Phosphorus-Nitrogen Compounds. Part 64. Comparative Reactions of Spiro and Ansa(N/O) Cyclotetraphosphazenes with Bulky (4-Fluorobenzyl) N/N and N/O Donor Type Bidentate Reagents: Structure, Stereogenic Properties and Cytotoxic activity Studies

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SUPPORTING INFORMATION

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Section S1. Determination of the cytotoxic activity with MTT assay.

In this study, the cytotoxicities of the phosphazenes were tested by colorimetric MTT assay[1] against L929 mouse fibroblast, Caco-2 colorectal adenocarsinoma and A549 non-small lung cancer cell lines. Cultures were grown and seeded (10^4 cells per well in the 96-well cell culture plates) in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (DMEM-10), 1% glutamine, 50 U/mL penicillin and 50 µg/mL streptomycin in an atmosphere of 5% CO_2 and 100% humidity in air. Cells were incubated for 24. The compounds to be tested were dissolved in DMSO (10%) and applied on cells in five different concentrations (begins from 1665µM and two fold dillutions). The solvent DMSO (10%), cisplatin for comparison, DMEM medium (blank) were used as controls. After 24 h incubation of seeded cells, 50 µL MTT (1mg/mL) solution was added to each well. And later 2 h of incubation at 37 °C, 100 µL of isopropanol was added to the wells, and the absorbance values of the 96-well plate were read at 570 nm in a microplate reader to determine cell viability. The cytotoxicity studies were made in triplicate and the data were given as mean ± standard deviation (SD).

Based on the control groups, the percent viability was calculated by the following formula:

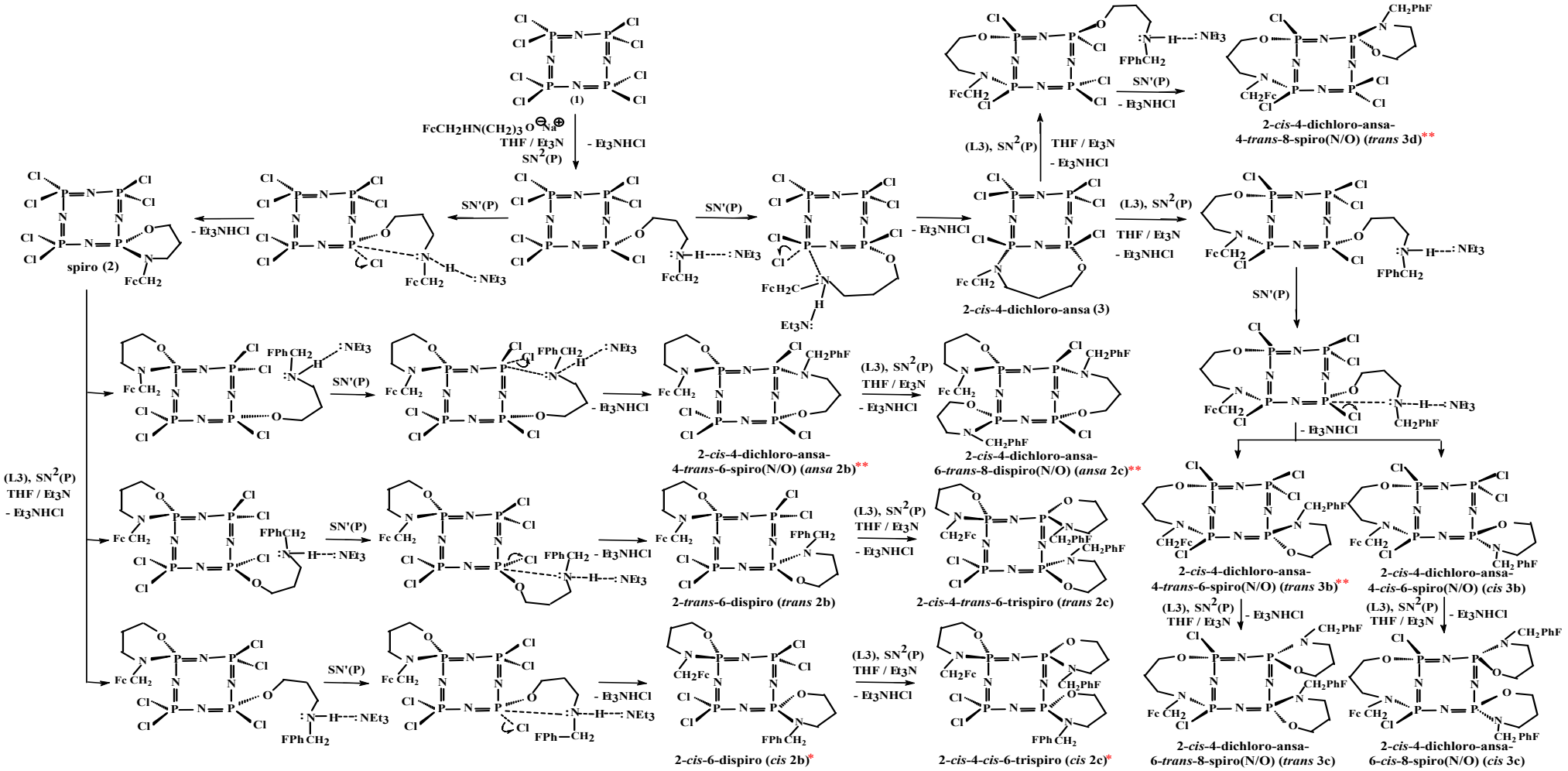
$$Cell\ Viability\ \% = \frac{Compound\ OD}{Control\ OD} x100$$

