

CAN SUPERCRITICAL CO₂ BE USED AS A REVERSIBLE BLOCKING GROUP IN SELECTIVE REACTIONS WITH AMINES?

Dijkstra, Z.J.*⁺, Doornbos, A.R., Weyten, H⁺, Keurentjes, J.T.F.

Process Development Group, Department of Chemical Engineering and Chemistry,
Eindhoven University of Technology,

⁺ Vito (Flemish Institute for Technological Research), Process Technology

INTRODUCTION

In conventional catalysis, substantial effort is put into developing reversible blocking groups for amines, especially in the use of peptide synthesis. The main problems encountered are the low binding efficiency and the reversibility of these blocking groups. Most present methods use additional catalysts and/or coupling agents to prepare or remove these protecting entities. These steps add up to the number of steps that have to be performed, which complicates the total synthesis process. Therefore, simplifying this procedure is important.

Using CO₂ as a protecting group could be a way to solve these problems and simplify the procedure. An amine in the presence of CO₂ forms a carbamate (Figure 1)[1,2]. This carbamate formation is fast and reversible which is a good starting point for the intended use. As CO₂ is a solvent with tuneable properties, changing temperature and pressure will have its influence on the equilibrium in the formation of the carbamate. Preliminary experiments with amines in CO₂ showed us that enzymatic conversion of the amines in CO₂ still occurs. Therefore, to use CO₂ as protecting group, a good understanding of the mechanism and kinetics of carbamate formation is necessary.

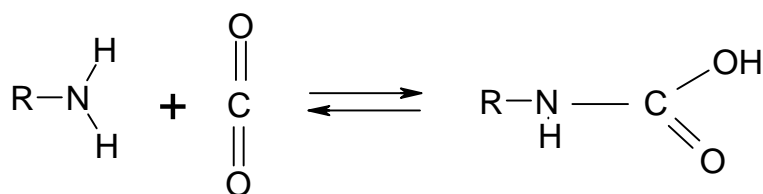


Figure 1 Carbamate formation of amine and carbon dioxide

In this study, the conditions of carbamate formation are investigated with atmospheric and high pressure NMR at different temperatures and pressures. To test the strength of the carbamate binding, two different catalysts are added to test the reactivity.

MATERIALS AND METHODS

Isopropyl amine and hexyl amine were used to evaluate the formation and behaviour of carbamates in scCO₂ and dimethylsulfoxide(DMSO). Atmospheric ¹H NMR and ¹³C NMR experiments with isopropyl amine and hexyl amine were performed in DMSO on a 200 MHz Varian instrument. High pressure NMR experiments were performed with a specially designed high pressure cell on a 500 MHz Varian Oxford instrument [3]. In the reactions,

CLEC-Candida Antarctica Lipase B and Zeolite Y were used. Analysis of reaction products was performed with Gas Chromatography.

RESULTS

Low pressure experiments

The first experiments are performed to test if carbamate formation occurs. The upper parts of both figure 2 and figure 3 show the ^1H NMR and ^{13}C NMR spectra of isopropyl amine in DMSO respectively. After bubbling CO_2 through this solution, the lower spectra appear. With these spectra, it becomes clear that carbamate formation occurs. Additionally, the rising peak in the ^{13}C NMR spectrum comes exactly at the predicted shift that was calculated with ACD Labs NMR Predictor. Another indication of carbamate formation, which is a strongly exothermic reaction, is the increase in temperature when CO_2 is bubbled through the liquid.

