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α-Nitro Ketone as an Electrophile and Nucleophile: Synthesis of 3-Substituted 2-Nitromethylenetetrahydrothiophene and -tetrahydrofuran as *Drosophila* Nicotinic Receptor Probes

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3-(6-Chloropyridin-3-yl)methyl-2-nitromethylenetetrahydrothiophene **2** and -tetrahydrofuran **3** were synthesized through novel approaches using α-nitro ketone intermediates as an electrophile and nucleophile, respectively. The 2-nitromethylenetetrahydrothiophene **2** was formed exclusively as the *Z*-isomer through intramolecular attack by a thiol substituent at the carbonyl group of an α-nitro ketone, in which the α-nitro ketone served as an electrophile. In contrast, the corresponding 2-nitromethylenetetrahydrofuran **3**, not accessible by the above route due to limited stability, was prepared as a mixture of *E*- and *Z*-isomers by intramolecular attack of the α-nitro ketone enol anion in which the deprotonated α-nitro ketone served as a nucleophile. These compounds, together with the corresponding 2-nitromethylenepyrrolidine (**1**), were used to probe the *Drosophila* neonicotinoid−nicotinic acetylcholine receptor interaction.

Introduction

Neonicotinoids represented by imidacloprid are the only major new class of insecticides introduced in the past three decades. They act as selective agonists at the insect nicotinic acetylcholine receptor (nAChR) and are therefore highly toxic toward important insect pests but relatively safe to mammals.[1] The nitroguanidine/nitromethylene moiety is an important structural requirement of these neonicotinoid insecticides (Figure 1). Three models (Figure 2) have been proposed to account for the high binding affinity of neonicotinoids. The first two models (**a** and **b**) suggest a primary role for N1 of the neonicotinoid equating it to the pyrrolidine nitrogen of nicotine. In addition, the pyridine nitrogen of nicotine is superimposed on that of the neonicotinoid in model **a**[2] and on an oxygen of the nitro group in model **b**.[3] The third and most recent model (**c**) involves a crucial role for the nitro group, an important contribution from the pyridine nitrogen and a supplemental role for N1.[4]

To refine the binding model, in particular the role of N1, we synthesized three nitromethylene analogues of imidacloprid to further explore the structure−activity relationships. Specifically, compounds **1**−**3** were prepared with N1 replaced by CH comparing nitrogen, sulfur, and oxygen heterocycles (Figure 3). While compound **1** was made by a conventional method,[5] the substituted 2-nitromethylenetetrahydrothiophene **2** and substituted 2-ni-

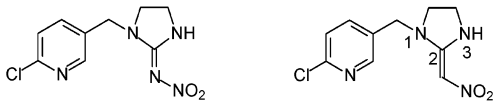

FIGURE 1. Imidacloprid (a nitroguanidine) and its nitromethylene analogue.

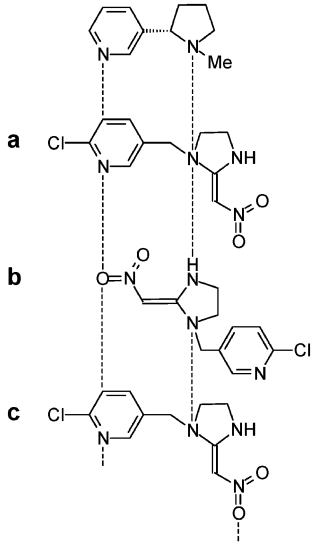

FIGURE 2. Three proposals for neonicotinoid sites involved in high-affinity binding.

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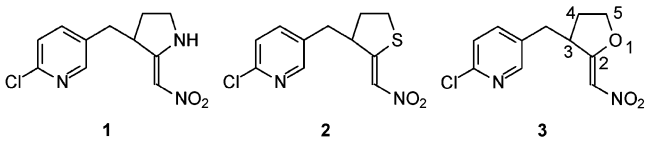
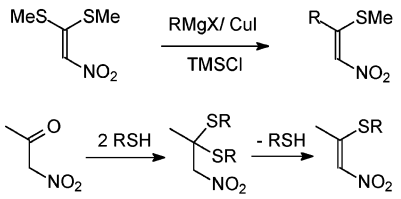


FIGURE 3. Probes to study the neonicotinoid−nAChR interaction.

