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SYNTHESIS OF CALIX[4]ARENES WITH FIXED CONFORMATION AS POTENTIAL INHIBITORS OF FIBRIN POLYMERIZATION

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Intravascular thrombus formation is one of the main causes of such deadly diseases and pathologies as ischemic heart disease, ischemic stroke, pulmonary embolism, atrial fibrillation, and venous thromboembolism [1, 2].

That is why the development of new approaches for preventing intravascular blood coagulation is an important task for biochemistry and biotechnology. Most of biomolecules that target clotting factors. However, in our study we focused on another type of molecules, which are direct inhibitors of fibrin polymerization.

As a scaffold, we have used a calix[4]arene molecule because of its tunable diversity at both upper and lower rims, its pre-organized nonpolar cavities, pre-organized ion-bonding sites and well-defined conformations [3]. Previously we have shown that calix[4]arenes are active inhibitors of fibrin polymerization process. Binding to the 'A'-knob of fibrin desAB molecule calix[4] arenes prevents knob-hole interactions between different fibrin molecules thus suppressing clotting. Recent studies proved the efficacy of sodium salt of 5, 11, 17, 23-bis(dihydroxyphos phoryl)methylcalix[4]arene (C-145) as an effective anticoagulant agent [4, 5].

Molecular dynamic studies of C-145 demonstrated the importance of conformation of the macrocycle for its fibrin-binding activity. In particular calyx[4]arene has two different conformations of its cup (the macrocycle) that can be described as '*1,3-alternate*' (opened) or '*conus-conformation*' (fixed).

Aim. The purpose of the present study was to develop a method for the fixation of 'fixed' conformation for estimation of the impact of calix[4]arene structure on the efficacy of its anticoagulant activity. This was achieved by substitution of the lower rim of C-145 analogue.

Metods. Calix[4]arene C-145 was obtained according to the method described in [6] with one additional step that included Mitsunobu reaction in order to obtain disubstituted calixarene C-145F (Fig. 1).

Modeling of 3D-structure of calix[4]arene C-145 and its analogue C-145F was performed in Maestro, Schrodinger software.

Calix[4]arene C-145F (compound 6) was obtained in 4 steps starting with Duff reaction. Calix[4]arene methylene-bis-phosphonic ester 3 was prepared via addition of diisopropylphosphite in presence of metallic sodium to the parent calix[4]arene aldehyde 2. Further steps included Mitsunobu reaction, that afforded dipropoxycalix[4]arene 5 with rather good yields (80%), following the hydrolysis step that resulted in compound 6 in almost quantitative yield.