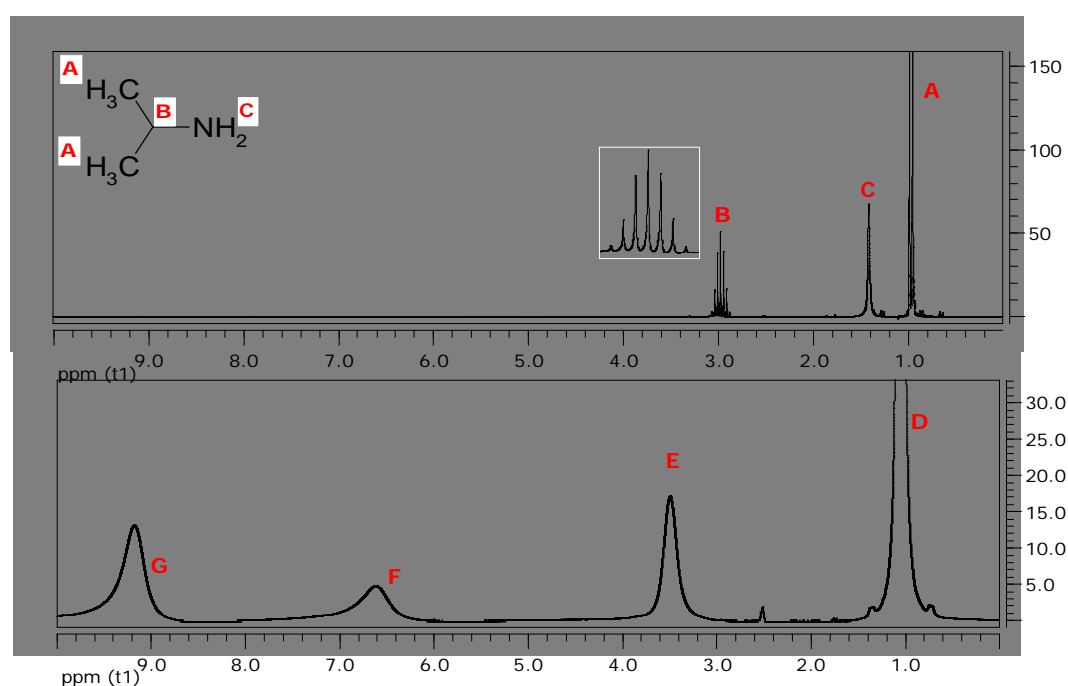


Figure 2 ^1H NMR spectra of isopropyl amine without (upper part) and with CO_2 bubbling (lower part)

The experiments with hexyl amine confirm these results. The proof that a stable chemical bond is formed between the amine and CO_2 is given by TGA-DSC measurements that were combined with a mass spectrometer. The results of these measurements show that the carbamate of hexyl amine and CO_2 becomes unstable at 102°C and CO_2 is liberated. This means that as long as the temperature of the reaction stays below 102°C , the amine is present in the form of carbamate and should not react. However, as soon as the enzyme or zeolite was added (at atmospheric pressure), reaction occurred and CO_2 escaped in the form of gas bubbles. Analysis of the reaction as a function of time does not show significant difference in the initial reaction rate between the reaction performed with and without bubbling CO_2 through the liquid. Apparently, the catalyst can react with both the amine and the carbamate with the same reaction rate or the carbamate is not as stable as expected.

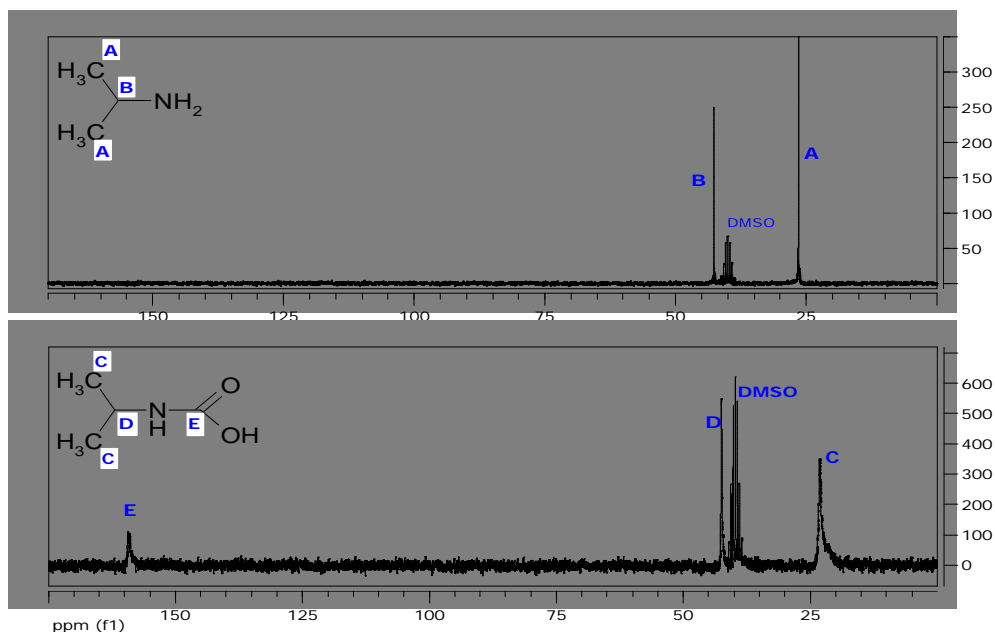


Figure 3 ^{13}C NMR spectra of isopropyl amine without (upper part) and with CO_2 bubbling (lower part)

