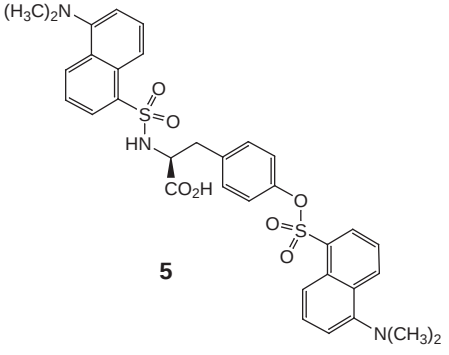
One analogue discovered, didansyltyrosine (**5**), has a K_i value of 1.3 μM, demonstrating that combinatorial chemistry and parallel synthesis techniques are complementary to structure-based methods for the discovery of novel enzyme inhibitors that have no obvious similarity to the enzyme substrate.



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Potential use of infrared microspectroscopy to study drug-related structural changes in bone

Bone tissue is a complex structure resulting from the intense activities of many cell lineages (e.g. osteoblasts, osteoclasts). The different cell types are interconnected and communicate via soluble or membranous mediators. Thus, a very large cytokine network provides for bone development and allows bone integrity to be conserved during life. Such mediators (cytokines, hormones and ions) can be released from the bone matrix as a result of osteoclastic bone resorption, a component of the normal remodelling process necessary to maintain bone integrity.

Calcium phosphate (Ca-P)-mineral phases of bone are composed of a poorly carbonated apatite. These phases play a key role in the mechanical properties of the bone as well as in several biological processes (such as homeostasis of phosphocalcic metabolism and cell regulation), and can be altered by Ca-P mineral metabolism disorders. Bisphosphonates, analogues of endogenous pyrophosphates, are highly potent inhibitors of osteoclastic bone resorption and decrease tumour cell adhesion and represent an important class of drugs for the treatment of patients with bone diseases. However, bisphosphonates are known to be avidly bound to hydroxyapatite crystals and can alter the chemical composition and structure of biological apatite crystals of bone. In addition, the long biological half-life of bisphosphonates might be detrimental to the metabolism of the skeleton.

Although bone is one of the most common metastatic sites in human breast and prostate cancers, the molecular mechanisms by which tumour cells induce osteolytic metastases are still not fully understood. It is accepted that tumour cell adhesion to the bone matrix and the release of soluble mediators from tumour cells stimulate bone resorption. Newly synthesized drugs, hormones and some cytokines that could be used for therapeutic purposes in bone disease might affect bone crystal structure and consequently its mechanical and biological functions.

Infrared spectroscopy has provided important information on the fine structure and the physicochemical characteristics of biological apatite-like bone. Information can be obtained concerning the nature of the mineral phases and their mineral content (i.e. phosphate, carbonate), mineral crystallinity and maturity, and the content of the organic matrix (secondary structure of proteins).

The application of resolution-enhancement techniques and curve-fitting methods has led to qualitative and semi-quantitative information regarding PO_4^{3-}, HPO_4^{2-} and CO_3^{2-} molecular environments. This information has been found to provide very sensitive indicators of bone mineral maturation and alterations in bone metabolism [Rey, C. *et al.* (1995) *Cells Mater.* 5, 345–356] and in the organic matrix structure [Paschalis, E.P. *et al.* (1997) *J. Bone Miner. Res.* 12(Suppl.), 229]. Similar information is also available at the microscopic level using infrared microspectroscopy. This information provides valuable molecular information on the chemistry of tissues and their spatial variations (i.e. mapping) at defined anatomical and morphological locations from tissue sections [Bohic, S. *et al.* (1998) *C. R. Acad. Sci. Paris Life Sci.* 321, 865–876; Boskey, A.L. *et al.* (1998) *Bone* 23, 187–196; Kalasinsky, V.F. *et al.* (1996) *App. Spectrosc. Rev.* 31, 193–249; LeVine, S.M. *et al.* (1994) *Am. J. Pathol.* 145, 1041–1047].

The combination of Fourier-transform infrared microspectroscopy (FT-IR) spectroscopy and light microscopy enables infrared transmission analysis of sections (0.5–5.0 μm thick) of embedded biological samples placed on suitable non-absorbing infrared windows, or examination, by reflectance, of the bulk sample placed on a gold mirror. The sample microstructure can be viewed from histology sections through the microscope and sample areas are selected prior to infrared analysis. Chemical mapping can be generated with 10 μm spatial resolution using a motorized stage moving the sample to sequential sites while the infrared beam remains stationary. Infrared spectra can also be recorded from discrete sites of the sections. The methodology for infrared microspectroscopic analysis can be obtained from the papers described later. The utility of infrared microspectroscopy relative to other methodologies for investigating calcified tissues, is that these tissues do not need to be homogenized. In fact, with infrared spectroscopy and X-ray diffraction,

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pulverized material would lead to a loss of spatial resolution, as all the types of bone crystals would become mixed together. Moreover, the commonly used technique of high-resolution transmission electron microscopy requires the analysis of a large number of microscopic sections and fields to obtain information on spatial variation of bone-crystal structure. Consequently, infrared microscopy is a good alternative method for providing insights into spatial and temporal changes in the chemical composition of the bone mineral and bone matrix through the analysis of multiple adjacent sections.

It has recently been demonstrated, using infrared microspectroscopy, that some cytokines (Leukaemia Inhibitory Factor and Oncostatin M) influence bone mineralization *in vivo* [Bohic, S. *et al.* (1998) *J. Bone Miner. Res.* 13, 1619–1632] and *in vitro* [Bohic, S. *et al.* (1998) *Biochem. Biophys. Res. Commun.* 253, 506–513]. In particular, changes in the crystallinity and maturity of bone apatite, in the carbonate environment and in the relative carbonate content were observed. Infrared microspectroscopic analysis has also been used for the evaluation of alendronate, an aminobisphosphonate, for the treatment of osteogenesis [Camacho, N.P. *et al.* (1997) *J. Bone Miner. Res.* 12(Suppl.), 389]. Furthermore, this technique has been used to monitor the decrease in bone quality in beagles following calcitonin treatment [Pienkowski, D. *et al.* (1997) *J. Bone Miner. Res.* 12, 1936–1943].

In these few examples, an alteration in the chemical composition of the bone has been found, probably leading to changes in bone mineral crystal size, solubility or interaction with cells, and proteins that control bone formation and resorption. This important physicochemical information is not detected by routine histology. In addition, infrared microspectroscopy imaging (mapping) appears to be a promising technique to enable a deeper understanding of bone physiopathology and might be useful for the evaluation of the effects of drugs on the bone [Marcott, C. *et al.* (1998) *Cell. Mol. Biol.* 44, 109–115; Miller, L.M. *et al.* (1999) *Synchrotron Radiation News* 12, 21–27].

Work in this field has led to the development of new approaches for studying the mineral and organic phases of bone to investigate the possible influence of drugs such as bisphosphonates, sex steroids, cytokines and other promising new molecules on skeletal disorders. Information gained from infrared microspectroscopy supplements that generated using other techniques in the study of bone material, by providing information on the fine structural changes in bone mineral phases or organic matrix.

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