Electronic Supplementary Material (ESI) for New Journal of Chemistry.
This journal is © The Royal Society of Chemistry and the Centre National de la Recherche Scientifique 2014

Electronic Supplementary Material (ESI) for New Journal of Chemistry.
This journal is © The Royal Society of Chemistry and the Centre National de la Recherche Scientifique 2014

Supplementary information for

Solid-phase synthesis and analytics of 3,6-dihydro-2*H*-1,2-oxazines of their stereo and regioisomers mixture.

Aidan Harrison[a], Michele Melchionna[a], Pilar Franco[b], Jan Hlaváč[a*]

e-mail: hlavac@orgchem.upol.cz

[a] Institute of Molecular and Translational Medicine, Palacký University, Faculty of Science, 17. Listopadu 12, 771 46, Olomouc, Czech Republic;

[b] Chiral Technologies Europe, Parc d'Innovation, Bd. Gonthier d'Andernach, BP 80140 F-67404 Illkirch Cedex, France

1. Materials and Methods

1.1 Synthesis

Dichloromethane (DCM) and dimethylformamide (DMF) were purchased from Penta Chemicals, Dimethylsulfoxide was purchased from Lach-ner and all were used without further purification. Chemicals were obtained from Sigma-Aldrich. Rink Amide AM resin (100-200 mesh, 0.49 mmol/g) and amino acids were purchased from AAAPTec. Syntheses were carried out on Domino Block Synthesiser (Torviq, USA) in disposable polypropylene reaction vessels.

Solid-phase synthesis of the oxazines followed procedure previously reported by Krchnak et. al.[7]

1.2 UHPLC/MS analyses

Samples for UHPLC/MS analysis during synthesis were prepared by cleaving approximately 10 mg of resin in TFA/DCM (50 vol %) for 30 min and evaporating the solvent with a stream of nitrogen. The samples were then extracted into 1 mL of MeOH/H_2O (50 vol %) for analysis.
The analyses were performed on an Acquity UHPLC system in combination with SQ Detector 2 mass detector from Waters Inc. and using a X-Select C18 column at 30 °C. The APCI source operated with a discharge current of 5 μA, vaporizer temperature of 350 °C and capillary temperature of 200 ^{o}C. Mobile phase: A gradient was formed over 2.5 minutes (0.6 mL/min). Solvent A: 10mM of ammonium acetate in water (HPLC grade). Solvent B: Acetonitrile (HPLC grade). (Table 1).

Table 1. HPLC methods used for purification of products

% acetonitrile	Sample
20-80	**8a, 8b, 8c, 8d, 8g, 16**
5-95	**13**
0-50	**8e, 8f**

1.3. HPLC purification

For purification of final compounds, the resin-bound compound (300 mg) was placed in a fritted polypropylene reaction vessel and treated with TFA/DCM (5 mL, 50 vol %) for one hr. The solution was collected and the resin washed 3 x with TFA/DCM (5 mL, 50 vol %) and these solutions also collected. The combined solution was evaporated with a stream of nitrogen and dissolved in acetonitrile (10 mL), or acetonitrile/10 mM aqueous ammonium acetate solution (10 mL, 33 vol %) in the case of compounds 8e. This solution was then purified by HPLC using an acetonitrile/10 mM aqueous ammonium acetate gradient over 6 min, or an isocratic method over 15 min as described in Table 2.

Table 2. HPLC methods used for purification of products

Product	Purification method	% acetonitrile
8a, 8b, 8c, 13	Gradient	20 to 50
8d	Gradient	30 to 60
8e	Isocratic	7.5
8f	Gradient	10 to 30
8g	Gradient	40 to 70
16	Gradient	30 to 60

HPLC purification was performed on a Waters 1500 series HPLC equipped with Autosampler 2707, a Binary HPLC pump 1525, Waters Photodiode Array Detector 2998 and Waters Fraction Collector III using a YMC C18 reverse phase column, 20 x 100 mm, 5 µm particles.

1.4. Preparation of crude samples for chiral analyses

Analytical samples were prepared by cleaving 20 mg of dried resin in TFA/DCM (50 vol %) for 30 min and evaporating the solvent with a stream of nitrogen. The samples were then extracted into 1 mL of HPLC grade MeOH for MS analysis and filtered before being used for chiral analysis.

1.5. Chiral HPLC

The chromatographic instrument used for chiral analysis of purified samples was a HPLC Dionex equipped with a quaternary pump (P680) with vacuum degasser, PDA detector (PDA-100) and Chromeleon software.

The chiral analytical columns, sized 250 x 4.6mm (i.d.), were supplied by Daicel Corporation (Tokyo, Japan). Figure 1 shows the chemical structures of the chiral selectors contained respectively in CHIRALPAK IA, CHIRALPAK IB, CHIRALPAK IC, CHIRALPAK ID, CHIRALPAK IE and CHIRALPAK IF. All these amylose or cellulose derivatives are chemically immobilized on 5 µm silica particles. The mobile phases for liquid chromatography were prepared from HPLC grade solvents supplied by Carlo-Erba (Val de Reuil, France), Merck (Darmstadt, Germany) and VWR Chemicals (Fontenay-sous-Bois, France). Diethylamine (DEA) and trifluoroacetic acid (TFA) were mainly obtained from Sigma-Aldrich (Saint Quentin Fallavier, France).

Different mobile phase systems were investigated in this study. The eluting strength of the mobile phases was adjusted in such a way so that each racemic compound could be eluted within a reasonable time window. The proportion of each mobile phase component or mobile phase additive was always measured by volume. The chromatographic runs were performed at a flow rate of 1.0 ml/min and at a column temperature of 25°C, if not otherwise indicated. Pure samples were prepared at a concentration of 1.0 mg/mL in HPLC grade MeOH, and analytical samples prepared from 20 mg of dry resin (Section 2.3). Sample injections were 10 µL in all cases.