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АРМЕНИЯ

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TRANSITION METAL-MEDIATED RADICAL REACTIONS AND DEVELOPMENT OF NOVEL THERAPEUTIC MEANS FOR BREAST CANCER TREATMENT

GAGIK G. MELIKYAN

Department of Chemistry and Biochemistry,
California State University Northridge, Northridge CA 91330, USA

Introduction

In the last few decades the scientific community has witnessed a rapid multifaceted development of radical chemistry.[1] Three major factors have contributed to this success. First, an elaboration of novel transition metal-induced methods of radical generation[2] allowed to significantly improve the selectivity for known species, and also to generate new types of radicals inaccessible by traditional means. Second, remarkable efficiency has been achieved in various types of chemical transformations, such as inter- and intramolecular processes, addition-cyclizations, conjugate additions, tandem and polycyclizations. Third, substantial contributions have been made in the synthesis of natural products having complex structures and unusual topology.[3] In spite of this success, the stereocontrol in radical reactions remains one of the major research thrusts in modern synthetic chemistry.[4] Both conceptually and experimentally, the most developed domain is a *substrate control in intramolecular cyclizations*, while *intermolecular radical reactions* are much less advanced demonstrating a variable selectivity both under *substrate control* and *chiral auxiliary control.* Overall, the existing methodology, although efficient and selective in some cases, has its drawbacks, such as a limited scope of the reactions, commonly observed low to moderate stereoselectivities, inaccessibility of some chiral auxiliaries, and limited applicability of the intramolecular strategies to controlling the stereoselectivity of the intermolecular reactions. Thus, efficient stereocontrol in radical reactions, in both inter- and intramolecular variants, requires new strategies and innovative approaches. Herein we report most recent developments in the field of radical organometallic chemistry, in particular (a) novel method for generation of cobalt-complexed propargyl cations under neutral conditions, (b) THF-mediated, highly stereoselective intermolecular coupling reactions, and also (c) design and synthesis of novel generation of aromatase inhibitors for breast cancer treatment.

I. Cobalt-complexed propargyl cations: Generation under neutral conditions and spontaneous, high temperature conversion to propargyl radicals

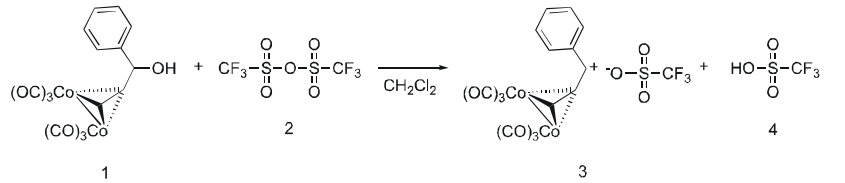
Transition metals are well known for their ability to stabilize π-bonded organic carbocations located alpha to the metal core.[5] To the contrary to their organic counterparts, metal-coordinated carbocations can be easily isolated, stored, and spectrally characterized, even by means of X-ray

crystallography. Their exploration over the last several decades has substantially enriched synthetic chemistry providing for novel approaches to a wide array of organic molecules, otherwise hardly accessible. Conventional protocol for generating carbocations dates back to 60s-70s and involves treatment of the respective alcohols, ethers, or acetates with acidic reagents, such as H_2SO_4, HBF_4, or BF_3. While efficient with chemically robust substrates, the use of strong acids, usually in large excess, imposes severe limits on the substrate base. The existing protocols do not apply to substrates bearing acid-sensitive – benzyloxy, acetal, 1,3-dioxolane -moieties, and also the functional elements susceptible to protonation, such as carbonyl, cyano, amino or imino groups. Thus, development of the novel method for cation generation under *neutral conditions* would drastically expand the substrate base and allow to use the functionalities that would otherwise be either removed under acidic conditions, or being protonated, and altered, structurally.

