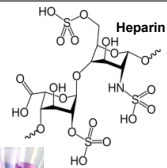
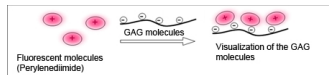




Problem statement/ background:

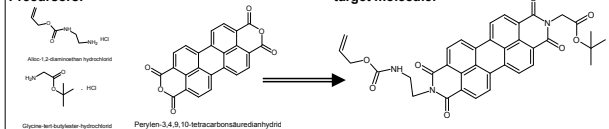
Fluorescent marking of Heparin (glycosaminoglycan, GAG) for better detection in blood! Heparines are therapeutically used substances as blood clotting inhibitors. Chemically they are polysaccharides (glycosaminoglycans), consisting of a variable number of aminosugars. Aspired changes through the asymmetric Perylenediimide:



Addition of the amino acid groups to the GAG-molecules.

Production of a perylenediimideaminoacid through insertion of protected amino acids.

Precursors:



target molecule:

Strategy for solution of producing Alloc-Py-Boc

1. Checking reactivity and selectivity of the reaction of the Alloc- and Boc amino acid onto the perylene, respectively.
2. Analogical checking synthesis instruction out of the doctorate of Fritz Thomas Wetzel from Freising. Revision of 3 reaction channels:

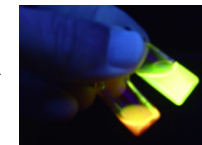
Reaction	Micro approach in testing tubes	Macro approach in testing tubes
Boc-Py-Boc-Syn	2.2 parts Boc 1.0 part Perylen in imidazole 140 °C/ 1h Extraction: extract with CH ₂ Cl ₂ /2M HCl in silica gel 60 column	2.2 parts Boc 1.0 part Perylen in imidazole 140 °C/ 1h Extraction: extract with CH ₂ Cl ₂ /2M HCl in silica gel 60 column
Alloc-Py-Boc-Syn	1.2 parts Boc 1.0 part Alloc in imidazole 140 °C/ 1h Extraction: extract with CH ₂ Cl ₂ /2M HCl in silica gel 60 column	1.2 parts Boc 1.0 part Alloc in imidazole 140 °C/ 1h Extraction: extract with CH ₂ Cl ₂ /2M HCl in silica gel 60 column
Alloc-Py-Alloc-Syn	2.2 parts Alloc 1.0 part Perylene in imidazole 140 °C/ 1h Extraction: extract with CH ₂ Cl ₂ /2M HCl in silica gel 60 column	2.2 parts Alloc 1.0 part Perylene in imidazole 140 °C/ 1h Extraction: extract with CH ₂ Cl ₂ /2M HCl in silica gel 60 column

Performed production steps:

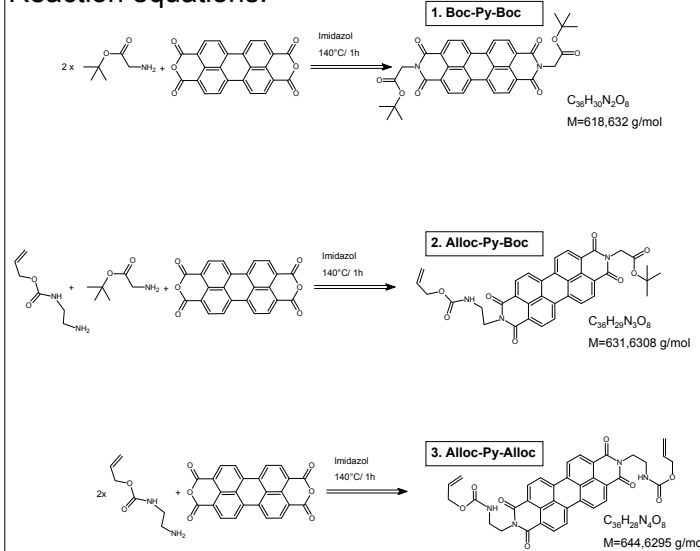
- Collecting of information for the procedure (internet research)
- Creation of reaction equation and stoichiometric computation
- Determination of the properties of the educts (safety indication etc.):
 - perylene
 - Alloc protecting group
 - Boc protecting group
 - imidazole
 - needed solvents
- Creation of synthesis instruction
- Ordering chemicals and laboratory equipment
- Performing reaction in compliance with safety- and hazard statements
- Processing with silica gel
- MS-Analytics and evaluation
- Documentation of results

Conclusions:

- detection of Boc-Py-Boc and Alloc-Py-Boc was successful through MS structure determination.
- I.) **Synthesis of pure Boc-Py-Boc was successful!**
- II.) **Synthesis of pure Alloc-Py-Alloc is not possible!**
- III.) **Synthesis of a Boc-Py-Boc and Alloc-Py-Boc mixture was successful!**
- Separation of both substances from III.) did not succeed completely with the used silica gel 60 column.
- IV.) **Full separation eventually possible with different eluent and different column material.**