SCHEME 1



tromethylenetetrahydrofuran **3** were synthesized by novel approaches using an α-nitro ketone as either an electrophile or a nucleophile, as we report here.

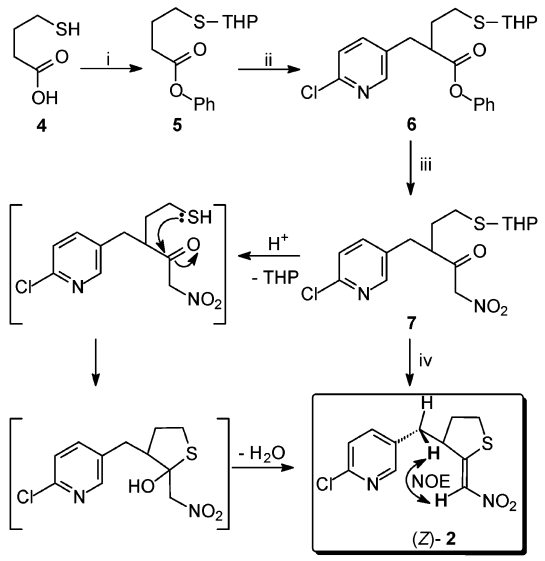
Results and Discussion

Our initial synthesis focused on 2-nitromethylenetetrahydrothiophene **2**, a 1,1-disubstituted 2-nitroethylene. There are only two reports of 2-nitroethylenes with both carbon and thio substituents at the 1 position (Scheme 1). One reacts the commercially available 1,1-bis(methylthio)-2-nitroethylene with an organocopper reagent in the presence of TMSCl to introduce a carbon substituent.[6] The other converts an α-nitro ketone to a thioacetal followed by elimination of a thiol to produce the nitroethylene moiety with a sulfur substituent.[7] Although these procedures are not directly applicable to the synthesis of compound **2**, we envisioned an α-nitro ketone precursor of the nitroethylene moiety; i.e., an intramolecular attack of a thiol group could produce a hemimercaptal and following elimination of water could generate the nitroethylene moiety with a thio substituent at the 1-position.

The synthesis of **2** involving α-nitro ketone **7** as the critical intermediate is shown in Scheme 2. The thiol group of 4-mercaptobutyric acid (**4**), prepared from hydrolysis of γ-butyrothiolactone,[8] was protected with a THP group, and the acid moiety was coupled with phenol and DCC to give phenyl ester **5** useful for further transformation to the α-nitro ketone.[9] The 6-chloropyridin-3-ylmethylene moiety was introduced by deprotonation of **5** with LDA followed by reaction with 2-chloro-5-iodomethylpyridine (CIMP). The resulting phenyl ester **6** was then converted to α-nitro ketone **7** in excellent yield by its reaction with the anion generated by treatment of nitromethane with potassium *tert*-butoxide.[9]

The next step was to remove the THP protecting group of α-nitro ketone **7** to provide a free thiol for intramolecular attack at the carbonyl site to form a hemimercaptal. Although the THP-protected thiol group is relatively stable toward acids and usually cleaved by metal-catalyzed procedures, the S-THP linkage can also be

SCHEME 2 [a]



[a] Reagents and conditions: (i) (a) dihydropyran, pyridinium *p*-toluenesulfonate, CH_2Cl_2, rt, overnight, (b) DCC, DMAP, PhOH, CH_2Cl_2, rt, overnight, overall 53%; (ii) LDA, CIMP, HMPA, THF, −78 °C to rt, overnight, 62%; (iii) CH_3NO_2, KOBu^t, DMSO, <20 °C, overnight, 79%; (iv) 37% HCl, rt, 30 min, 86%.

