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ARTICLE

Furan-2,5-dimethylene-Tethered Bis-imidacloprid Insecticide Conferring High Potency

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Bis-imidacloprid (bis-IMI) analogues with suitable alkylene spacers have plant-systemic insecticidal properties. The alkylene-tethered bis-IMI binds in a unique mode to the insect nicotinic acetylcholine receptor (nAChR) wherein the chloropyridine moieties are embraced by two distinct and distant domains. The heptamethylene spacer optimally bridges these two subsites, yet the linker itself binds in a relatively nonspecific manner. This investigation examines the hypothesis that a bis-IMI analogue with a heteroaromatic tether, which undergoes specific interaction(s) with the newly recognized receptor cavity, may enhance the potency relative to those of the alkylene-tethered derivatives. Remarkably, a novel bis-IMI with a furan-2,5-dimethylene fulcrum showed highest receptor potency and insecticidal activity among the analogues with various chemotype spacers. The nAChR structural model, simulating the binding site interactions of the furan-2,5-dimethylene-tethered bis-IMI, reveals that the furan ring is nestled in a hydrophobic pocket, consisting of three aromatic amino acids, and is stabilized via hydrogen bonding.

KEYWORDS: Acetylcholine binding protein; bis-imidacloprid; N^3,$N^{3'}$-furan-2,5-dimethylene-tethered bis-imidacloprid; nicotinic acetylcholine receptor

INTRODUCTION

The insect nicotinic acetylcholine receptor (nAChR) is the target molecule for the action of neonicotinoid insecticides exemplified by the premier compound imidacloprid (IMI) (**Figure 1**) (*1*−*5*). Defined nAChR structure in neonicotinoid-bound state (*6*−*9*) has accordingly facilitated the receptor structure-guided ligand design quest for more effective insecticides with unique biological properties (*10*−*14*). The bis-pharmacophore drug design approach has been applied to neonicotinoid insecticide, yielding N^3,$N^{3'}$-alkylene-tethered bis-imidacloprid (bis-IMI) derivatives (**Figure 1**) (*15*−*17*). The dimeric IMI analogues with hexamethylene, heptamethylene, and octamethylene linkers have plant-systemic insecticidal properties (*18*). The number of bis-IMI methylene units determines the binding affinity to the insect nAChR (*15*−*17*, *19*), and the heptamethylene analogue showed optimal potency (*19*). The IMI dimer binds in a unique manner to the insect nAChR model wherein the chloropyridine moieties are embraced by two distinct and distant domains and the heptamethylene moiety suitably bridges these two subsites (*19*). This observation suggests that the optimal configuration of alkylene units, although relatively nonspecific, is determined by regional binding domains involving various stabilization interactions. The present investigation examines the hypothesis that a bis-IMI analogue with a heteroaromatic tether moiety (**Figure 2**), which undergoes specific interaction(s) as a fulcrum in the unique receptor cavity, may enhance the potency relative to the alkylene-tethered derivatives. We fortuitously identified a novel bis-IMI with a furan-2,5-dimethylene fulcrum showing outstanding receptor potency and insecticidal activity, thereby prompting us to establish a possible binding site interaction model featuring the furan linker nestled in a distinct hydrophobic pocket.

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MATERIALS AND METHODS

Chemistry. Compounds **1**, **2**, and **10** were available from our previous studies (*15*). All melting points (mp) are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded using a JEOL ECA-500 spectrometer at 500 and 125 MHz, respectively. The chemical shifts were recorded in δ (ppm) and the coupling constants J_{H-H} in hertz unless otherwise stated. Mass spectra were recorded at 70 eV with the JEOL JMS-700 instrument. Compounds **3**−**6**, **8**, **9**, and **11**−**16** were prepared according to the published procedure (route 1 in **Scheme 1**) (*15*−*17*), and the analytical data for structural confirmations are given in the Supporting Information.

