

SHORT
COMMUNICATIONS

Synthesis of New Carboxylate Ligands for Simulation of Binuclear Active Centers of Nonheme Oxygenases*

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In binuclear active centers of nonheme iron-containing enzymes activating molecular oxygen: methanemonooxygenase (MMO), ribonucleotide-reductase (RNR), Δ -decarboxylase, toluenemonooxygenase etc the coordination surrounding of iron is characterized by the presence of four carboxylate donors (from Asp and Glu). Therewith one or two among these carboxylates form bridges between iron atoms, and the remaining together with two imidazoles (from His) complete the coordination sphere of each iron atom as terminal ligands [1].

Two approaches were suggested for increasing the kinetic stability of carboxylate complexes. One approach consists in application of a polydentate ligand whereas the kinetic stability increases due to chelate effect [2, 3].

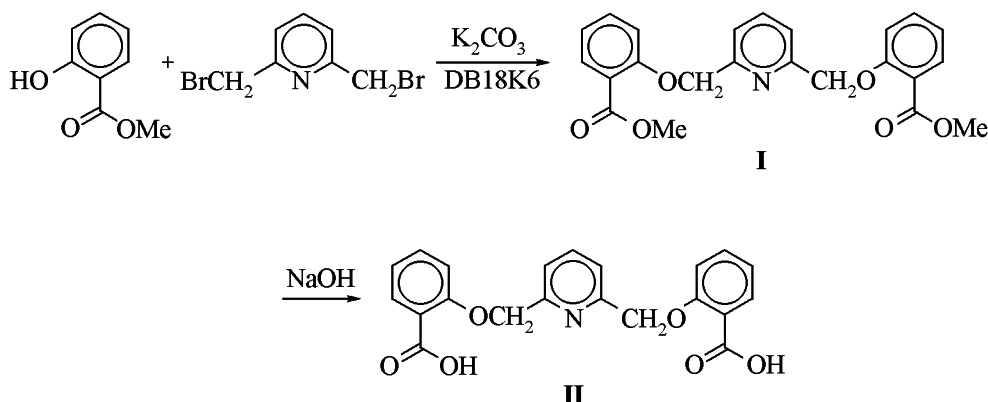
It was shown recently [4, 5] that the use in the model systems of sterically hindered benzoates enhanced the kinetic stability of the carboxylate ligand by its shielding with bulky *ortho*-substituents of the benzoate. At the same time this approach

permits the synthesis of binuclear iron(II) complexes with low coordination number, affords complexes of higher solubility in nonpolar solvents, and provides a possibility of modelling the hydrophobic surrounding of the binuclear center.

Taking into account that the models with the sterically hindered benzoates are promising it seems advantageous to increase the range of these ligands using *ortho*-substituents of various size, form, and conformational mobility and to develop new synthetic routes to such structures.

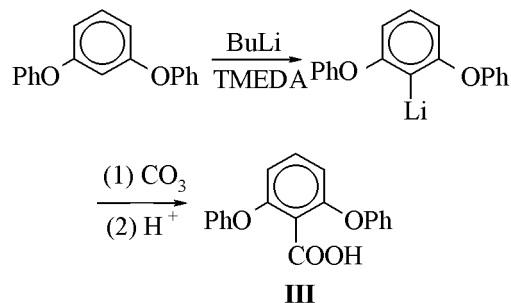
Thus the problem consists in the synthesis of new carboxylate ligands with bulky substituents of various size and flexibility. It is presumable that skilful combination of sterical effects from nitrogen and carboxylate donors should provide a possibility of varying iron complex structures in order to attain the structure of model complexes which would best reproduce the structure and dynamics of the active center.