hydrolyzed under strong acidic conditions.[10] Thus, α-nitro ketone **7** was treated with concentrated HCl (37%, 12 M) at room temperature, leading to an immediate dark solution and precipitation after several minutes; the precipitate was collected after 30 min and identified as target compound **2** by NMR, MS, and elemental analysis. In this one-pot synthesis, three consecutive reactions are efficiently carried out under the strong acidic conditions, i.e., removal of the THP group, intramolecular attack at the carbonyl group, and elimination of water. The α-nitro ketone acts as an electrophile and is attacked by the thiol group. The elimination produced the desired *Z*-isomer exclusively, as evidenced by the NOE effect between the nitromethylene proton and one of the pyridinylmethylene protons, which is in contrast to the mixture of *E*- and *Z*-isomers obtained from the elimination of an alkylthiol from an α-nitro thioacetal.[7]

With the successful synthesis of 2-nitromethylenetetrahydrothiophene **2**, it was expected that tetrahydrofuran analogue **3** could be synthesized analogously to build the O1−C2 bond where O1 came from the hydroxyl group (Scheme 3). The synthesis started from TBS-protected 4-hydroxybutyric acid (**8**), which was readily prepared from the sodium salt of 4-hydroxybutyric acid with TBSCl in DMF by a novel silyl-transfer process.[11,12] Compound **8** was converted to its phenyl ester **9** with DCC, and the 6-chloropyridin-3-ylmethylene moiety was similarly introduced by treatment with LDA and then CIMP to give **10**. Further reaction with deprotonated nitromethane provided the α-nitro ketone **11**. Compound **11** was subjected to the same conditions as that of **7** by mixing with 37% HCl. However, although all starting material

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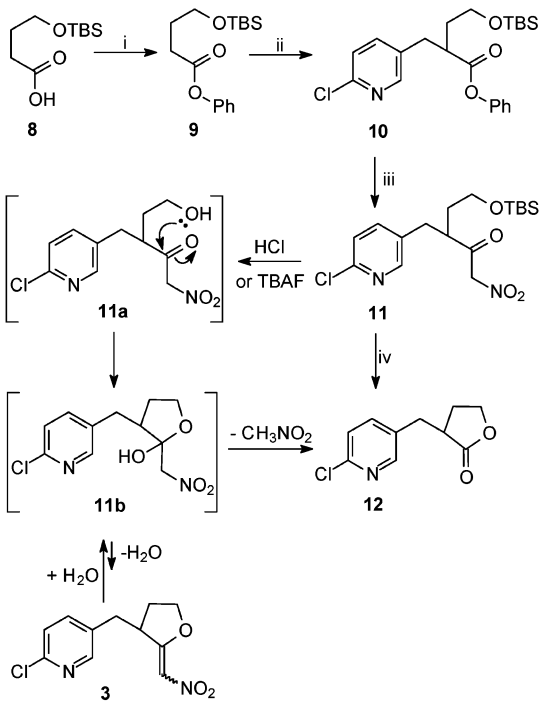
SCHEME 3[a]



[a] Reagents and conditions: (i) DCC, PhOH, DMAP, CH_2Cl_2, rt, overnight, 80%; (ii) LDA, CIMP, HMPA, THF, −78 °C to rt, overnight, 30%; (iii) CH_3NO_2, KOBu^t, DMSO, <20 °C, overnight, 44%; (iv) 37% HCl, rt, 30 min, 80% or TBAF, rt, 4 h, 98%.

was consumed, no desired product was found and instead lactone **12** was the major product isolated by silica gel chromatography. A rationalization for the production of **12** is that the hydroxy group released under strong acidic conditions attacks the carbonyl group (**11a**) to form a hemiacetal (**11b**), which is subjected to a retro-Henry reaction with elimination of the nitromethylene moiety (Scheme 3), in contrast to the elimination of water in the synthesis of tetrahydrothiophene **2**. Since the TBS group can be usually removed with TBAF,[10] a reaction of **11** with TBAF will presumably give the deprotected alcohol **11a**. However, upon chromatography of the reaction mixture, the major isolated product was again **12**. It seems that α-nitro ketone **11a** is unstable and once the hydroxy group is freed, it will attack the carbonyl site simultaneously and retro-Henry reaction gives lactone **12**, no matter whether acidic or basic conditions are used.