Compound **7** was prepared via the intermediate *2,5-bis-(2-nitroiminoimidazolidin-1-ylmethyl)furan* (**17**) by following procedure route 2 in **Scheme 1**. A solution of ethylenediamine (484 mg, 8.1 mmol) in ethanol (0.5 mL) was added to a solution of 2,5-diformylfuran (200 mg 1.6 mmol) in ethanol (2 mL). The mixture was stirred at the reflux temperature for 9 h. The reaction solution was concentrated in vacuo, and the residue was dissolved in ethanol (10 mL) again. To the solution was added $NaBH_4$ (190 mg, 5.0 mmol) portionwise, and the mixture was stirred for 12 h at room temperature. Ethanol was removed under reduced pressure. The residue was suspended with acetonitrile (20 mL), the undissolved solid

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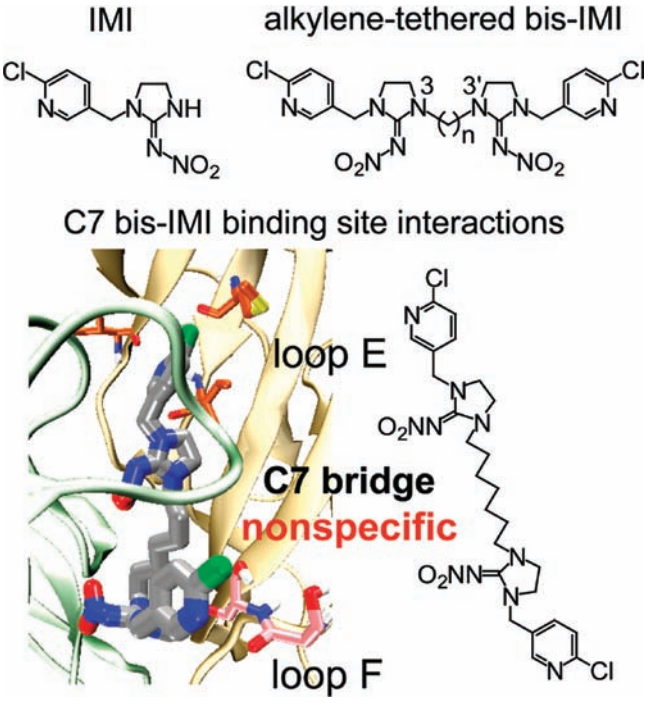


Figure 1. (Top) Neonicotinoid insecticide IMI and the IMI dimers connected with an alkylene spacer through the imidazolidine N^3 position. (Bottom) Predictive binding site interactions of the optimal compound heptamethylene-linked (C7) bis-IMI with the *Aplysia californica* acetylcholine binding protein (AChBP) (*19*) as a suitable structural surrogate for the insect nAChR extracellular ligand-binding domain (*5*−*8*). The IMI dimer binds to the AChBP wherein the chloropyridine moieties are embraced by two distinct and distant domains; loop E and F relevant amino acids are shown in orange and pink, respectively.

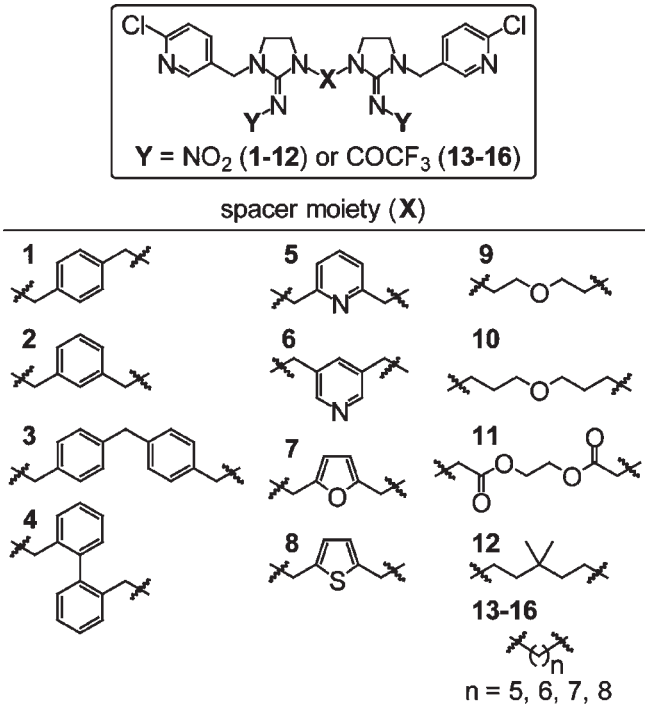


Figure 2. Structures of bis-IMI analogues with various chemotype spacer moieties.