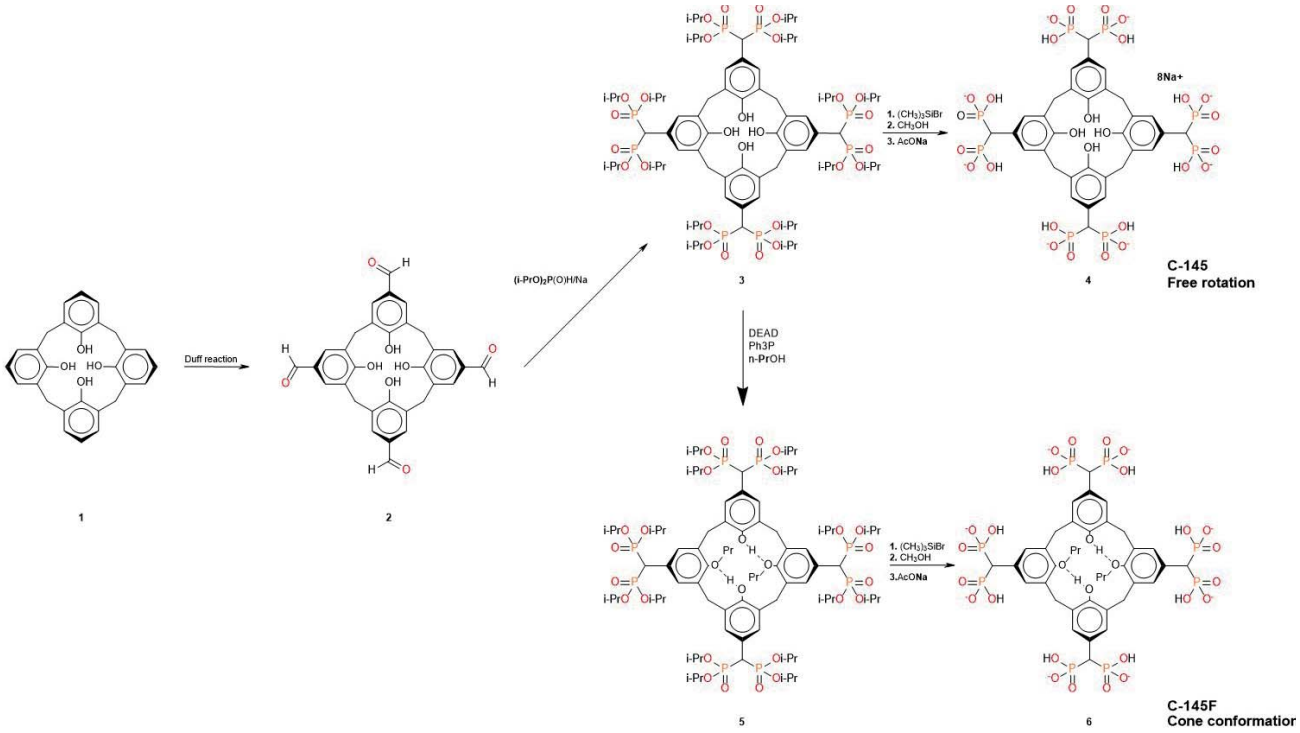

Fig. 1. **Synthesis of calix[4]arenes C145 and its analogue C145F with lower rim substitution**