Another option to form the tetrahydrofuran ring is to build the O1−C5 bond (see Figure 3), possibly by attack of the enol anion of the α-nitro ketone at C5 bearing a good leaving group. Although halides and tosylates qualify for this role, in our synthesis (Scheme 4) we introduced a methylthio group at C5 to simplify the protection−deprotection process. The methylthio substituent is usually not a leaving group, but it can be activated by methylation since a dimethyl sulfide is a good leaving group. The synthesis started from 4-methylsulfanylbutyric acid (**13**), prepared from γ-butyrolactone with sodium methylthioate.[13] Similar to the previous syntheses, the acid **13** was converted to the phenyl esters **14** and then **15** which was further transformed into the α-nitro ketone **16** by reaction with nitromethane. Compound **16** was treated with iodomethane and potassium *tert*-butoxide successively to finish the cyclization in one pot. The major product was the desired (*Z*)-**3** with lesser amounts of (*E*)-**3** and a double bond shifted isomer **17**. The NOE effect was observed between the nitromethylene proton and one of the pyridinylmethylene protons for (*Z*)-**3** but not (*E*)-**3**.

The final one-pot cyclization includes three consecutive reactions: methylation at the sulfur atom, generation of the enol anion of the α-nitro ketone, and intramolecular attack of the enol anion at C5 (Scheme 4). Selective methylation at the methylthio group was done with 10 times excess of iodomethane using methanol as the solvent; simply reacting the α-nitro ketone **16** with iodomethane without solvent or using DMF as solvent complicated the reaction with significant methylation at the pyridine nitrogen. The addition of 18-crown-6 is crucial for the cyclization; without it, little (*E*/*Z*)-**3** was formed.

The biological activities of the new compounds were determined at the *Drosophila* nAChR with [^{3}H]imidacloprid binding assays. The nitromethylene analogue of imidacloprid is one of the most potent agonists known for the *Drosophila* nAChR, in this assay with a K_i of 0.13 nM versus 2.2 nM for imidacloprid[14] (Table 1). Compound **1** has very high activity (K_i 1.2 nM) followed by the sulfur analogue **2** (K_i 180 nM). However, (*E*)-**3** and (*Z*)-**3** have significantly lower potencies than **1** and **2**, and interestingly, there is no big difference between their activities, suggesting hydrolytic instability. The stability of compounds (*E*)-**3** and (*Z*)-**3** was examined under the binding assay conditions, revealing half-life times of 9.1 and 8.6 min, respectively, in pH 7.4 buffer. Lactone **12** was identified as the product from hydrolysis. The hydrolysis of **3** to **12** could go through the hemiacetal intermediate **11b** possibly formed by a Michael-type addition, as shown in Scheme 3. Thus, the measured apparent activity of **3** is probably actually that of compound **12**. In addition, a check of the stability for compound **2** under the same condition showed no hydrolysis.

The high binding affinities of ligands **1** and **2** suggest that N1 is not absolutely essential for activity, but the considerably reduced activity of **1** relative to that of the imidacloprid nitromethylene analogue indicates that N1 distinctly enhances potency. This finding favors binding model **c** (Figure 2).

