



## Synthesis of Leu-enkephalin using AmphiSpheres™ Resin

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### Introduction

In order to demonstrate that no polyethyleneglycol leaches from AmphiSpheres resins during use, leucine enkephalin, H-Tyr-Gly-Gly-Phe-Leu-NH<sub>2</sub>, was synthesized using AmphiSpheres 20 RAM resin and the peptide cleaved and then subjected to NMR analysis.

### Sample Preparation

The peptide was prepared using a peptide synthesizer on a 0.1 mmol scale using 10 fold excess Fmoc-AA-OH (with standard side chain protection as required) and coupling reagent (HBTU) for coupling reactions. Single 9 minute couplings were used throughout. Deprotection reactions (20% piperidine in DMF) were monitored by conductivity meter. Following peptide assembly and cleavage of the final Fmoc group on the synthesizer the peptide was cleaved from the resin by treating with TFA / TIPS / water 95:2.5:2.5 (v/v) for 2-3 h. Cleavage reagents were removed by rotary evaporation followed by trituration of the resultant solid with

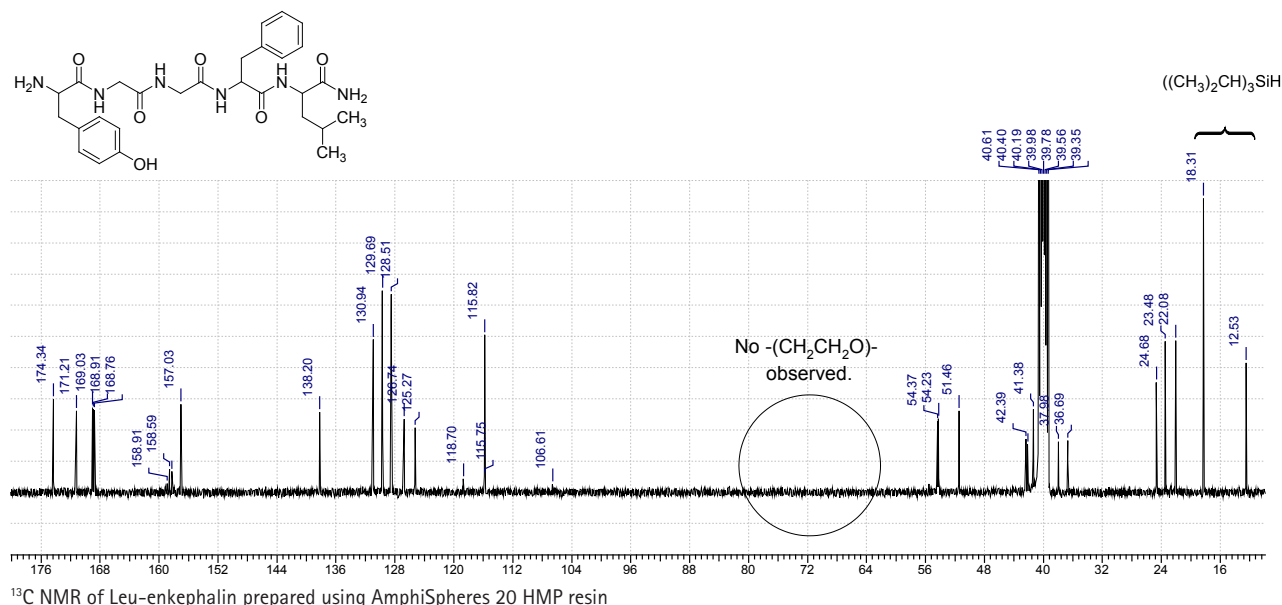
cold ether. The peptide was then lyophilized from water or water / acetic acid mix.

### Results and Discussion

Although the NMR spectrum does show some residual triisopropylsilane (TIPS) used as a scavenger during the cleavage reaction, there is absolutely no sign of PEG chains arising from the resin.

### Conclusions

The excellent stability of AmphiSpheres resin was demonstrated by the complete absence of PEG chains in leucine end product. AmphiSpheres is a significant addition to the StratoSpheres product line from Varian, Inc. AmphiSpheres resins contain hydrophobic polystyrene and hydrophilic polyethyleneglycol components. This imparts a change in the swell characteristics of this amphipathic resin that can help improve synthesis of "difficult" peptide sequences.



These data represent typical results.  
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