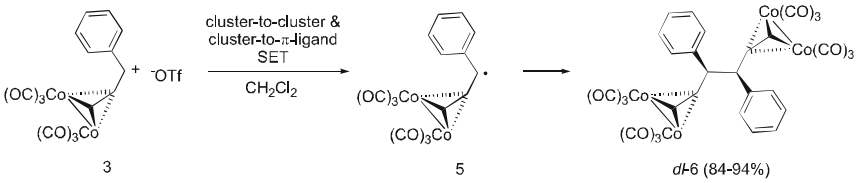
Spontaneous generation of cobalt-complexed propargyl radicals occurs slowly at ambient temperature when respective alcohols are treated with a two-fold excess of triflic anhydride.[6a] The most attractive feature of this reaction is its ability to form propargyl triflates *in situ*, rendering the laborious isolation of cations unnecessary. Cobalt-complexed propargyl alcohols could function as precursors to triflates, undergoing, albeit slow, a spontaneous C-O bond heterolysis (23h). Besides the reaction rate, another drawback is the formation of triflic acid, as a by-product, that creates a high acidity environment and makes the whole process inapplicable for substrates with acid-sensitive functionalities. *In the current study, the dual objectives were to make the reaction faster by carrying it out at elevated temperatures, and second, to avoid an acidic medium altogether in order to enhance the synthetic potential of the parent reaction and to expand its substrate base.*

Borrowing the lessons from the recently reported high-temperature reaction of pre-isolated propargyl cations,[6b] cobalt-complexed propargyl cation **1** was treated with a two-fold excess of Tf_2O (**2**) and heated in 1,1,2,2-tetrachloroemane at 147°C for 1min (Scheme 1).

Scheme 1. **Generation of the propargyl triflate 3 under acidic conditions: Method A.**



Scheme 2



An initial stage involves an *in situ* generation of propargyl triflate **3** and triflic acid **4**. Given its ionic nature, the former is analogous to the cobalt-complexed propargyl tetrafluoroborate salts and undergoes spontaneous conversion to the radical **5**, which further dimerizes to bis-cluster **6** (Scheme 2). While *d*,*l*-diastereoselectivity observed was excellent *(d,l:meso*, 94:6), an isolated yield of bis-cluster **6** was only 21.0% (Table 1; entry 1). To establish if the presence of an excess of Tf_2O (2) could be detrimental to the reaction outcome, only one equivalent of Tf_2O was applied (Table 1, entry 2). Careful monitoring of the reaction, by NMR, revealed some retardation (6min vs Imin), accompanied with a significant decline in stereoselectivity of the radical coupling *(dl*-**6***:meso*-**6**, 86:14). Meanwhile, the yield increased

noticeably, from 21.0% to 30.2%, clearly indicating that an excess of reagent substantially affects the amount of product that survives high-temperature treatment, albeit relatively brief.

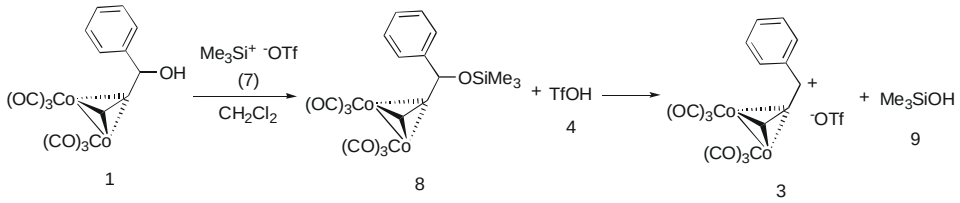
Table 1. **Spontaneous, high temperature radical coupling of cobalt-complexed propragyl triflates.**