In conclusion, we synthesized the biologically important imidacloprid analogues 3-(6-chloropyridin-3-yl)-methyl-2-nitromethylenetetrahydrothiophene (**2**) and -tetrahydrofuran (**3**) via α-nitro ketone intermediates which served as an electrophile and nucleophile, respectively. The 2-nitromethylenetetrahydrothiophene **2** was formed through intramolecular attack by a thiol substituent at the carbonyl group of the α-nitro ketone, serving as an electrophile, providing the *Z*-isomer exclusively. In contrast, the corresponding 2-nitromethylenetetrahydrofuran **3**, not accessible by the aforementioned route due to limited stability, was prepared by intramolecular attack of the enol anion in which the deprotonated α-nitro

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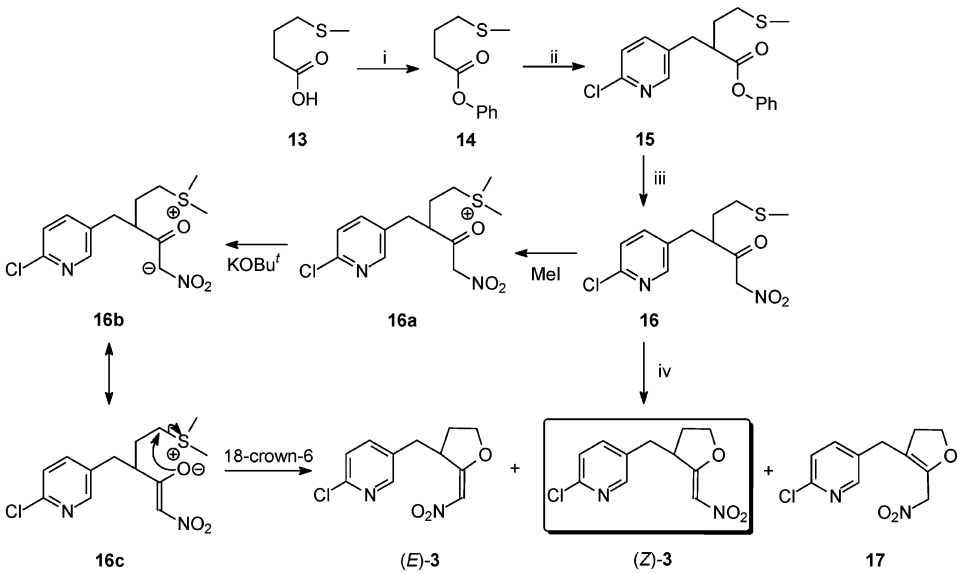
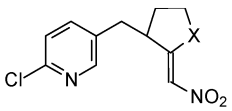
SCHEME 4[a]



[a] Reagents and conditions: (i) DCC, PhOH, DMAP, CH_2Cl_2, rt, overnight, 91%; (ii) LDA, CIMP, HMPA, THF, −78 °C to rt, overnight, 75%; (iii) CH_3NO_2, KOBu^t, DMSO, <20 °C, overnight, 87%; (iv) MeI, MeOH, rt, overnight; then KOBu^t, 18-crown-6, DMF, rt, 2h, 27% for (*Z*)-**3** and 21% for (*E*)-**3**.

TABLE 1. Binding Affinities of 1−3 to the *Drosophila* nAChR



compd no.	X	K_i (nM ± SD, $n = 3$)[a]
1[b]	NH	1.2 ± 0.3
2	S	180 ± 15
(*Z*)-**3**[c]	O	24000 ± 1800
(*E*)-**3**[c]	O	48000 ± 3700

[a] K_i values are calculated with the equation of Cheng and Prusoff,[15] i.e., $K_i = IC_{50}/(1 + [L]/K_D)$ with [L] = 3 nM and K_D = 2.4 nM. [b] For comparison, the corresponding K_i values for imidacloprid and its nitromethylene analogue are 2.2[14] and 0.13 ± 0.01 nM, respectively. The 2-nitromethylpyrrolidine analogue of **1** (synthesis not described here) has a K_i value of 600 ± 18 nM. 2-Nitromethyleneimidazolidine, an imidacloprid analogue without the chloropyridinylmethyl moiety, has a K_i value of 110000 ± 8500 nM compared with 2-nitromethylene[1,3]thiazinane (nithiazine) with a K_i value of 2400 ± 300 nM. [c] Hydrolytically unstable so the reported activity is probably for the hydrolysis product **12**.

ketone served as a nucleophile. These compounds and the corresponding 2-nitromethylenepyrrolidine (**1**) establish a distinct role but not absolute necessity for N1 in conferring high affinity at the *Drosophila* nAChR.