OD: Optical density

Reference:

[1] A. Okumuş, H. Akbaş, K. Kılıç, L. Y. Koç, L. Açık, B. Aydın, M. Türk, T. Hökelek and H. Dal, *Res. Chem. Intermed.*, 2016, **42**, 4221-4251.

Scheme S1. The Tentative Reaction Routes of spiro (**2**) and 2-*cis*-4-ansa (**3**) with the Sodium Salt of **L3**.



* Compounds *cis* 2b and *cis* 2c are expected products. But, they are not obtained.

** Compounds *ansa* 2b, *ansa* 2c, *trans* 3b and *trans* 3d are also expected products. But, they are not isolated purely.

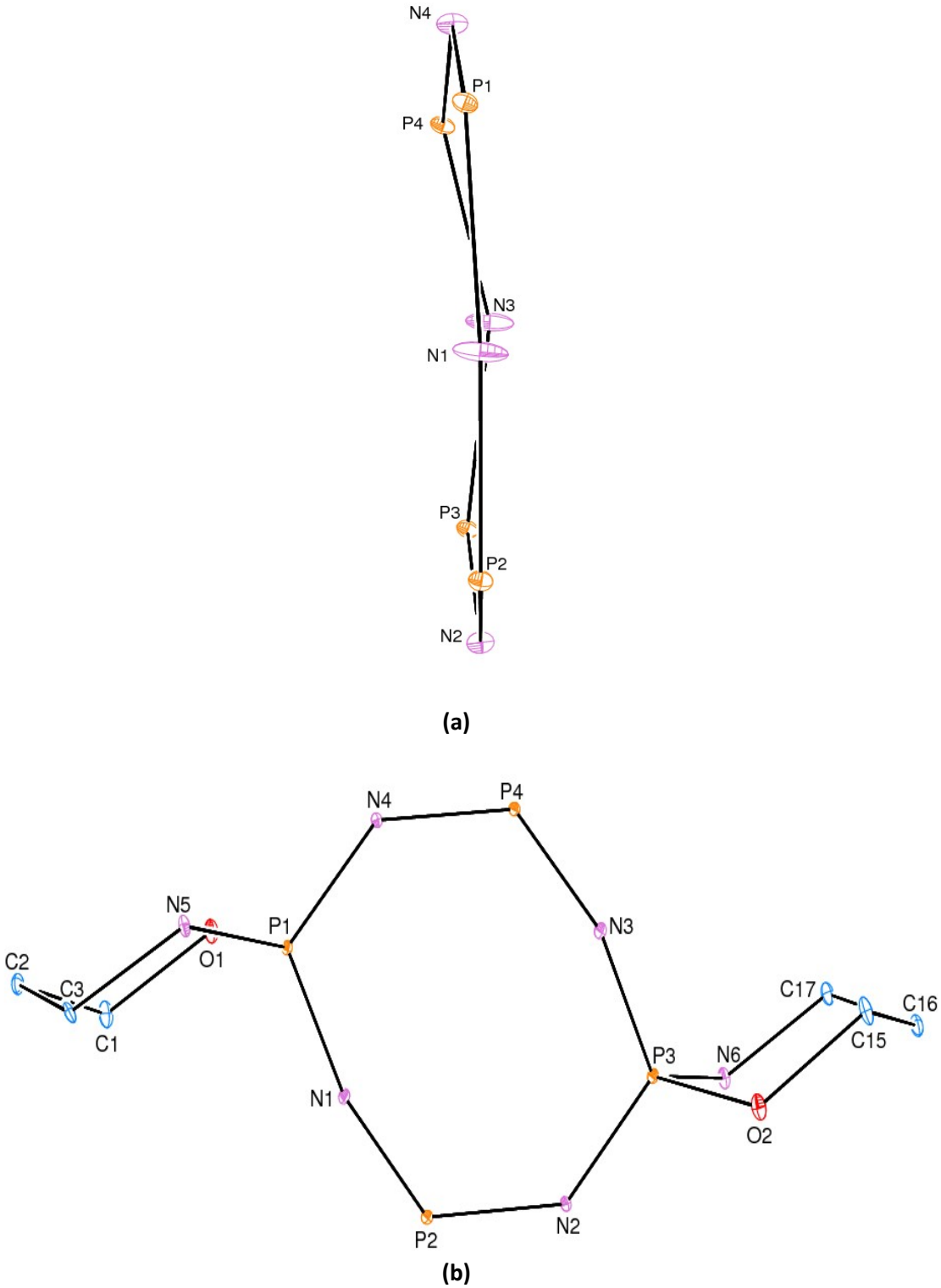
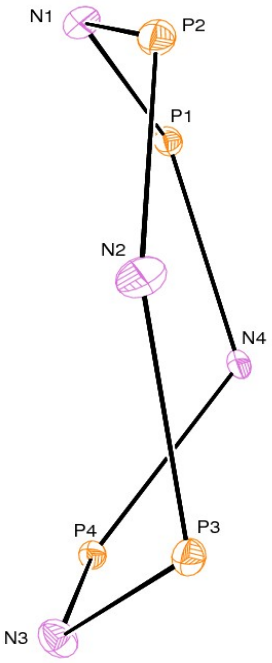
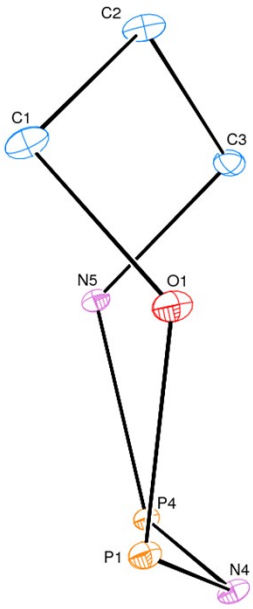


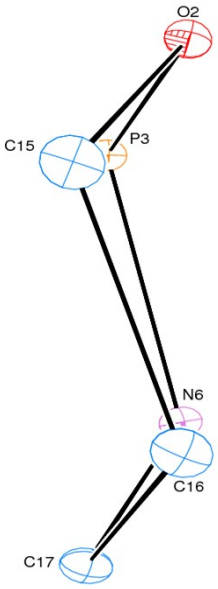
Fig. S1 The conformation of (**a**) the tetramer ring, (**b**) the six-membered NO spiro rings with the tetramer ring of **trans-2b.**