was filtered off, and the filtrate was concentrated in vacuo. The residue was treated as above again. Finally, the residue was dissolved in ethanol (15 mL), and dimethyl-*N*-nitrodithioiminocarbonate (313 mg 1.9 mmol) was added. The mixture was stirred at 70 °C for 5 h. The concentrated residue was subjected to column chromatography on a silica gel ($CHCl_3$/MeOH 10:1) to give **17**: yield, 123 mg (22%); mp, 100−102 °C; NMR ($CDCl_3$), δ_H 3.61 (4H, m), 3.78 (4H, m), 4.50 (4H, s), 6.27 (2H, s), 8.15 (2H, bs); δ_C 41.4, 41.6, 45.7, 110.1, 149.1, 161.4. FAB-HRMS for $C_{12}H_{18}N_8O_5$: calcd ($+H^+$), 353.1322; found, 353.1332.

2,5-Bis-[*1-*(*6-chloro-3-pyridinylmethyl*)*-2-nitroiminoimidazolidine-3-ylmethyl*]*furan* (*7*). To an ice-cooled solution of **17** (84 mg, 0.3 mmol) in DMF (10 mL) was added sodium hydride (60% oil dispersion; 40 mg, 1.0 mmol) in portions. The mixture was stirred at room temperature for 0.5 h and cooled again in an ice−water bath when a solution of 2-chloro-5-chloromethylpyridine (162 mg, 1.0 mmol) was added slowly. The mixture was stirred overnight at room temperature. The reaction was quenched by adding of a drop of acetic acid, and the solution was concentrated in vacuo. The residual liquid was purified by chromatography on a silica gel column using EtOAc/MeOH 5:1 as eluent to give **7** as a white solid: yield, 55 mg (34%); mp, 58−60 °C; NMR ($CDCl_3$), δ_H 3.63 (4H, m), 3.78 (4H, m), 4.48 (4H, s), 4.49 (4H, s), 6.32 (2H, s), 7.37 (2H, d, J = 8.2 Hz), 7.69 (2H, dd, J = 8.2 Hz, J = 2.0 Hz), 8.32 (2H, d, J = 2.0 Hz); δ_C 43.5, 45.4, 46.3, 47.6, 111.2, 124.9, 129.0, 139.1, 148.4, 149.4, 151.8, 161.0. FAB-HRMS for $C_{24}H_{24}Cl_2N_{10}O_5$: calcd ($+H^+$), 603.1386; found, 603.1406.

Biology. Binding potencies of compounds were evaluated with native housefly (*Musca domestica*) brain nAChR using [^{3}H]IMI radioligand (*20*, *21*). IC_{50} values (molar concentrations of test compounds necessary for 50% inhibition of specific [^{3}H]IMI binding) were determined by iterative nonlinear least-squares regression using SigmaPlot software (SPSS Inc., Chicago, IL). IC_{50} values were finally converted to inhibition constants (K_i) using the equation of Cheng and Prusoff (*22*). Insecticidal activity was evaluated with adult female houseflies via intrathoracic injection in the presence of a cytochrome P450 inhibitor [*O*-propyl *O*-(2-propynyl) phenylphosphonate], which serves as a synergist by reducing the oxidative detoxification rate (*23*).