	Protocol	Reactant composition	Medium	T, °C	Reaction time	crude **6** *d,l:meso*	Yield,[a] %	isolated **6** *d,l:meso*
1.	method A	alcohol **1** + 2eqs Tf_2O	acidic (TfOH[b])	147°	1min	94:6	21.0	96:4
2.	method A	alcohol **1** + 1eq Tf_2O	acidic (TfOH[b])	147°	6min	86:14	30.2	84:16
3.	method B	alcohol **1** + 1eq Me_3SiOTf	acidic (TfOH[b]/ Me_3SiOH[b])	83°	3min	92:8	81.2	94:6
4.	method C	Me ether **10** + 1eq Tf_2O	**neutral**	83°	3min	94:6	82.0	95:5

[a]The yields are calculated on the basis of the reaction stoichiometry that requires two equivalents of propargyl cations to form an equivalent of respective radicals. b Compound determining the acidity of the reaction medium.

To minimize an impact of TfOH-induced acidity upon the main characteristics of the radical coupling reaction – *yield, d,l-diastereoselectivity* – triflic anhydride was replaced with trimethylsilyl triflate (**7**). The main difference between Tf_2O (**2**) and Me_3SiOTf (**7**) is the nature of the electropositive component, $CF_3SO_2^+$ vs Me_3Si^+. Combining the latter with a hydroxy group derives TfOH (**4**) and Me_3SiOH (**9**), the species drastically differing in their acidities (pK_a -15 vs 11, respectively). By being less acidic, by some 26 orders of magnitude, Me_3SiOH (**9**) could provide more benign environment for radical reaction, and its products, thus improving the yield and stereochemical outcome. An interaction of propargyl alcohol **1** with Me_3SiOTf (**7**) affords silyl ether **8**, along with triflic acid **4** (Scheme 3). The latter, analogous to HBF_4, protonates a trimethylsiloxy group, giving rise to propargyl triflate **3** and Me_3SiOH (**9**). The optimization of the experimental protocol – solvent, temperature, reaction time – allowed us to identify conditions by far superior to that in *method A* (Table 1, entry 3). When carried out in 1,2-dichloroethane, at 83°C, with equimolar quantities of Me_3SiOTf (**7**), the reaction came to completion in 3min, forming dimers **6** in high yield (81.2%) and diastereoselectivity *(d,l:meso,* 92:8).

Scheme 3. **Generation of the propargyl triflate 3 under acidic conditions: Method B.**

