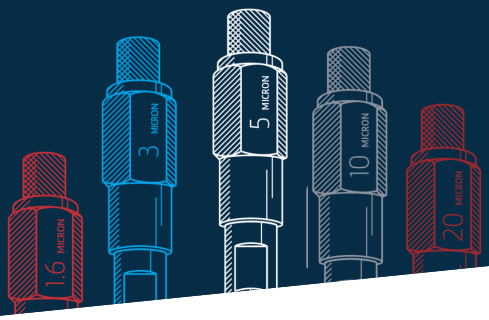
APPLICATION NOTE

Chiral Separation of Key Intermediate used for the Study of LSD1 Mechanism of Action

Dr. Weston J. Umstead, Dr. Chao Liu



DAICEL Chiral Technologies

INTRODUCTION

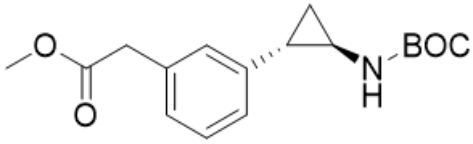


Figure 1: Key n-Boc protected intermediate

Lysine-specific demethylase 1 (LSD1) is an enzyme that removes methyl groups from specific lysine residues of histone H3. Histones are proteins that help guide DNA folding and regulate gene expression by controlling how tightly the DNA is packaged. Dysregulation of LSD1 activity, particularly its overexpression, has been implicated in a variety of cancers. As a result, LSD1 has become a high priority therapeutic target for many major pharmaceutical companies developing targeted small-molecule cancer therapies.

The compound shown in Figure 1, originating from the Liau group at Harvard University[1], represents a key intermediate in the library synthesis of several potential new therapies targeting LSD1. Previously studied compounds from Takeda[2] and Glaxo-Smith Kline[3] determined the shown trans (R,R) stereochemistry to be the most effective at targeting LSD1. Therefore separation from the less effective opposite trans (S,S) enantiomer is important for developing a highly-selective library and a robust set of test results.

This application is focused on establishing such a separation by using Daicel polysaccharide-based chiral stationary phases (CSPs). Method development was conducted in high-performance liquid chromatography (HPLC) mode with normal phase (NP) solvents, and further optimization performed to develop suitable conditions for preparative isolation.

EXPERIMENTAL

Chromatographic Conditions for Separation of n-Boc protected Intermediate	
Column	CHIRALPAK® IK (250 mm x 4.6 mm i.d., 5 µm) Part #: 91325
Mobile Phase	Hexane-Ethanol = 80-20
Flow Rate	1.0 ml/min
Detection	UV 254 nm ref. 450 nm
Temperature	25°C
Sample	1.0 mg/ml in ethanol
Injection Volume	5 µl

Dr. Chao Liu of the Liau Group at Harvard University kindly provided the key n-Boc protected intermediate. The solvents used were all purchased from Scientific Equipment Company (SECO), were HPLC-grade, and were used as-is. Specifically the hexane (hex) used contained 95% n-hexane.

Screening and optimization for HPLC mode was performed on an Agilent 1200 configured with high-pressure mixing quaternary mobile phase delivery system, vacuum degasser, autosampler, temperature controlled column compartment, and photodiode array UV detector. The instrument was controlled by an Agilent ChemStation Version RevB.04.03.

The chiral columns used for screening included CHIRALPAK® IA, IB N-5, IC, ID, IE, IF, IG, IH, IJ, IK, IM, and IN, and were 4.6 mm inner diameter (i.d.) by 250 mm length and a 5 µm particle size.

DISCUSSION

A retention check was performed prior to the start of the screening to ensure reasonable elution was achieved, before committing to a full screen. A single injection was made on IB N-5 with both Hex-EtOH and Hex-IPA = 70-30 (v/v). It was observed that this produced reasonable retention and was thus used for screening.

Several separations were observed with both EtOH and IPA mobile phases on IB N-5, IC, IG, IH, IJ, and IK, with the best separations (based on selectivity), coming on IK using EtOH. To improve the separation for better preparative throughput, a weaker eluting mobile phase of 80-20 = Hex-EtOH was used. This produced the separation seen in Figure 2.

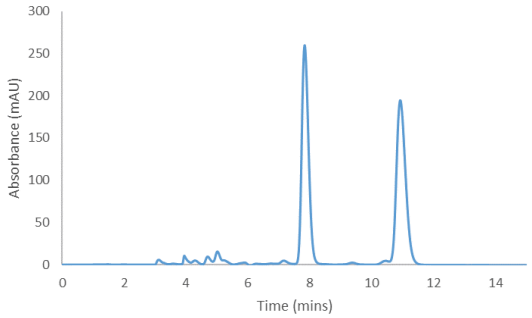


Figure 2: NP-HPLC separation of n-Boc protected intermediate on CHIRALPAK IK

To determine the productivity under preparative conditions, 20 milligrams of material was dissolved in the mobile phase, and a series of increasing injection volumes made (as shown in Figure 3), until the back of peak 1 touches the front of peak 2. Knowing the sample loading (500 µl @ 20 mg/ml) and the cycle time (6 mins), one finds the hourly productivity to be 100 mg on a 4.6x250 mm analytical column.

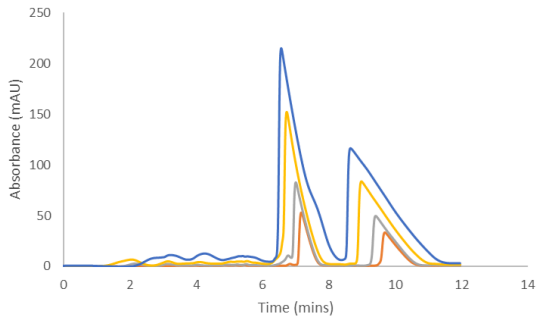


Figure 3: Overloading of n-Boc protected intermediate on CHIRALPAK IK

CONCLUSIONS

Suitable conditions for the separation of the key n-Boc protected intermediate were established using CHIRALPAK IK and a simple normal phase mobile phase. With an hourly productivity of 100 mg on an analytical column, this method is readily scalable to larger preparative columns, enabling the production of hundreds of grams of material.

In parallel, the authors of the Harvard paper identified important structural features that govern a previously undocumented LSD1 deactivation mechanism. By incorporating these structural features into candidate therapeutics, new molecules can be developed to achieve permanent enzymatic deactivation. This approach offers a promising strategy for durable, targeted cancer therapeutic development.

REFERENCES

1. Sugawara T. et al. Covalent adduct Grob fragmentation underlies LSD1 demethylase-specific inhibitor mechanism of action and resistance. *Nat Commun*. 2024; 15:3992. doi:10.1038/s41467-025-57477-3

2. Ishikawa Y., et al. A Novel LSD1 Inhibitor T-3775440 Disrupts GFI1B-Containing Complex Leading to Transdifferentiation and Impaired Growth of AML Cells. *Mol Cancer Ther*. 2017; 16(2):273-284. doi: 10.1158/1535-7163.MCT-16-0471.

3. Cusan M., et al. LSD1 inhibition exerts its antileukemic effect by recommissioning PU.1- and C/EBPα-dependent enhancers in AML, *Blood*, 2018; 131(15):1730-1742, https://doi.org/10.1182/blood-2017-09-807024.