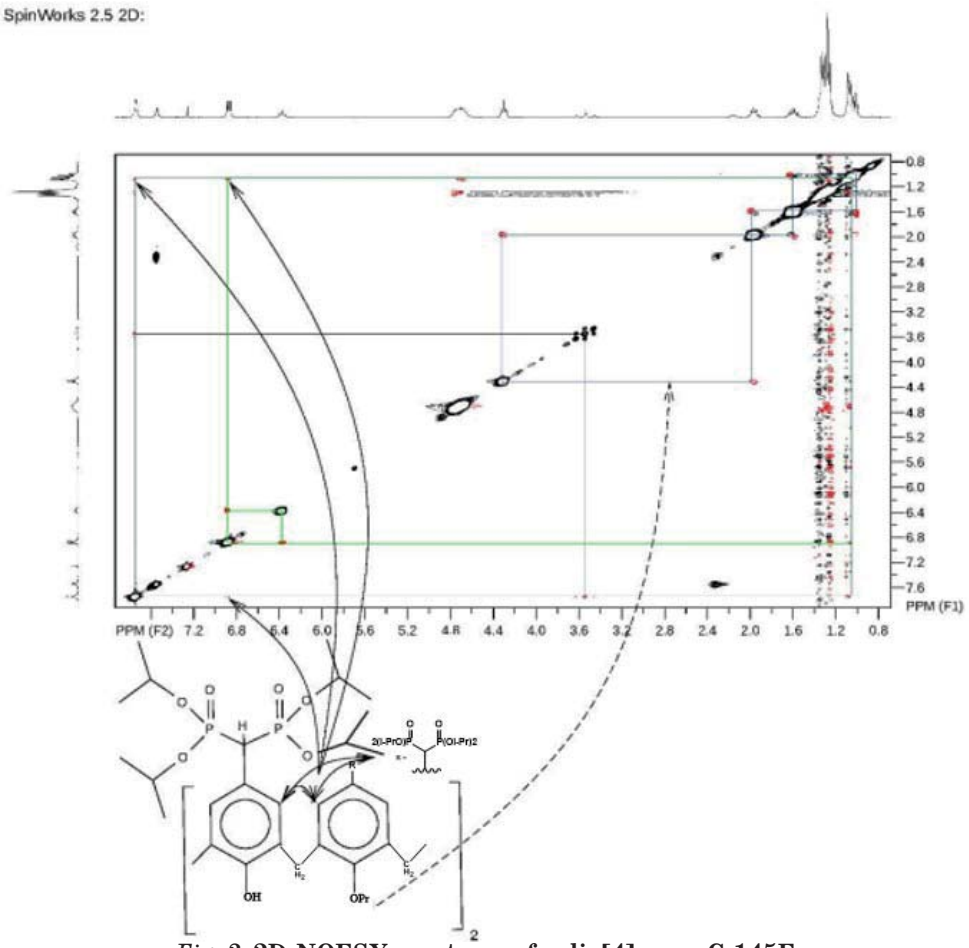

Fig. 2. **2D-NOESY spectrum of calix[4]arene C-145F**

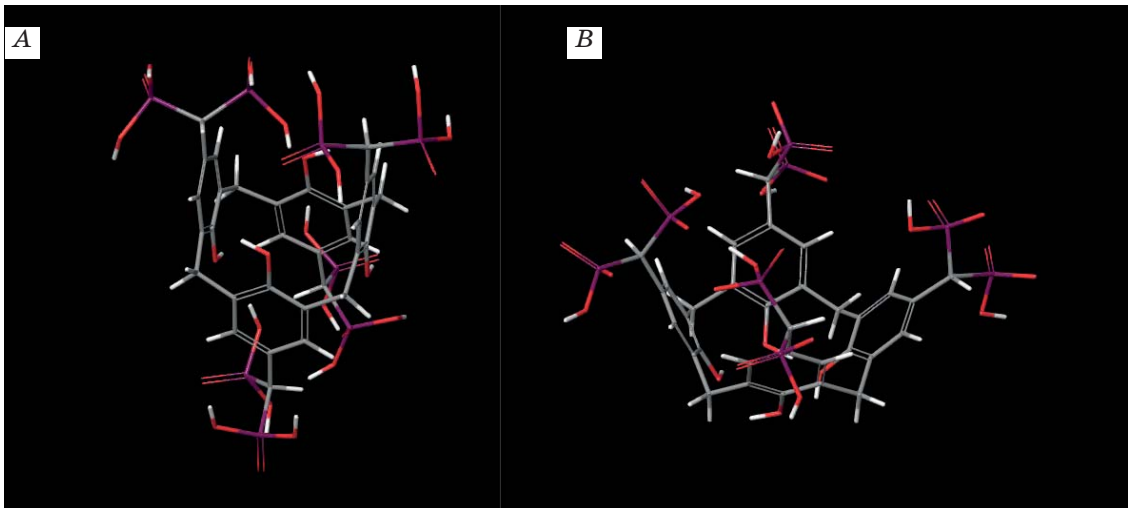

Fig. 3. **Structure of calix[4]arene C-145 and it's analogue with fixed conformation of a calix[4]arene cup:**
A — C145; *B* — C145F

Results. Using a 2D NMR-NOESY spectroscopy, we can observe a distinct cross-peak between an aromatic singlet with a chemical shift on 7.72 ppm and protons of isopropyl group with a chemical shift on 1.62 ppm, which are moved in the strong field (Fig. 2). This correlation proves us their steric proximity, that can be observed only in conus-conformation (Fig. 3, *A*), not in the *1,3-alternat*e. So, in this work we confirmed that disubstituted calix[4]arenes (low-rim modification) have fixed conus conformation.

NMR spectroscopy proves us that calix[4]arene C145F exists in fixed conus-conformation.

Discussion. Presented experiment represents how easily we can modify calix[4]arene cup in order to obtain molecule with new chemical and physical properties. The fine-tuning and incorporation of different ligating sites in the calix[4]arene scaffold may be used to produce numerous molecules, forming a library of compounds.

Comparison of the action of fibrin polymerization of calix[4]arene molecules with fixed and opened conformations will allow to estimate the role of different conformation in fibrin recognition and will support targeted design of even more effective calix[4]arene-based inhibitors of blood clotting in future.

Conclusions. The easy method of the fixation of conus conformation of calix[4]arene cup will be useful for synthesis of novel functionally active compounds. We believe that further development and study of different calix[4]arenes will help scientists to obtain bioactive molecules that could be prospective anti-thrombotic drugs.

Key words: calix[4]arene; fibrin polymerization; organic synthesis;, bioinformatics.

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Author's contribution. DA performed synthesis of calix[4]arenes, OH did molecular modeling.

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