S)*-**70**.

Scheme 11



66

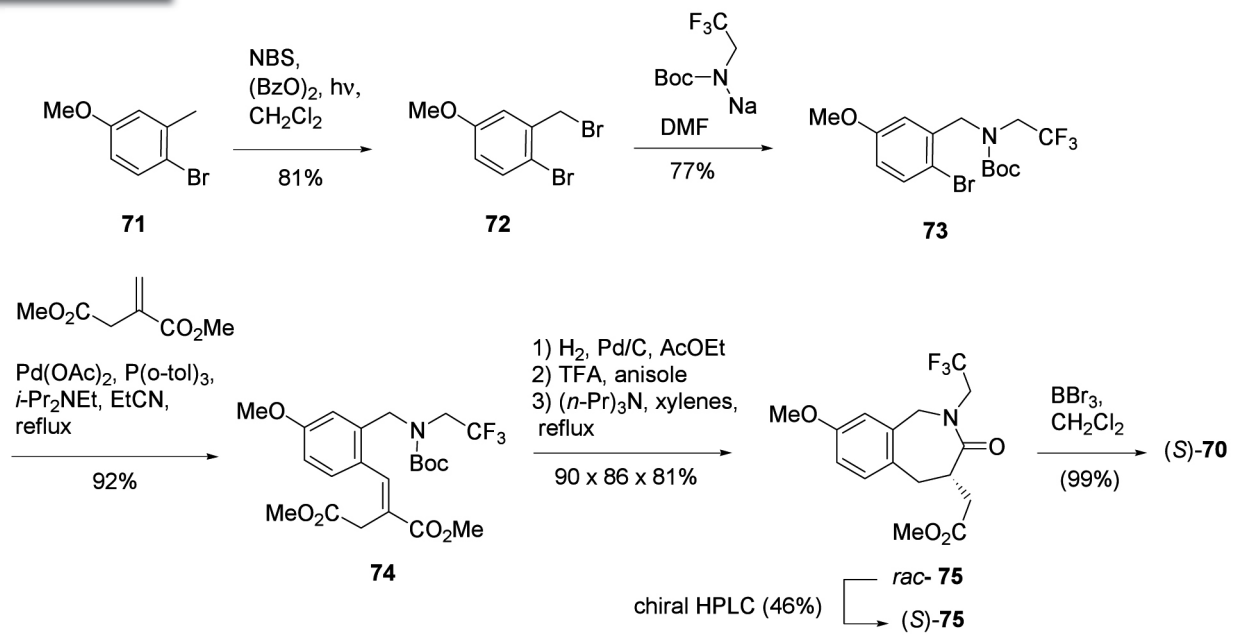
68

67

69

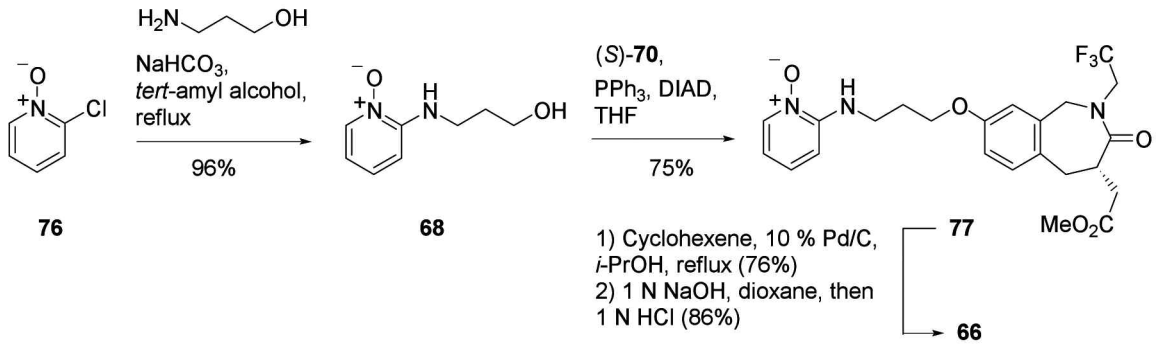
(S)- 70

Scheme 12

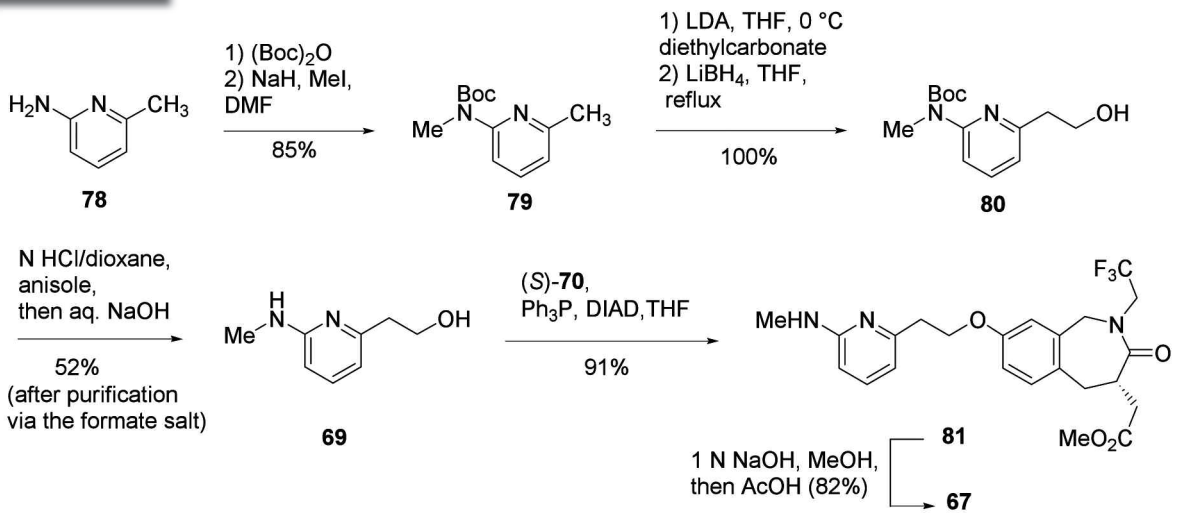


The synthesis of compound **66** (Scheme 13) began with a Mitsunobu reaction between *(S)*-**70** and alcohol **68** prepared from commercially available 2-chloropyridine *N*-oxide hydrochloride **76**. Reduction of the N-O bond of the resulting ether derivative **77** followed by ester saponification achieved the synthesis of **66**. For the synthesis of compound **67** (Scheme 14), the key aminopyridine-alcohol **69** was first prepared from 2-aminopicoline **78**. Thus, *N*-Boc protection of **78** followed by *N*-methylation gave aminopyridine **79**, next transformed to alcohol **80** through an acylation-reduction sequence. Reaction of **69** with *(S)*-**70** in a Mitsunobu reaction afforded **81**, the saponification of which delivered the target compound **67**.

Scheme 13



Scheme 14



Conclusion

The search for selective antagonists of the $\alpha_V\beta_3$ receptor has allowed the identification of several compounds with excellent in vitro profile, a significant unbound fraction in human plasma and good pharmacokinetics in rat, dog and monkey. Moreover, some of these compounds proved also to be effective at reducing bone resorption in a rat model of osteoporosis. The discovery of such orally active $\alpha_V\beta_3$ antagonists opens a new therapeutic route for the treatment of postmenopausal osteoporosis and one may believe that explorations in this domain will increase in a near future.

Acknowledgment

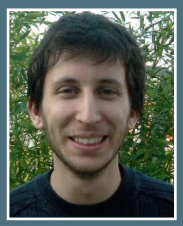
I would like to thank my colleague Pr. J. LEBRETON for his attentive and critical lecture of the manuscript.




Presentation of a chemistry biology team