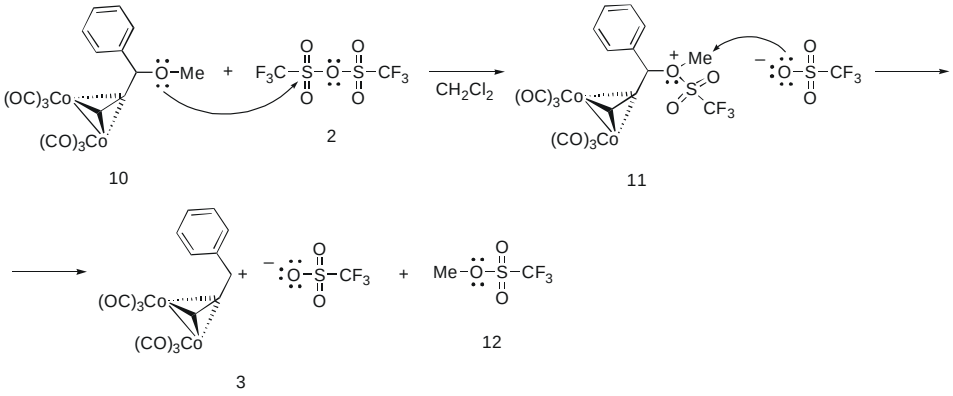


1 8 4 3 9

An ideal case scenario would have been the generation of propargyl triflate 3 under truly *neutral conditions* when the strong acids are not used, or generated, in the course of the reaction, either as final artifacts, or intermediate products. This objective was achieved by replacing propargyl alcohol **1** with Me-ether **10** (Scheme 4). Its treatment with an equimolar amount of Tf_2O (**2**), at 83°C, brought reaction to fruition in 3min, providing for high yield (82.0%) and restoring the level of diastereoselection *(d,l-***6***:meso-***6**, 94:6) (Table 1, entry 4). The mechanism of the process initially involves a nucleophilic attack upon electropositive sulfur in Tf_2O (**2**), affording an ionic pair **11** (Scheme 4). An oxonium counterpart then receives a nucleophilic attack by CF_3SO_2-anion, generating the requisite propargyl triflate **3** and methyl triflate (**12**), a *non-acidic molecule.* Its formation was unambiguously established by careful monitoring of the reaction by NMR. Given the chemical nature of methyl triflate (**12**), and also that of intermediates formed in the course of the reaction, the method is considered to be the first

generation of cobalt-complexedpropargyl cations under neutral conditions (Scheme 4). Among its attractive features are: (1) an *in situ* formation of propargyl cation, bypassing the laborious isolation step; (2) compatibility of the reaction, and its intermediates, with practically every acid-sensitive functional group; and (3) a high level of *d,l*-diastereoselection.

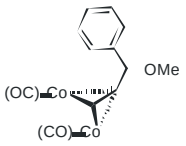
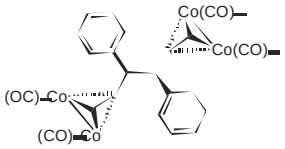
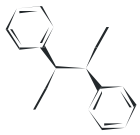
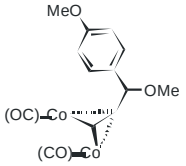
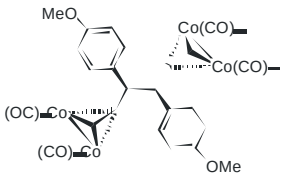
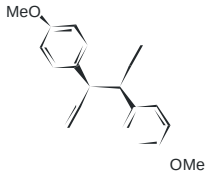
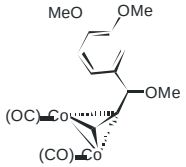
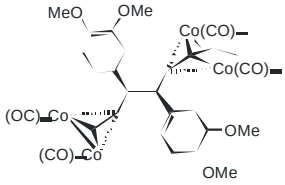
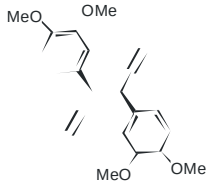
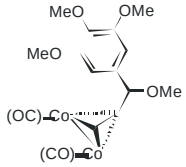
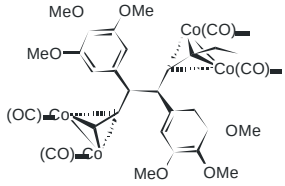
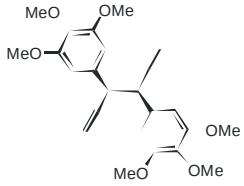
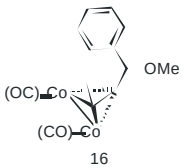
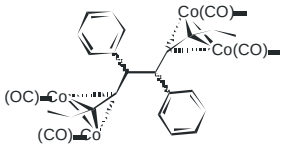
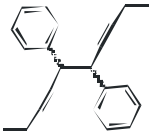
Scheme 4. **Generation of the propargyl triflate 3 under neutral conditions: Method C.**



The scope of the reaction was expanded by varying the topology and functionality of methyl propargyl ethers **13-16** (Table 2). The latter were synthesized either under acidic conditions from the respective propargyl alcohols (HBF_4/methanol), or, under non-acidic conditions, when the condensation product of sodium acetylide with the respective benzaldehydes was alkylated *in situ* with methyl iodide, followed by the complexation with $Co_2(CO)_8$. Introducing the MeO-substituents in the topologically diverse positions of the aromatic ring (**13-15**), as well as an Et-group in the γ-position of the acetylenic moiety (**16**) did not interfere with the course of the reaction, indicating, in particular, the compatibility of Tf_2O (**2**) with the substituents having a lone pair(s) of electrons. Under standardized conditions (83°C, 3min), radical dimerization products **17-20** were formed in high yields and excellent diastereoselectivity *(d,l*- 89-98%; Table 2). Decomplexation was carried out with eerie ammonium nitrate (8-10eqs) within the temperature range -78°÷-40°C, releasing 3,4-diaryl-l,5-alkadiynes in high yields as pure *d,l*-diastereomers (**21, 22**), or as diastereomeric mixtures (**23-25**).