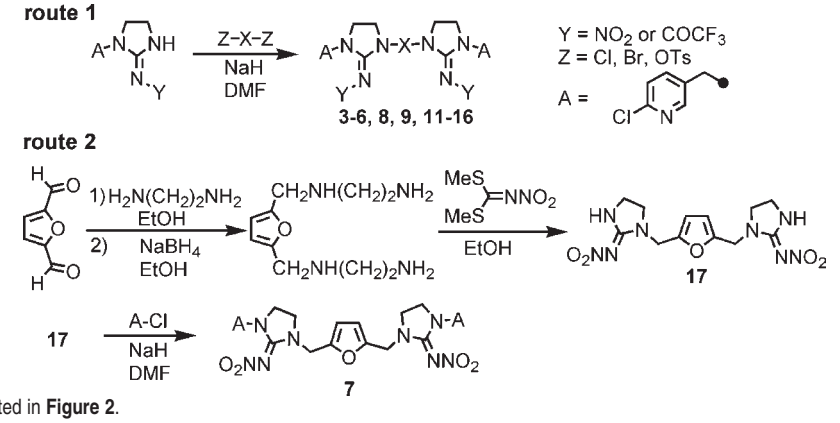
Calculations. Potential ligands were docked to the insect nAChR structural surrogate *Aplysia californica* acetylcholine binding protein (AChBP) (*6*−*8*) crystal structure 2WNJ chain A/E interface (*24*) using Glide as implemented in Maestro (Glide 5.5, Maestro 9.0, Schrödinger, LLC, New York, 2009) (*25*). We chose 2WNJ because it is an AChBP with a large bound ligand. It has a suitable starting state for the position of the Cys loop, with an open state of loop A Y93 and a rotation of loop D Y55, both opening the pocket for larger ligands. The protein was prepared for docking by the addition of hydrogen atoms and the removal of nonprotein moieties. Prior to docking, the protein was subjected to several cycles of minimization including optimization of hydrogen bonds. The Glide receptor grid was cubic and centered on the region occupied by the ligands in the original protein data bank structures. Due to the large conformational space associated with these ligands, they were initially manually fitted and minimized in the active site in binding modes consistent with the known neonicitinoid motifs. Ligands were then redocked and scored using standard precision.

To further explore the nature of the ligand binding interactions, the furan-2,5-dimethylene-tethered bis-IMI ligand (**7**) was subjected to cycles of molecular dynamics (MD) in the chain A/E binding pocket of 2WNJ. All residues within 6 Å of the ligand were allowed to move, whereas residues farther away were subjected to increasing layers of constraints. This is necessary because our model is a two-chain interface isolated from the functional structure. MD simulations were run using Macromodel (Macromodel 9.7, Schrödinger, LLC, 2009) (*26*) in 1 ns series with 100 ps equilibration at 300 K using a 1.5 fs step with SHAKE applied to bonds to hydrogen and the OPLS 2005 force field (*27*) with a constant dielectric water model. We included three starting configurations, one with both Y55 and Y188 OH moieties turned away from the furan oxygen, one with the Y55 OH oriented to the hydrogen bond to the furan oxygen, and one with the Y188 OH oriented to the hydrogen bond to the furan oxygen. All docking and MD simulations included a single water in the active site known to be critical to neonicitinoid binding (*8*, *12*).

RESULTS AND DISCUSSION

Synthesis. All tethered molecules were prepared by the coupling of IMI with the corresponding α,ω-bis-halide or bis-tosylate

Scheme 1. Preparation of Bis-IMI Analogues[a]



[a] Spacer moieties (X) are listed in **Figure 2**.

Table 1. SARs of Bis-IMI Analogues with Various Tether Moieties

compound			
no.	spacer moiety (X)	binding to *Musca* nAChR[a], $K_i \pm$ SD, nM ($n = 3$)	toxicity to *Musca*[b], LD_{50}, μg/g female
	nitroimine analogues		
1	$-CH_2-C_6H_4-CH_2-$ (*para*)	2270 ± 120	>1 (0%)[c]
2	$-CH_2-C_6H_4-CH_2-$ (*meta*)	2770 ± 420	>1 (0%)[c]
3	$-CH_2-C_6H_4-CH_2-C_6H_4-CH_2-$ (*para*−*para*)	17000 ± 1100	>1 (0%)[c]
4	$-CH_2-C_6H_4-C_6H_4-CH_2-$ (*ortho*−*ortho*)	800 ± 110	>1 (10%)[c]
5	$-CH_2-$pyridine$-CH_2-$ (2,6)	5200 ± 470	>1 (0%)[c]
6	$-CH_2-$pyridine$-CH_2-$ (3,5)	2300 ± 350	>1 (10%)[c]
7	$-CH_2-$furan$-CH_2-$ (2,5)	30 ± 1	0.14
8	$-CH_2-$thiophene$-CH_2-$ (2,5)	150 ± 11	1.0
9	$-(CH_2)_2O(CH_2)_2-$	530 ± 80	>1 (0%)[c]
10	$-(CH_2)_3O(CH_2)_3-$	2660 ± 70	>1 (0%)[c]
11	$-CH_2C(O)O(CH_2)_2OC(O)CH_2-$	1550 ± 310	>1 (0%)[c]
12	$-(CH_2)_2C[(CH_3)_2](CH_2)_2-$	1460 ± 160	>1 (0%)[c]
	trifluoroacetylimine analogues		
13	$-(CH_2)_5-$	16000 ± 1300	>1 (0%)[c]
14	$-(CH_2)_6-$	26000 ± 1200	>1 (0%)[c]
15	$-(CH_2)_7-$	2900 ± 510	>1 (0%)[c]
16	$-(CH_2)_8-$	26000 ± 1300	>1 (0%)[c]