High pressure experiments

Because CO_2 blocking of amines does not seem to work at low pressure, experiments were performed at elevated pressure. Figure 4 shows the ^1H NMR spectra of isopropyl amine in CO_2 with increasing temperature and pressure. It is difficult to assign all the peaks as the peaks are much smaller and because there are more peaks than would be expected. Interestingly, there is one peak (indicated with the arrow) moving across the spectrum. This movement is due to changes in the CO_2 environment as it keeps the same area and moves back when the temperature is decreased again. The fact that more peaks are present in the spectra than expected, indicates that not only the carbamate is formed. In the literature it is described that at higher temperatures urea derivatives and also isocyanates can be formed [4]. Therefore, it is assumed that the additional peaks in the high pressure spectra can be explained by the presence of these components. As most of the amine is present in its carbamate form, it is expected that the reaction with the amine cannot occur. However, after testing the reaction at high pressure CO_2 , there is again no significant difference in reaction rate compared to the same reaction at atmospheric pressure.

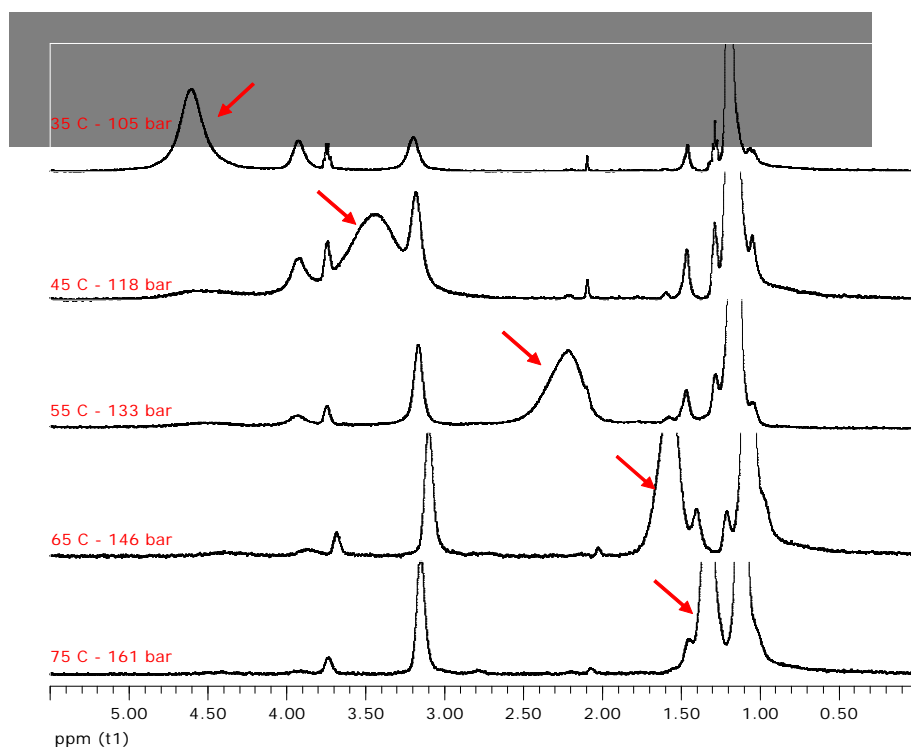


Figure 4 ^1H NMR spectrum of isopropyl amine in CO_2 with increasing temperature and pressure

CONCLUSION

From the experiments described above it becomes clear that carbamate formation occurs by simply CO_2 -bubbling through a solution containing an amine. At higher pressures carbamate formation also occurs. At both atmospheric and high pressure conditions, it is expected that the amine function is blocked for reaction. As reaction still occurs, the situation is more complicated than expected. At this moment, it is clear that carbamate formation cannot be used for blocking amines in reactions in the conditions that were used. However, CO_2 blocking of amine groups by carbamate formation should work when used with proper reaction conditions. Therefore, more effort will be put into finding and optimizing these conditions in such a way that this method can be used in future applications.

ACKNOWLEDGEMENTS

We would like to thank Prof C.J. Elsevier (University of Amsterdam) for his help with the high pressure NMR experiments

REFERENCES

- [1] CAPLOW, M. J of Am Chem Soc, Vol. 90, **1968**, p6795-6803
- [2] KIRSCH, M. KORTH, H., SUSTMANN, R., DE GROOT, H., Chem. Res. Toxicol., Vol.13, **2000**, p.451-461
- [3] GAEMERS, S., LUYTEN, H., ERNSTING, J.M., ELSEVIER, C.J., Magn. Reson. Chem., Vol 37, **1999**, p. 25-30
- [4] WALDMAN, T.E., MCGHEE, W.D., J. Chem. Soc., Chem. Commun, **1994**, p.957-958