It is worthy to mention that intermolecular radical dimerization reactions exhibit a low stereoselectivity in purely organic setting. The "classical" propargyl - propargyl coupling reaction exhibits a poor regioselectivity due to acetylene-allene rearrangement. Catalytic processes (Ru, Pd) suffers from a low regioselectivity because of the formation of isomeric allene-ynes. Also, they are inherently limited in scope with yields and diastereoselectivities drastically decreasing in the presence of electron-withdrawing (CF_3) and electron-donating aromatic substituents (Me; OMe). The alternative mediation of intermolecular coupling of propargyl alcohols with a $Ti(OiPr)_2Cl_2$ / Mg mixture suffers from a low diastereo selectivity, incomplete conversions (-70%), and poor regioselectivity with target 1,5-alkadiynes being accompanied by a comparable quantities of acetylenic allenes (45-50%).

Table 2. **Tf_2O-Mediated conversion of propargyl methyl ethers to dl-1,5-alkadiyne.**

Substrate	Bis-cluster	*dl* : *meso* ratio	Organic product[c]
		94 : 6[a]	
10	6		21
		97 : 3[a]	
13	17		22
		98 : 2[b]	
14	18		23
		97 : 3[b]	
15	19		24
		89 : 11[b]	
16	20		25

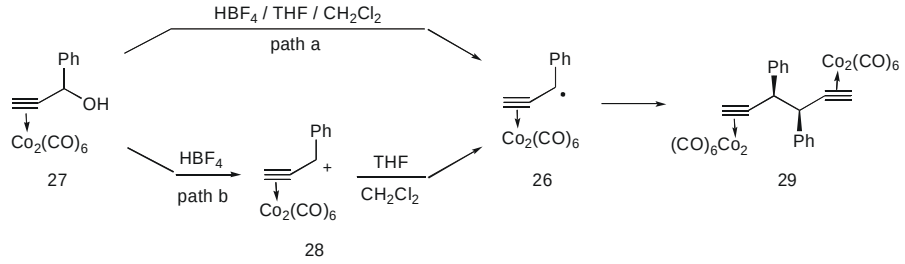
Chromatographically separable. Chromatographically inseparable.

II. THF-mediated, highly stereoselective intermolecular coupling reactions

A systematic study of the chemistry of transition metal-templated propargyl radicals and cations led us to the discovery of the novel THF-mediated generation, and coupling, of the $Co_2(CO)_6$-coordinated propargyl radicals.[7] *One-* and *two-step protocols* utilize metal-templated propargyl alcohols, as substrates, and include either *in situ* generation, or isolation, of respective cationic species. Thus, secondary radical **26**, derived from propargyl alcohol **27**, *via* cation **28**, was shown to dimerize to *d,l*-**29** with an excellent diastereoselectivity (de 90%, Scheme 5). The *path a* - one-step protocol - is considered to be more efficient than *path b*, since it bypasses a laborious isolation of the requisite cation **28**. The synthetic versatility of the reaction was proven by the stereoselective construction of eight- and nine-membered 1,5-cycloalkadiynes,[7b] and *d,l*-hexestrol, an inhibitor of microtubule