Alexandrine LUSEAU, Assistante chef de projet chez ATLANTIC BONE SCREEN depuis Juin 2010, est titulaire d'un doctorat en Immunocancérologie. Dans le cadre de cette formation, elle a travaillé au sein d'une équipe de recherche INSERM s'intéressant de près à une tumeur spécifique de la moelle osseuse, le Myélome Multiple. Après de multiples expériences autour de la recherche sur le cancer, elle a rejoint ABS où elle est en charge du développement de modèles tumoraux cellulaires et participe à la réalisation technique des études clients.

Alexandrine LUSEAU *, Assistant Project Manager at ATLANTIC BONE SCREEEN Company since June 2010, has a PhD degree in Immunology and Cancer Research. During these studies, she worked in a research team at INSERM involved in a specific bone marrow tumor, the Multiple Myeloma. After several experiences concerning Cancer Research issues, she joined ABS where she is in charge of tumor models development and takes part to the technical realization of customers' studies.*



Eden OCHOA, est Technicien de Recherche et Développement en Chimie chez ATLANCHIM PHARMA depuis Septembre 2010. Titulaire d'une licence professionnelle Recherche et Développement option chimie fine, il a connu dans son parcours deux expériences professionnelles marquantes dans le domaine de la synthèse chimique à façon. A son actif, il compte ainsi comme réalisations la synthèse totale de deux dérivés de pyrazolotriazine et la synthèse à façon de produits phytosanitaires. Il maîtrise à la fois la réalisation des synthèses organiques, leur purification ainsi que les analyses.

Eden OCHOA*, is Technical expert in Research and Development in Chemistry at ATLANCHIM PHARMA Company since September 2010. Holder of a bachelor in Research and Development with a specialization in fine chemistry, he achieved two main professional experiences in the field of custom chemical synthesis. To his credit, he realized total synthesis of two pyrazolotriazine derivatives and custom synthesis of phytosanitary products. He has full command of realization of organic synthesis, of their purification and of the analysis.*

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