[a] Assayed with [^{3}H]IMI. $K_i = IC_{50}/(1 + [L]/K_d)$ with radioligand concentration [L] = 5 nM and dissociation constant K_d = 5.4 nM (*20*, *21*). Control neonicotinoid IMI has K_i = 4.6 nM. [b] Fifty percent lethality doses were evaluated 24 h after toxicant administration via intrathoracic injection into flies, which were pretreated with a cytochrome P450 inhibitor [*O*-propyl *O*-(2-propynyl) phenylphosphonate] (*23*). IMI gives LD_{50} = 0.03 μg/g female. [c] Mortality percent at the indicated dose.

linker (route 1 in **Scheme 1**) except for compound **7**, which was obtained by the double introduction of the chloropyridinyl group to 2,5-bis-(2-nitroiminoimidazolidin-1-ylmethyl)furan (**17**), which was available by a three-step reaction starting from furan-2,5-dialdehyde (route 2 in **Scheme 1**). The final products thus obtained were purified through column chromatography with ethyl acetate as the eluent, where the bis-IMI derivatives were completely separated from fast-eluted IMI.

Biological Activities (Table 1). The compound with a *p*-xylenyl linker (**1**) showed moderate nAChR potency, and the *o*-isomer (**2**) was similarly active. Compound **3** with a bulky methano-dibenzyl (*para*−*para*) spacer, corresponding to an undecamethylene unit in spacer carbon length, had a greatly diminished potency, whereas the *ortho*−*ortho* biphenyl-dimethylene linker (**4**), equivalent to a hexamethylene linker, was 21-fold more active than the former one. These structure−activity relationship (SAR) findings related to the methylene unit number are similar to that of alkylene-tethered bis-IMI analogues (*19*). Pyridinyl-dimethylene isomers (**5** and **6**) had moderate to low receptor potencies. Intriguingly, bis-IMI with a furan-2,5-dimethylene fulcrum (**7**) was outstandingly potent, and the thiophene analogue (**8**) also showed relatively high affinity. The compound with a diethylene ether spacer (**9**), the acyclic analogue of **7**, showed higher binding potency than compound **10** with a dipropylene ether linker, suggesting that an oxygen atom in an optimal position may play an important role in molecular recognition. Compounds with two carbonyl esters and a branched alkylene linker (**11** and **12**) had moderate affinity. Neonicotinoids with a trifluoroacetylimino group are consistent in biological potency with the nitroimino pharmacophore insecticide (*13*), leading to the design of bis-IMI derivatives with a CF_3 substituent. The heptamethylene-tethered compound (**15**) had the best potency among the pentamethylene, hexamethylene, and octamethylene compounds (**13**, **14**, and **16**), similar to the SARs of the equivalent nitroimino bis-IMI analogues (*19*). Most compounds were not effective as insecticides, whereas the furan-2,5-dimethylene compound (**7**) gave high insecticidal activity, and the thiophene-2,5-dimethylene compound (**8**) also had some potency. Therefore, the suitable