We report here on the synthesis of new carboxylate ligands for simulation of binuclear active centers of

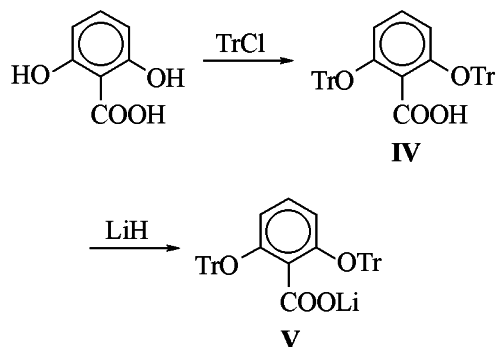


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The lithium 2,6-diphenoxybenzoate was obtained by passing a flow of dry carbon dioxide through a solution of lithium derivatives of 1,3-diphenoxybenzene. A free acid **III** was obtained by controlled acidification of the salt. Its pure lithium salt was prepared for complexation study by boiling of the acid in toluene with lithium hydride.



We failed to prepare ligand **IV** by direct tritylation of resorcylic acid with trityl chloride under conditions applied to tritylation of phenols. The ditrityl derivative thus obtained was used in the synthesis of 2,6-ditrityloxybenzoic acid by the procedure of ligand **III** preparation (yield 23% with respect to resorcinol). However the best results were obtained at direct tritylation of the resorcylic acid under conditions of the phase-transfer catalysis formerly developed for tritylation of five-membered nitrogen-containing heterocycles [7]. At the treating of the resorcylic acid with trityl chloride in a system dichloromethane-aqueous NaOH in the presence of Bu_4NBr the corresponding ditrityl derivative was obtained in 56% yield.



We carried out preliminary investigation of new ligands complexing with Fe(II) under argon atmosphere. A reaction of sodium salt of ligand **II** with $\text{Fe}(\text{ClO}_4)_2 \cdot 10\text{H}_2\text{O}$ in methanol furnished a yellow complex that turned green at oxidation in air. Basing

on UV spectra the complex was assigned a structure of binuclear μ -oxocomplex of trivalent iron.

The lithium salt of ligand **III** and salt **V** with $\text{Fe}(\text{OTf})_2(\text{MeCN})_2$ in a mixture acetonitrile-toluene gave rise to pale straw-colored complexes of Fe(II) that by analogy to complex of ligand **II** prepared under the same conditions [5] was assigned a structure of binuclear complexes $\text{Fe}_2(\text{OCOR})_4(\text{MeCN})_2$.

The final conclusions on the structure of Fe(II) complexes with ligands **I-V** should be based on the X-ray diffraction analysis whose results will be published elsewhere.

2,6-Bis(2-methoxycarbonylphenoxy)methylpyridine (I). A mixture of 8.5 g (79 mmol) of 2,6-lutidine, 32.5 g (182 mmol) of N-bromosuccinimide, and 0.15 g of azobisisobutyronitrile in 300 ml of benzene was stirred at 50°C for 24 h at irradiation with a lamp of etched glass of 200 W power. Then the reaction mixture was treated with 10% solution of Na_2CO_3 (2×100 ml), washed with water (100 ml), benzene was removed under reduced pressure, and the residue was extracted with hexane (2×50 ml). On distilling off hexane under reduced pressure we isolated 6.7 g (32%) of 2,6-bis(bromomethyl)pyridine as a yellowish crystalline substance that was used further without additional purification. The repeated bromination with smaller charge resulted in 15% yield.

To a solution of 3.0 g (11.3 mmol) of 2,6-bis(bromomethyl)pyridine in 25 ml of anhydrous acetone was added a solution of 3.5 g (23 mmol) of methyl salicylate in 25 ml of acetone, 1.5 g of anhydrous potassium carbonate, and 50 mg of dibenzo-18-crown-6. The mixture was stirred at 45–50°C for 15 h. On completion of the reaction acetone was evaporated at reduced pressure, the residue was treated with benzene (100 ml), filtered, and on evaporating the benzene at reduced pressure we obtained 3.26 g (71%) of crude 2,6-bis(2-methoxycarbonylphenoxy)methylpyridine (**I**). The pure compound was obtained as colorless crystals after two recrystallizations from acetone. mp 120°C. ^1H NMR spectrum (CDCl_3), δ , ppm: 7.91 d (2H, β -H-P-), 7.83 t (1H, γ -H-P-), 7.74 d (2H, C_6H_4 , *p*-), 7.45–7.51 m (C_6H_4 , o -), 7.01–7.06 m (4H, C_6H_4 , *m*-), 5.03 s (4H, OCH_2N), 3.96 ©(6H, OCH_3). Found, %: C 67.76; H 5.35; N 3.45. $\text{C}_{23}\text{H}_{21}\text{NO}_6$. Calculated, %: C 67.80; H 5.20; N 3.44.