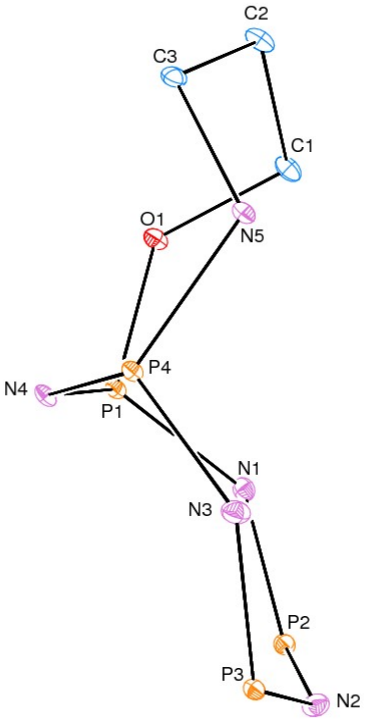
(a)



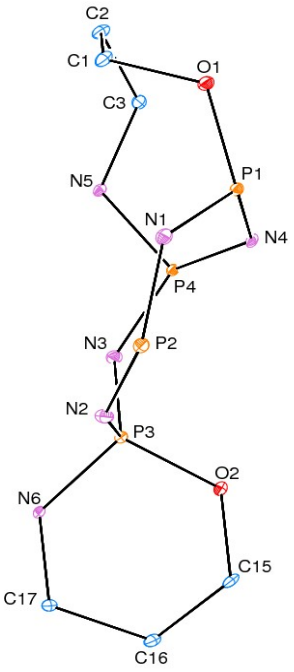
(b)



(c)



(d)



(e)

Fig. S2 The conformation of (**a**) the tetramer ring, (**b**) the eight -membered NO ansa ring, (**c**) the six-membered NO spiro-ring,(**d**) the eight -membered NO ansa-ring with the tetramer ring (**e**) the six-membered NO spiro-ring with the eight -membered NO ansa-ring with the tetramer ring of **cis-3b**.

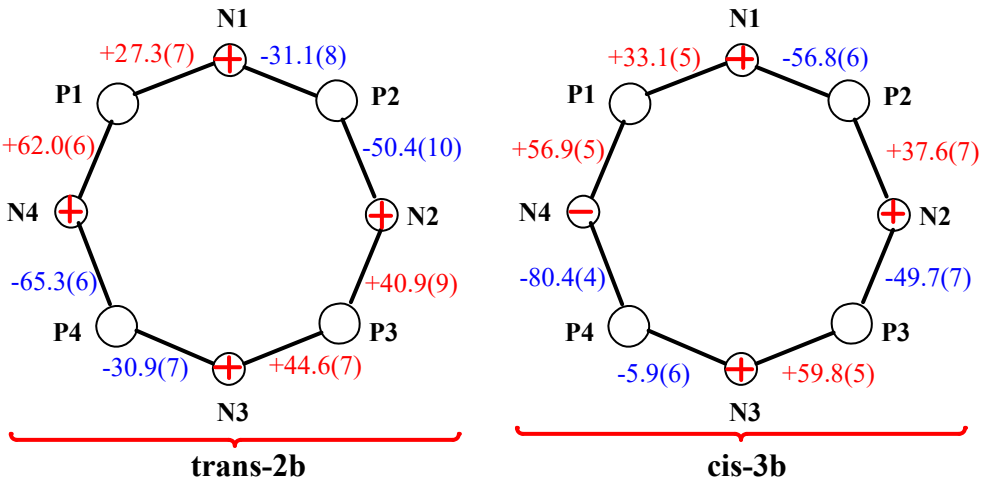


Fig. S3 The shapes of the phosphazene rings in **trans-2b** and **cis-3b** with torsion angles (deg) given.