A Novel Isolation Method of a Stable Crystalline Salt of a Cyclic RGD Peptide Zwitterion[1]

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INTRODUCTION

Cyclic[D-2-aminobutyryl-N^2-methyl-L-arginylglycyl-L-aspartyl-3-(aminomethyl)-benzoic acid (XL118) is the zwitterion of a cyclic RGD peptide that is a potent glycoprotein IIb/IIIa receptor antagonist (Figure 1) (1,2). A significant body of literature has existed on the optimal characteristics of salts of non-peptide organic molecules (3). However, little information is available on the preparation or chemical and physical characterization of salts of peptides. The ability of salts of zwitterions to improve solubility has been presented (4). The authors also describe the general tendency of salts of zwitterions to be quite hygroscopic and the ability to overcome the behavior by forming hydrates (4). As part of the preclinical development of XL118, a novel isolation of a cyclic RGD peptide was identified.

MATERIALS AND METHODS

Materials

XL118 was prepared by Chemical Sciences Section of the DuPont Merck Pharmaceutical Company at the Kilo Lab, Experimental Station, Wilmington, Delaware and was used as received. The water was house-deionized water that was passed through a Nanopure II (Barnstead) ion-exchange cartridge system and had a specific resistance of greater than 17 MΩ-cm. All solvents were HPLC grade. All other reagents were of analytical grade.

Mesylate Salt Preparation

XL118 was suspended in a minimal amount of hot isopropanol. Methanesulfonic acid in isopropanol was added to the XL118 isopropanol suspension in a 10% molar excess. The XL118 dissolved in the acidified isopropanol. As soon as the last of the XL118 dissolved into the acidified isopropanol, continuous boiling of the solution resulted in the nucleation of the white crystalline mesylate salt. If boiling of the isopropanol is not continued after solubilization of the zwitterion, a glue-like material was produced.

Thermal Analysis

The thermal properties were characterized with differential scanning calorimetry (DSC 910, TA Instruments) and thermogravimetric analysis (TGA 2950, TA Instruments) with data analysis via a thermal analyzer (Analyzer 2100, TA Instruments). Heating rates of 5°C/min or 10°C/min were employed for the techniques over a temperature range of 25–300°C for DSC and 25-150°C for TGA.

Polarized Light Microscopy

Microscopic observations were performed by suspending the sample in silicone immersion oil and examining with a polarized light microscope equipped with cross polars (Aristomet Microscope, Wild Leitz).

X-ray Powder Diffraction

X-ray powder patterns were recorded using an automated X-ray diffractometer (APD 3720, Phillips) with copper tube K alpha radiation over a 2θ range of 2–60 degrees with the samples run in a dry state without solvent fixing.

Hygroscopicity

The water content was measured with direct coulometric analysis (Coulometer 684KF, Metrohm). The drug substance was incubated at 85% relative humidity (RH). The humidity was maintained in sealed chambers (Dry Keeper, Samplatec Corporation) with a layer of saturated aqueous potassium chloride solution in contact with excess solid for 85% RH.

RESULTS AND DISCUSSION

The mesylate salt underwent a rapid phase change via hot crystallization. XL118 was insoluble in boiling isopropanol. As soon as XL118 was solubilized in boiling isopropanol following the addition of methanesulfonic acid, a white crystalline salt nucleated instantaneously while isopropanol continued to boil. Elemental analysis of the mesylate salt was in agreement with theoretical values and HPLC found

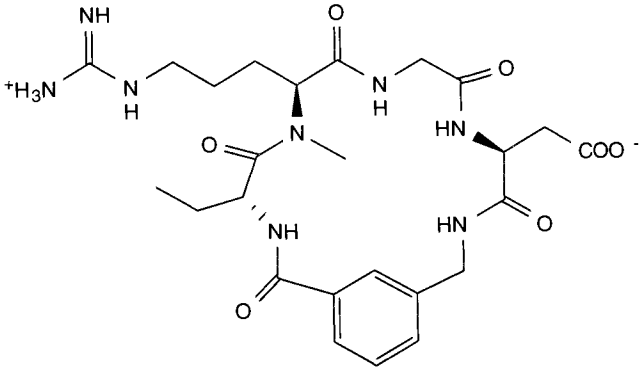

Fig. 1. Chemical structure of cyclic[D-2-aminobutyryl-N^2-methyl-L-arginyl-glycyl-L-aspartyl-3-(aminomethyl)-benzoic acid (XL118).