2,6-Bis(2-carboxyphenoxy)methylpyridine (II). Reagent **I** (1 g) was boiled for 15 h in 50 ml of 2% water solution of NaOH, then the mixture was cooled,

neutralized with 5% HCl till pH 6, the separated colorless precipitate was filtered off and dried in air (0.91 g, 97%). After recrystallization from 2-propanol we obtained 0.7 g of compound **II** as colorless crystals, mp 210°C. ¹H NMR spectrum (CD₃CN), δ, ppm: 8.16 d (2H, β-H-Py), 7.85 t (1H, γ-H-Py), 7.55 t (2H, C₆H₄, *o*-), 7.44 d (2H, C₆H₄, *p*-), 7.11–7.16 m (4H, C₆H₄, *m*-), 5.48 s (4H, OCH₂N). Found, %: C 66.42; H 4.74; N 3.64. C₂₁H₁₇NO₆. Calculated, %: C 66.48; H 4.52; N 3.69.

2,6-Diphenoxybenzoic acid (III). To a solution of 22 g (0.08 mol) of 1,3-diphenoxybenzene and 15 ml (0.105 mol) of tetramethylethylenediamine in 200 ml of anhydrous hexane at 10°C was added dropwise under argon atmosphere a solution of 0.12 mol of butyllithium in 150 ml of hexane. In 3 min after completion of butyllithium addition the reaction mixture became turbid, and a separation of granular colorless precipitate gradually started. The stirring was continued for 1 h at 10–15°C, then at cooling through the reaction mixture a flow of dry carbon dioxide (35 l, 1.5 mol) was passed. After that the stirring was continued for 1 h more, and at vigorous stirring was added 400 ml of water. The water layer was separated and thrice washed with 200 ml of hexane, then it was acidified till pH 5, the separated precipitate was filtered off and dried in a vacuum desiccator over P₂O₅. Yield 19 g (77%), mp 67–68°C. ¹H NMR spectrum (CDCl₃), δ, ppm: 7.29 t (4H, Ph, *m*-), 7.13 t (1H, C₆H₃), 7.05 t (6H, Ph, *o*- + *p*-), 6.55 d (2H, C₆H₃). Found, %: C 74.35; H 4.78. C₁₉H₁₄O₄. Calculated, %: C 74.50; H 4.61.

2,6-Bis(trityloxy)benzoic acid (IV). To a mixture of 3.08 g (0.02 mol) of resorcylic acid, 0.5 g of tetrabutylammonium bromide, 50 ml 20% NaOH, and 15 ml of dichloromethane under argon atmosphere was gradually added at slight stirring 12.27 g (0.044 mol) of trityl chloride in 35 ml of dichloromethane. The mixture was stirred for 3 h, the organic layer was separated, washed with water, dried with anhydrous potassium carbonate, the dichloromethane

was evaporated at reduced pressure, the solid residue was washed with ether and dried in a high vacuum. After recrystallization from benzene we obtained 7.15 g (56%) of acid **IV**, mp 224–226°C (decomp). IR spectrum, ν, cm⁻¹: 750, 850, 1038, 1078, 1244, 1391, 1444, 1487, 1594. ¹H NMR spectrum (CDCl₃), δ, ppm: 7.29 s (1H, C₆H₃, *p*-), 7.05 (2H, C₆H₃), 6.96–7.03 m (20H, tr). Found, %: C 84.43; H 5.54. C₄₅H₃₄O₄. Calculated, %: C 84.62; H 5.37.

IR spectra were recorded on spectrometer UR-20 from KBr pellets, NMR spectra were registered on spectrometers Varian-300 and Tesla at operating frequencies 300 and 100 MHz respectively.

Reagents from Aldrich and Lancaster Companies were used without additional purification. Fe(OTf)₂(MeCN)₂ was obtained by procedure [8].

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