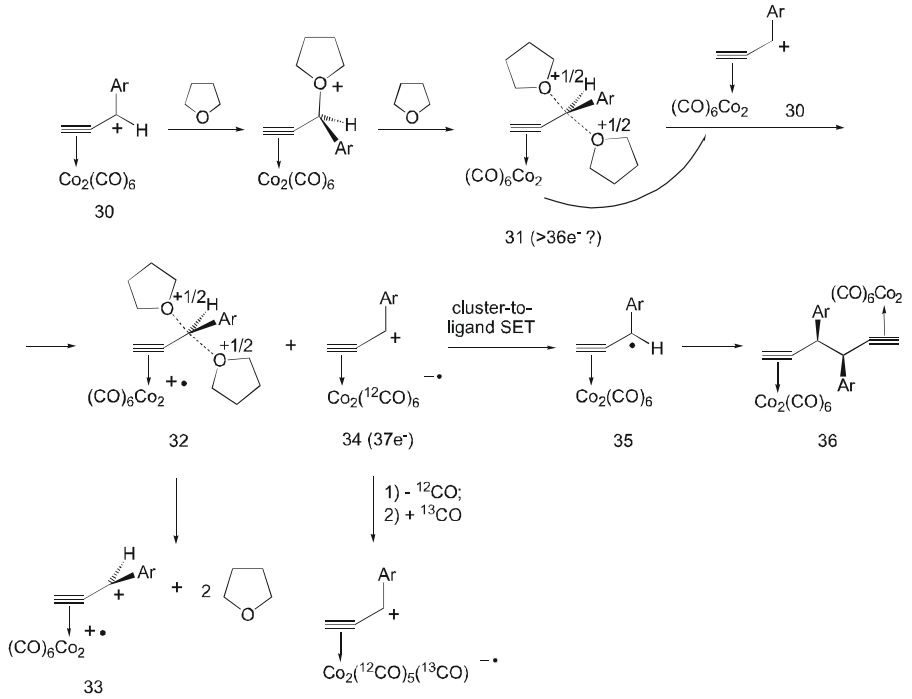
assembly. The intimate details of the THF-mediated process - *a mode of radical generation, the genesis of a single electron converting the cations to the respective radicals, the fate of THF molecule* - remained nevertheless unclear. It is worthy to mention that an alleged mediation of the radical reaction is not quite consistent with THF's synthetic profile. As a mild Lewis donor, it is widely used in organic chemistry as a solvent with an enhanced solvating power. As a reagent, it is known to donate H-atoms and hydride ions, but not to mediate, or directly participate, in a single-electron transfer (SET) processes. The current study was undertaken to shed light upon the mechanism of the THF-mediated radical process.

Scheme 5.