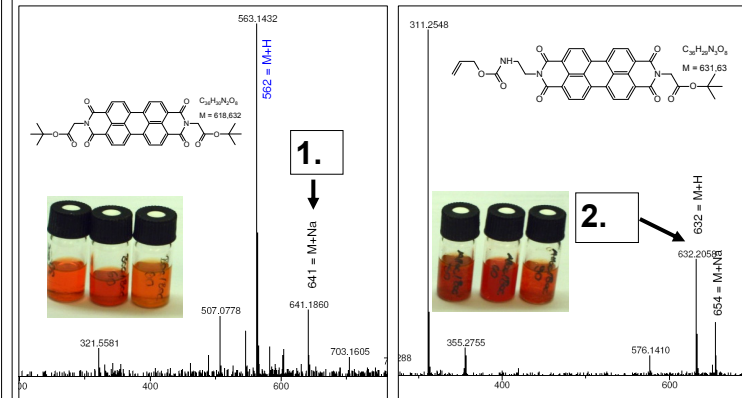


Reaction equations:



Analytics:

LC: Dionex Ultimate 3000RS UHPLC
Column: Phenomenex Kinetex C18, 2.1x100mm, 1.7µ
Eluent: A= H₂O + 0,1% formic acid, B= ACN + 0,09% formic acid
Flow: 0,550ml/min
Temp: 40°C
Gradient: 0 min A 100% B 0%, 22min A 5% B 95%, 27min A 5% B 95%
UV: 200-500 nm
MS: Bruker micrOTOF II
Method: ESI +
Masses: 70-1600



Synthesis instruction Alloc-Perylen-Boc:

1. Add 195,4mg of perylene to 4g of imidazole molten at 110°C and stir until a suspension is formed. Then add 100mg (0,6mmol) G-Boc and 108mg (0,6mmol) D-Alloc, heat the suspension to 140°C. TLCs are taken for reaction observation (dichloromethane/methanol 20:1 used as eluent).
After 90 min the reaction is stopped, cooled to room temperature, the solid reaction product is collected in 40 ml of dichloromethane and the suspension is stirred in the ultrasonic bath and filtrated. Edulcorate the filtrate 2x with 40 ml of a solution of NaH₂PO₄ (10g in 120ml H₂O) each in the separatory funnel. The product dissolves in the organic phase. Red slurry forms between the phase boundaries which can be flushed out with the organic phase. Edulcorate the organic phase 3x with 20ml of water each. The organic phase is dried through sodium sulphate without the slurry and the dichloromethane is removed with the rotary evaporator at 50°C without vacuum.
2. Dissolve the solid in dichloromethane and separate in a column filled with silica gel 60. Mobile phase (80:1 dichloromethane : methanol). The products appear as two red bands, whereas the Boc-Py-Boc substance is the highest and underneath it is the Boc-Py-Alloc substance on the TLC. In the particular elutions the solvent is removed with the rotary evaporator and the different products are dried in vacuum!

Synthesis instruction Boc-Perylene-Boc:

1. Initial weight: 50 mg (0,3mmol) glycine-tert.-butylester hydrochloride (G-Boc)
Initial weight: 45,24 mg (0,12 mmol) perylene added to 1 g imidazole molten at 110°C in a 25ml pear shaped flask. Stir the mixture until a uniform solution/suspension is formed. Add D-Alloc at stirring and at given temperature and heat up to 140°C. TLCs for reaction observation after 30, 60 and 90 min (dichloromethane/methanol 20:1 used as eluent). Stop reaction after 90 min. Cool down to room temperature and add 40ml dichloromethane while stirring the suspension. Add NaH₂PO₄ (10g in 120ml H₂O) and extract in separatory funnel. The product is dissolved in the organic phase and the imidazole in the water phase. Edulcorate the organic phase 3x with 20ml water each; dry through sodium sulphate. Filtration and following concentration of the organic phase with rotary evaporator (without vacuum at 45°C bath temperature).
2. Dissolve the solid in dichloromethane. Separate in column filled with silica gel 60. Cleaning off byproducts in the sample (dichloromethane/methanol 80:1 used as eluent). Qualitative control with TLC: products give off red bands.

Literature cited:

1. <http://www.sigmaaldrich.com/safety-center.html>
2. http://www.chemiedidaktik.uni-wuppertal.de/alte_seite_du/material/milch/milchweiuss/aminosauren.pdf
3. Langhals, Heinz; Jona, Wolfgang: Synthese für substituierte Perylene. Universität München 1997
4. Langhals, Heinz; Jona, Wolfgang: The synthesis of perylenebisimide monocarboxylic acids. European Journal of Organic Chemistry (1998), (5), 847-851.
5. Büttner, Jörn: Neue Synthesewege zu NIR-Farbstoffen auf Basis der Quaterrylenbisimid. Universität München 2006
6. Wetzel, Fritz Thomas: Synthese von neuen Verbindungen auf Basis der Perylen-3,4,9,10-bis(dicarboximide) für Anwendungen im Flüssigkristall-Bereich und als optische Schalter. Universität München 2003
7. Onken, Kristian: Synthese und Charakterisierung neuartiger, ambipolarer organischer Halbleiter, basierend auf Perylen-carboximid-Systemen kombiniert mit funktionalisierten Spirofluoreneinheiten. Universität Kassel 2007
8. Kampner, Michaela: Schulversuche mit Milch und Einsatzmöglichkeiten im Chemieunterricht. Universität Wuppertal 2005
9. Kuhn, Isabelle: Verbesserung: DC-Trennung von Aminosäuren. Phillips-Universität Marburg 2006
10. Krämer, Prof. R.: Mix-and-measure fluorescence assays for the selective detection of glycosaminoglycans. Universität Heidelberg Gesetze/ Schlußordnung der Georg von Langen BBS