

### Introduction:

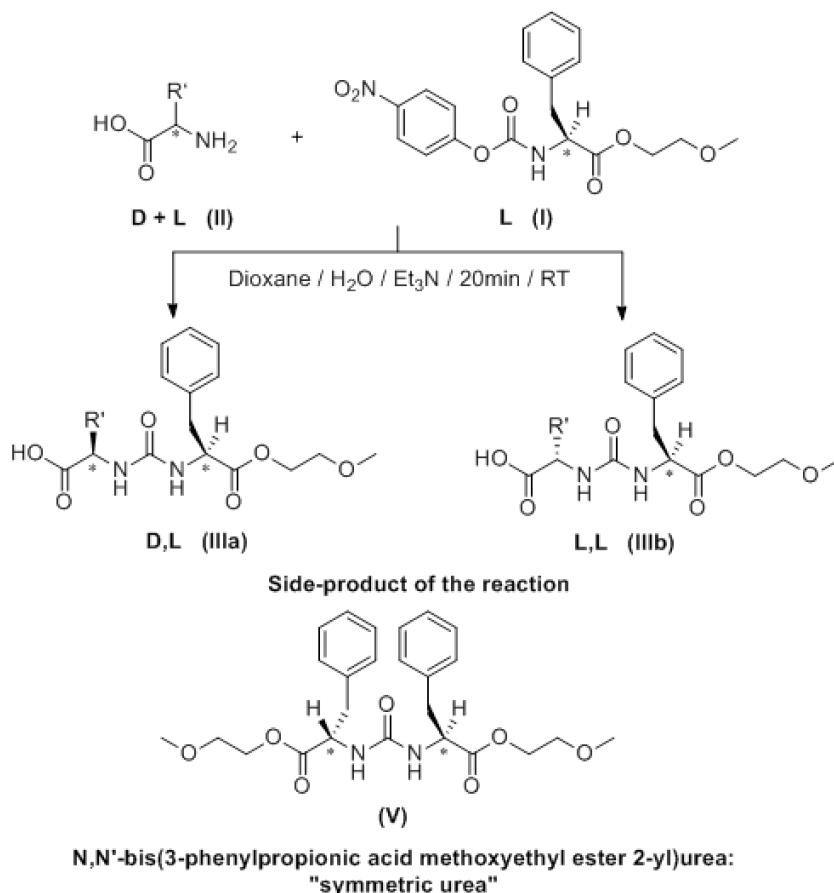
S(NIFE) was designed in the research lab's of Peptisyntha/Solvay as a generally applicable CDA and has become their standard reagent to test all incoming and home made non-natural amino acids for optical purity.

### Chemistry (cfr. Reaction scheme 1)

(S)NIFE (I) reacts with the amino function of an amino acid (II) to form a urea derivative (III) with two chiral centers with concomitant liberation of one mol of 4-nitrophenol (IV). Excess (S)NIFE decomposes to the symmetrical urea compound (V)

It possesses all the characteristics a good CDA should have:

- Good resolution of the diastereoisomers on the most common non-chiral RP-HPLC columns with routinely used eluents
- Quantitative reaction with a small excess of reagent in a short time
- Low limit of detection (LOD) thanks to the presence of the Phe moiety
- No racemization and dynamic resolution in the course of the reaction



Reaction Scheme 1

### Literature data:

Shortly after the invention of (S)NIFE the compound was intensively tested in the laboratories of prof. Tourwé in Brussels (B) and prof. Peter in Szeged (H) and they reported their results in a series of papers.