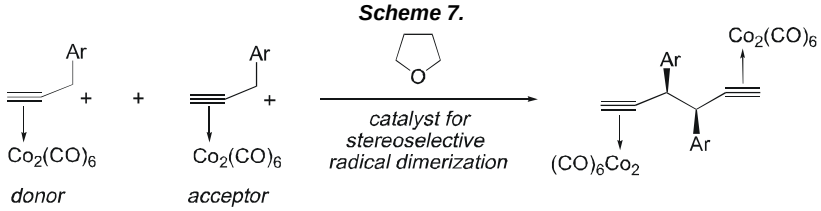
Based on the stoichiometry of the process, the measurement of the kinetic isotope effect (KIE) in the competitive and noncompetitive settings (THF-d_0 and THF-d_8), ligand substitution experiments with ^{13}CO, kinetic studies with model compounds - $Co_2(CO)_6$-complexed 1-phenyl-2-propyne, tetrahydrothiophene, cobalt-alkyne anchored tetrahydropyran - as well as *ab initio* calculations, the mechanism of the reaction was proposed (Scheme 6).[7c] The initial stage of the process includes the coordination of two molecules of THF with an electrophilic center in cation **30**. Several lines of supporting evidence include: a) kinetic data on THF and its 2,5-dimethyl-and 2,2,5,5-tetramethyl derivatives with reaction rates declining with an increase in a steric hindrance; b) stoichiometry studies with an empirically found optimal ratio of cation : THF equal to 1 : 2; c) calculation data on propargyl cation - THF complexes; and also d) literature precedence on the formation of the stable and structurally characterizable proton - heterocycle clusters. Complex **31**, a tentative key intermediate, would represent a significant departure from the original species **30**, both in steric and electronic terms. The electron density on the cluster in complex **31** should be higher (>36e^-) than that in cation **30** thus enabling the former to act as a reducing agent and transfer an electron toward a second molecule of cation **30**. The complex **31** is analogous to *radical anions* which can be generated from metal carbonyls by reduction with Na/Pli$_2$CO, or electrochemically, and exhibit a significant stability - in air, up to several hours -and increased reactivity in ligand displacement reactions. Conceptually, it is reminiscent of the (18+δ) complexes with an electron density higher than that of traditional 18-electron complexes and known to act as strong reducing agents. An oxidized complex **32** might undergo decomposition releasing bis-cationic species **33** and THF molecules, the process mimicked by a high recovery of cobalt-anchored THP derivative. The formation of anion-radical **34** accounts for an extensive ligand replacement observed in the presence of "heavy" carbon monoxide. This observation is fully consistent with an ample literature evidence that reduced transition metal complexes are susceptible to ligand displacement by a dissociative mechanism. The conversion of anion-radicals **34** to key radicals **35** might occur through an intramolecular cluster-to-ligand reduction reminiscent of the spontaneous, albeit slow, dimerization of the related species yielding dimer **36**.[6a] *Thus we conclude that THF acts as a catalyst that accelerates the process by forming the electronically enriched, and structurally altered, transient species* **31,** *and then breaks away, upon reaction completion, from the organometallic scaffold, in a chemically unchanged form.*

Scheme 6.



The proposed mechanism, by its very nature, is unorthodox for transition metal π-complexes: the THF molecule accelerates cluster-to-cluster electron transfer by structurally and electronically modifying a π-bonded ligand and converting cobalt-complexed propargyl cation into a source of electrons. Although used in a two-fold excess, THF acts *as a catalyst* altering the requisite $Co_2(CO)_6$-complexed cations, and separating from the organometallic scaffold in a chemically unaltered form. Phenomenologically, the new reaction represents a disproportionation between cobalt-complexed propargyl cations with THF - known in organic chemistry as a Lewis donor, donor of H-atoms and hydride ions - acting in an unusual capacity of a *radical mediator* (Scheme 7).

Scheme 7.



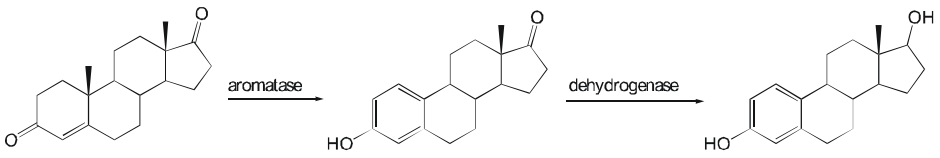
III. Novel Aromatase Modulators for a Breast Cancer Cure

The people of the United States continue to be faced with alarming numbers of breast cancer diagnoses and with a devastation of nearly 5,000 deaths a year in California alone. Although some therapeutics, like tamoxifen, are widely used in the medical practice, there is an urgent need for discovery of the new, highly efficient, non-toxic, tissue-specific and low cost drugs. In the proposed study, a *conceptually new approach to the discovery ofaromatase inhibitors - most promising class of therapeutics for breast cancer treatment - was developed.* The working hypothesis is that in order to achieve a high tissue specificity and low toxicity, a drug candidate should be chemically transformed

into its precursor, *a prodrug*, which could travel in the body without damaging healthy cells. Having come into contact with cancer cells in which an aromatase is expressed, a drug candidate would oxidatively convert itself into *an active form* capable of interacting with enzyme constituents and inhibiting estrogen biosynthesis. The design and construction of the target molecules – *prodrugs* – was carried out by using novel, cobalt-mediated radical C-C bond forming reactions developed in our laboratory.[6,7]