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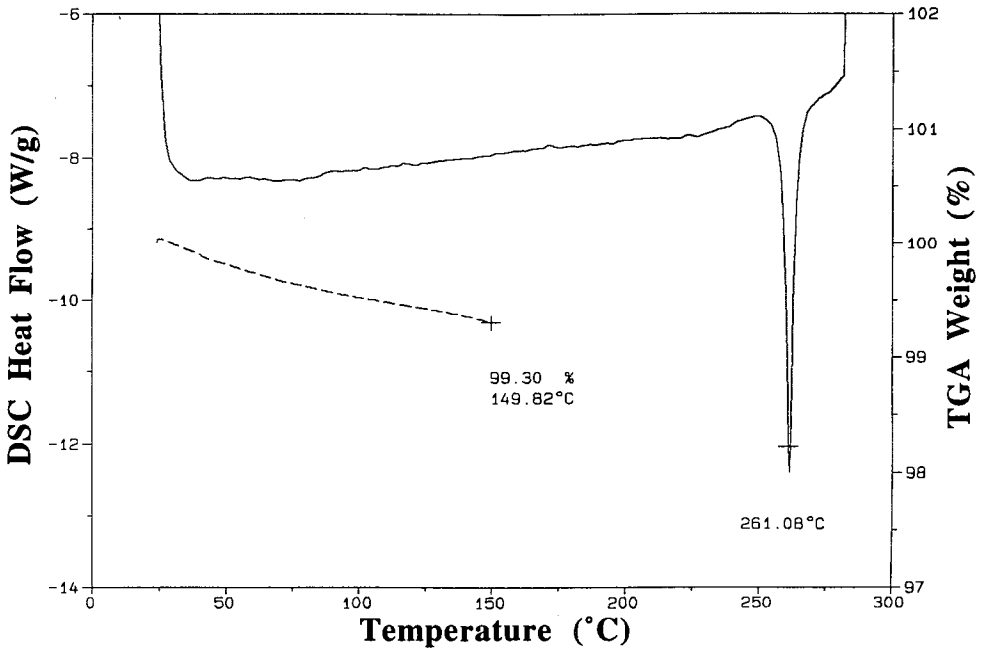

Fig. 2. DSC thermogram (solid line) with a single melt peak at 261.1°C and TGA profile (dashed line) with a weight loss of 0.7% at 150°C of the mesylate salt at 10°C/min.

no degradation or esterification. The mesylate salt was insoluble in its recrystallization solvent, isopropanol. As far as we know, this is the first report of a hot crystallization.

DSC of the mesylate produced a single melt transition at 261.1°C (Figure 2). TGA to 150°C found a weight loss of 0.7% for the mesylate salt (Figure 2). Polarized light microscopy found the mesylate salt to be birefringent with distinct extinctions when rotated under cross polarization consistent with a crystalline material. X-ray powder diffraction pattern found the material to be crystalline with no peaks at 2θ above 40 degrees. The x-ray powder diffraction pattern from 2–40 degrees is presented in Figure 3. The mesylate was nonhygroscopic with the initial water content of 1% remaining unchanged on storage at 85% RH.

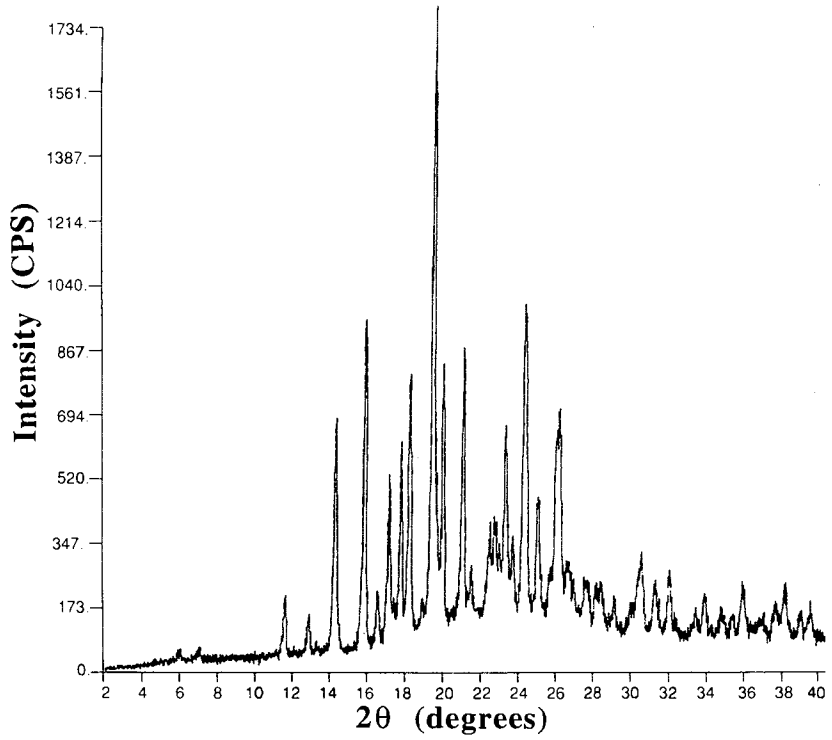

Fig. 3. X-ray powder diffraction pattern of the mesylate salt from 2-40 degrees.

In conclusion, the mesylate salt was crystallized via hot crystallization. The preparation was amenable to large scale synthesis resulting in a drug substance that contained a minimal amount of water. It was crystalline, nonhygroscopic, and water soluble. The mesylate was the preferred crystalline form that entered clinical development.

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