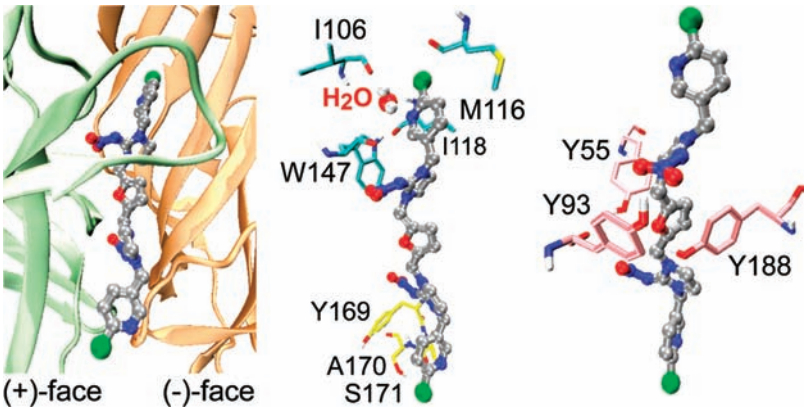


Figure 3. Binding site interactions of furan-2,5-dimethylene-tethered bis-IMI (**7**) with the *Aplysia* AChBP. (Left) Compound **7** is embraced by the interfacial binding pocket between the (+)-face (lime) and the (−)-face (orange) subunits. (Middle) MD snapshot shows the interactions of two IMI counterparts with the relevant amino acids for upward IMI (cyan) and downward IMI (yellow). The water molecule (*8*, *12*), bridging between pyridine nitrogen and related amino acids, is also displayed. (Right) Hydrophobic interactions of the furan fulcrum with the aromatic niche consisting of loop A Y93, loop C Y188, and loop D Y55 are illustrated. Loop D Y55 of *Aplysia* AChBP is replaced by tryptophan in the nAChRs (the Y55 of AChBP is functionally consistent with the tryptophan of receptor in making hydrophobic interactions with the furan ring of compound **7**). The furan oxygen atom forms a hydrogen bond with the Y188 OH.

configuration and/or spacer atom numbers of the bis-IMI chain-linker moiety are determined by the appropriate interactions of chloropyridine moieties with the two distant subsites, yet the linker itself binds in a relatively nonspecific manner. On the other hand, unique and specific interactions are expected for a rational fulcrum in addition to the two chloropyridine moieties, consequently consolidating the binding interactions conceivably via the three major receptor cavities.

Binding Site Interactions (Figure 3). In silico binding site interactions for the most active compound, **7**, were explored with the insect nAChR structural surrogate *Aplysia* AChBP. In a representative MD snapshot, the furan-2,5-dimethylene tethered bis-IMI (**7**) fits at the interface between the principal or (+)-face subunit and the complementary or (−)-face subunit (with an energy of −9.89 kcal/mol). One of the two IMI moieties (the upward one) binds to the known location where a single IMI molecule is accommodated (*5*, *6*, *8*). The upward chloropyridine nitrogen atom forms a water bridge with the loop E I106 backbone carbonyl oxygen and the I118 amide NH, whereas chlorine is nestled in a pocket formed by I106 and M116 backbone atoms. The nitro group of this IMI moiety possibly also hydrogen bonds to loop A Y93 OH. The upward guanidine plane may undergo hydrophobic interactions with loop B W147. The alternative IMI portion of the bis compound faces toward the loop F subsite, wherein the downward chloropyridine moiety shows a range of similar conformations involving hydrogen bonding and/or hydrophobic interactions with Y169/A170/S171 region. Other interactions in this field may be mediated via water(s) as these residues are near the protein surface. These observations are similar to those of the heptamethylene-tethered bis-IMI analogue (*19*). Uniquely, the furan moiety is embraced by a hydrophobic pocket consisting of three aromatic side chains, that is, loop A Y93, loop C Y188, and loop D Y55. More importantly, the furan oxygen favorably hydrogen bonds to Y188 OH, in this manner stabilizing the ligand in the hydrophobic cavity. Therefore, these interactions are presumably responsible for the high receptor potency of compound **7**.

Concluding Remarks. The present investigation identified a novel highly potent bis-IMI analogue with a furan-2,5-dimethylene fulcrum, which undergoes specific interactions within a unique receptor niche. Accordingly, simultaneous chemorational and biorational molecular designs may expedite discovery of novel insecticides.

ABBREVIATIONS USED

AChBP, acetylcholine binding protein; bis-IMI, bis-imidacloprid; IMI or [^{3}H]IMI, imidacloprid or its tritiated radioligand; K_i, inhibition constant; MD, molecular dynamics; nAChR, nicotinic acetylcholine receptor; SAR, structure−activity relationship.

Supporting Information Available: Analytical data for structural confirmation of compounds **3**−**6**, **8**, **9**, and **11**−**16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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