Human enzymes have long been studied to establish their functionality and relevance in identified pathologies. Aromatase enzyme was selected by us for further study due to the extensive documented research on its role in estrogen biosynthesis[8] and the structural similarity between known aromatase inhibitors and *d,l*-hexestrol, a nonsteroidal hormone. The enzyme is responsible for key chemical transformations: an aromatization of A-ring in androstene-3,17-dione (**37**) and testosterone yielding estrone (**38**) and estradiol (**39**), respectively. Two distinctive strategies have been formulated in the quest for the chemicals capable of inhibiting aromatase activities. The first group, *steroidal inhibitors*, is best represented by exemestane and formestane which mimic natural precursors and cause an irreversible enzyme inhibition. *Nonsteroidal inhibitors*, such as aminoglutethimide and fadrozole representing the second group, are widely available in today's pharmaceutical market. Among many disadvantages of known therapeutics is a toxicity and lack of selectivity: being intrinsically less enzyme specific, nonsteroidal inhibitors interfere with hydroxylation caused by other cytochrome P-450 enzymes.

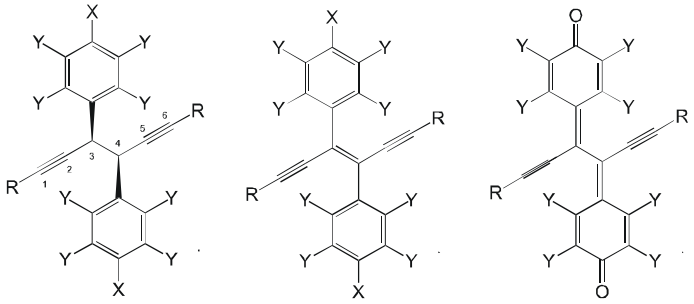
Scheme 8.



37 38 39

The conceptually novel approach features *a prodrug -d,l*-3,4-diaryl-l,5-hexadiyne **40** - that is synthesized, stored and administered in its *inactive, "sleeping" form.* The key transition toward *active form* - pseudo-bis-quinone **41,** via enediyne **42** - will occur by an oxidative interaction with aromatase well expressed in cancer cells. In the absence of aromatase-positive cells, a prodrug will be able to exist in a non-oxidizing biological environment without converting to its active form, thus exhibiting a lower toxicity and better selectivity. While activated, the potential drug - pseudo-bis-quinone **41** - will interact with enzyme, at least, by two distinct mechanistic pathway, i. e. by formation of Fe-O bond between oxygen-centered radical and iron(II)-species, and by H-atom abstraction from protein chain bearing the suitable functionalities, such as SH- and OH-groups. Both processes would result in an irreversible damage to enzyme rendering it incapable of interacting, and aromatizing, androstene-3,17-dione and testosterone.

Figure 1.



40 42 41

R = H, CH_2OH, CH_2NH_2; X = OH, OMe; Y = H, OH, OMe.

Topologically and functionally diverse *d,l*-3,4-diaryl-l,5-alkadiynes were screened against commercial aromatase assay. The standard procedure includes an exposure of testosterone to HumanCYP19 and P450 Reductase with the latter acting as a source of electrons. At pH 7.4, an incubation at 37°C for 20 min converts ca. 20% of testosterone to β-estradiol. The conversion is monitored by HPLC on a reverse-phase column (Zorbax, C_{18}) providing a baseline separation for both analytes. Most promising synthetic compounds with an inhibition rate of higher than 50% were then tested against breast cancer cell lines, such as MCF7. *In vitro* tests allowed to do lead optimization and undertake preliminary steps for preclinical and toxicological trials.

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