Synthesis of peptidic bola-dendrimers

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Nano2Clinic
Cancer Nanomedicine - from the bench to the bedside
Glioblastoma

- The most common, gene-related brain tumor.
- Arises from neuroglial cells, which nourish and support nerve cells.
- Since the diagnosis of the disease patients survive less than 15 months.
- Despite surgical resection, radiation and aggressive chemotherapy, the tumor returns, grows rapidly and gradually impairs the activity of additional parts of the brain.

“Bola” type peptide dendrimers

- provide a great chemical diversity,
- better stability of the structures,
- similarity to biomacromolecules,
- biocompatible and biodegradable,
- non-toxic, non-immunogenic.


Synthetic strategy I

Scheme 1 Reagents and conditions: (a) Boc-Lys(Boc)-OH, DCC/HOBT, DMF, 3h at -5 to 0°C and 48h at rt; (b) HCl/EtOAc, 10h at rt; (c) Boc-Lys(Boc)-OH, DCC/HOBT, DMF, 3h at -5 to 0°C and 120h at rt; (d) HCl/EtOAc, 48h at rt; (e) Imd (1-methyl-1H-imidazole-5-carboxylic acid), DCC/HOBT, DMF, 3h at -5 to 0°C and 168h at rt, Final product was synthesised by Schotten-Baumann reaction for 44h at rt;
Application of Schotten-Baumann reaction for surface functionalization and for obtaining bola-type dimers significantly reduces reaction time. However, it strongly depends on size and stereochemistry of a dendron.

<table>
<thead>
<tr>
<th>Time</th>
<th>Solution Peptide Synthesis</th>
<th>Schotten-Baumann reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Step (a – d) 48 – 120h</td>
<td>48h – 168h</td>
</tr>
<tr>
<td></td>
<td>Step (e) - 168h</td>
<td>Step (e) 24h</td>
</tr>
<tr>
<td></td>
<td>Step (f) – no reaction</td>
<td>Step (f) 44h</td>
</tr>
<tr>
<td></td>
<td>progression</td>
<td></td>
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<table>
<thead>
<tr>
<th>Reagents</th>
<th>Solution Peptide Synthesis</th>
<th>Schotten-Baumann reaction</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>DCC/HOBt, DMF, Et₃N,</td>
<td>DCM, Et₃N,</td>
</tr>
<tr>
<td></td>
<td>coupling of amine and</td>
<td>coupling of amines and</td>
</tr>
<tr>
<td></td>
<td>acid groups – typical</td>
<td>dendrons in a form</td>
</tr>
<tr>
<td></td>
<td>peptide synthesis</td>
<td>of acid chlorides,</td>
</tr>
<tr>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Solution Peptide Synthesis</th>
<th>Schotten-Baumann reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Argon, 3h 0°C, then r.t.</td>
<td>Argon, dry solvents,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>r.t.</td>
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</table>
**Synthetic strategy II**

**Scheme 2** Reagents and conditions: (a,c) Boc-Orn(Boc)-OH, DCC/HOBT, DMF, 3h in -5°C and 48h-96h at rt.; (b) HCl/EtOAc, 16h at rt.; (d) 1 M NaOH, 12h at rt.; (e) linker, DCC/HOBt, DMF, 3h in -5°C i 90h at rt.;
Functionalization of the dendrimer

Bola dimer (68.2%)

Functionalized bola (49.94%)

Solid phase dendron synthesis

Scheme 3  Reagents and conditions: a. 20% piperidine, b. Fmoc-Lys(Fmoc)-OH, HATU, DIPEA, c. 20% piperidine, d. Fmoc-Lys(Fmoc)-OH, HATU, DIPEA, e. 20% piperidine, f. X-OH, HATU, DIPEA, g. 90% TFA/H₂O, Triisopropylosilane
### SPDS vs Synthesis in Solution

#### Resins:
- Wang resin
- 2-chlorotrityl resin
- Tenta Gel S PHB resin
- Low loaded 0.25 mmol/g

#### Key Comparisons:

<table>
<thead>
<tr>
<th></th>
<th>SPDS</th>
<th>Synthesis in solution</th>
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</thead>
<tbody>
<tr>
<td><strong>Amino acid</strong></td>
<td>Lysine</td>
<td>Ornithine</td>
</tr>
<tr>
<td><strong>Reaction time</strong></td>
<td>Shorter</td>
<td>Usually longer</td>
</tr>
<tr>
<td><strong>Intermediate products</strong></td>
<td>continuous synthesis, purification after resin cleavage</td>
<td>Step-by-step with purification of intermediate products</td>
</tr>
<tr>
<td><strong>Coupling reagents</strong></td>
<td>HATU, DIPEA or DIC/Oxyma, DIPEA</td>
<td>DCC/HOBt</td>
</tr>
<tr>
<td><strong>Monitoring</strong></td>
<td>Kaiser test</td>
<td>TLC</td>
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</table>
Spectroscopic characterization - UV
Secondary structure in solution – CD experiments

![Graph showing molar ellipticity vs wavelength for different concentrations of a compound]

- Molar Ellipticity $\theta$ [deg x cm$^2$ x dmol$^{-1}$]
- Wavelength [nm]

- D2 compounds at 60 μM, 90 μM, and 120 μM concentrations are depicted.

- Key peaks at 206.8, 209.4, and 215.4 nm for the 60 μM concentration.

- Additional peaks are observed at 206, 229, and 222.2 nm for the 90 μM concentration.

- For the 120 μM concentration, peaks are seen at 205 and 204.2 nm.

![Structural formulas of acetic acid and amide]

- Acetic acid
- Amide structure
Complexation experiments with anticancer drugs - HPLC

- Chlorambucil
- Cytarabine
- DMSO
- Temozolomide
Non-functionalized „bola” dimer does not form complex with the anticancer drugs!
HPLC complexation studies

Alkylation agent used as a treatment of some brain cancers; as a second-line treatment for astrocytoma and a first-line treatment for glioblastoma multiforme.
Complexation experiments

- Complexation experiments involving dimer D2B, temozolomide, cytarabine, and DMSO.
- Involves other dendrimers.
Summary of the synthetic strategies

Strategy when the SPPS or Schotten-Baumann reaction should be applied.

Lysine

Strategy when the active ester method for peptide synthesis should be applied.

Ornithine
Conclusions/Ongoing work

Generally, the most efficient synthesis of bola type peptide dendrimer was the second strategy (synthesis of the core in solution and functionalization in the final step),

Type of the synthetic strategy strongly depends of the branching units and the derivatives which should be found on the surface,

Synthesis and purification is very demanding,

„Bola” type dimers form complexes with anticancer drugs.

Synthesis of bola dimers with longer hydrofobic linkers,

Studies on mechanism of bola/drug complex formation (UV, NMR, MS spectroscopy),

Anticancer screening,

Molecular modeling.
Acknowledgments:

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Prof. Valentin Ceña

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