1. Application of (S)-N-(4nitrophenoxycarbonyl) L-phenylalanine-2methoxyethylester as a new chiral derivatizing agent for proteinogenic amino acid analysis by HPLC Peter,A. et al Chromatographia (2000), 52 (11/12) 821-826.  
In this first publication the optimized derivatization protocol was defined i.e 2fold excess of CDA gives quantitative conversion in 20min. Lys,Cys and Tyr are twice derivatized and need 5 fold excess to go to completion. All diastereoisomeric pairs were readily resolved on a C18 RP-HPLC column with MeOH performing somewhat better than CH<sub>3</sub>CN as organic modifier
2. HPLC enantioseparation of phenylalanine analogs by application of (S)N-(4nitrophenoxycarbonyl) L-phenylalanine-2methoxyethylester as a new chiral derivatizing agent Olajos,E. et al.Chromatographia (2001) 54 (1/2) 77-82.  
Six ring substituted phenylalanines and their alpha-Me counterparts were examined. Excellent results with the ring substituted Phe's and 5 out of 6 alpha-Me analogs. GITC\* required 150min reaction time at 40°C, Marfey's reagent\*\* didn't work at all!
3. Application of a new chiral derivatizing agent to the enantioseparation of secondary amino acids Peter,A. et al. Journal of Chromatography ,A (2002) 948 (1-2) 283-294.  
In all 19 different imino acids (proline, piperolic acid analogs etc.) were derivatized and easily separated on a C18 reversed phase column.
4. Liquid chromatographic enantioseparation of spin labelled beta amino acids Peter,A. et al. Journal of Chromatography A (2003), 1021 (1-2) 1-10.  
In this special very demanding case (S)NIFE gave acceptable results while the more commonly known CDA's like GITC (slow reaction with racemisation) and Marfey's Reagent (no reaction at all) gave unacceptable results.
5. HPLC separation of enantiomers of alpha substituted proline analogs by the application of (S)N-(4nitrophenoxycarbonyl) L-phenylalanine-2methoxyethylester as a chiral derivatizing agent Peter,A. et al.Journal of Liquid Chromatography and Related Technologies (2004) 27 (1) 17- 29.  
As already indicated sub (2) (S)-NIFE performs very well with highly constrained amino acids.
6. Direct and Indirect high-performance liquid chromatographic enantioseparation of beta amino acids Peter,A. et al. Journal of Chromatography, A (2004) 1031 (1-2) 171-178.  
The applicability of (S)NIFE to this class of compounds (18 examples) is clearly demonstrated.
7. High-performance liquid chromatographic separation of stereoisomers of beta amino acids and a comparison of separation efficiencies on Chirobiotic t and TAG columns Arki,A. et al.Chromatographia (2004) 60 (Suppl1) S43-S54.  
Same conclusion as sub(6).

\*GITC: 2,3,4 6-Tetra-O-acetyl-beta-D-glucopyranosyl isothiocyanate; CAS n° 14152-97-7

\*\*Marfey's reagent (FDAA): N-alpha(2,4- dinitro-5-fluorophenyl)-L-alanimamide; CAS n° 95713-52-3

**Since 2004 SAF-Fluka offers (S)N-(4nitrophenoxycarbonyl) L-phenylalanine-2methoxyethylester in their catalogue and other laboratories started reporting their experiences with the use of this CDA..**

1. Chirality determination of unusual amino acids using precolumn derivatization and liquid-chromatography-electrospray ionization mass spectrometry Hess,S. et al. Journal of Chromatography,A (2004) 1035(2) 211-219.  
Confirmation that (S)NIFE is easy to work with

2. High-performance liquid chromatographic enantioseparation of (R,S)-fluoxetine using Marfey's reagent and (S)-N-(4-nitrophenoxycarbonyl) L-phenylalanine-2-methoxyethyl ester as chiral derivatizing reagents along with direct thin-layer chromatographic resolution and isolation of enantiomers using L-tartaric acid as mobile phase additive Bhushan, R. et al. Biomed. Chromatogr. 2010 24 1152-1158
3. Application of (S)-N-(4-nitrophenoxycarbonyl) L-phenylalanine-2-methoxyethyl ester as a chiral derivatizing agent for reversed phase high-performance liquid chromatographic separation of diastereoisomers of amino alcohols, non-protein amino acids and PenA Bhushan, R. et al. Amino Acids (2010) 39 549-554.

In this article the author describes his results with the (S)-NIFE derivatization of 8 amino alcohols and 7 non-natural amino acids and his conclusion is clear: thanks to its robustness (no special precautions to be taken, quick reaction time, no racemisation and stable derivatization products) the reagent qualifies well for quality control in a pharmaceutical QC lab.

While SAF continues to supply small quantities for the frequent user, we sell 1g lots at €190. Should you need technical assistance with the use of (S)-NIFE please feel free to contact us.