Experimental Section

4-(Tetrahydropyran-2-ylsulfanyl)butyric Acid Phenyl Ester (5). To a solution of 4-mercaptobutyric acid (**4**) (2.20 g, 18.3 mmol) in CH_2Cl_2 (50 mL) were added dihydropyran (2.30 g, 27.4 mmol) and pyridinium *p*-toluenesulfonate (0.46 g, 1.8 mmol). The solution was stirred overnight at rt, after which time the solvent and excess dihydropyran were removed. The residue was mixed with DCC (4.20 g, 20 mmol), DMAP (0.44 g, 3.6 mmol), and PhOH (2.10 g, 22 mmol) in CH_2Cl_2 (40 mL). The solution was stirred overnight at rt, concentrated, dissolved in EtOAc, and filtered. The filtrate was concentrated and subjected to silica gel column chromatography with EtOAc and hexanes (1:6) to give **5** as an oil (2.0 g, 53%): ^{1}H NMR δ 7.41−7.07 (m, 5H), 4.89 (dd, 1H, J = 3.6, 6.2 Hz), 4.13−4.06 (m, 1H), 3.56−3.48 (m, 1H), 2.85−2.78 (m, 1H), 2.73−2.66 (m, 3H), 2.11−1.58 (m, 8H); ^{13}C NMR δ 171.6, 150.7, 129.4, 125.7, 121.5, 82.3, 64.6, 33.2, 31.4, 29.6, 25.6, 25.2, 21.7. Anal. Calcd for $C_{15}H_{20}O_3S$: C, 64.26; H, 7.19. Found: C, 64.15; H, 7.20.

2-(6-Chloropyridin-3-ylmethyl)-4-(tetrahydropyran-2-ylsulfanyl)butyric Acid Phenyl Ester (6). To a stirred solution of LDA (2.0 M, 4.5 mL, 9.0 mmol) in THF (30 mL) was added dropwise a solution of **5** (1.68 g, 6.0 mmol) in THF (10 mL) at −78 °C, and the mixture was stirred for 30 min at the same temperature. CIMP (2.28 g, 9.0 mmol, prepared from 2-chloro-5-chloromethylpyridine by reaction with NaI in acetone) and HMPA (1.6 mL, 9.0 mmol) were added. The mixture was then stirred at rt overnight, and the reaction was quenched with saturated NH_4Cl solution and extracted with EtOAc. The extract was washed with water and brine, dried, and concentrated. Silica gel column chromatography of the residue with CH_2Cl_2 and hexanes (30:1) provided **6** (pair of diastereomers) as an oil (1.50 g, 62%): ^{1}H NMR δ 8.31 (d, 1H, J = 2.5 Hz), 7.57 (dd, 1H, J = 2.5, 8.2 Hz), 7.38−7.19 (m, 4H), 6.91 (dd, 2H, J = 1.5, 7.7 Hz), 4.90−4.82 (m, 1H), 4.08−4.01 (m, 1H), 3.51−3.46 (m, 1H), 3.13−2.75 (m, 5H), 2.25−1.58 (m, 8H); ^{13}C NMR δ 172.8, 150.4, 150.1, 150.0, 139.4, 133.1, 129.5, 126.3, 126.0, 124.1, 121.3, 82.5, 82.2, 64.6, 46.0, 34.4, 34.3, 32.4, 31.3, 28.0, 27.8, 25.6, 21.7. Anal. Calcd for $C_{21}H_{24}ClNO_3S$: C, 62.14; H, 5.96; N, 3.45. Found: C, 62.30; H, 5.98; N, 3.30.

3-(6-Chloropyridin-3-ylmethyl)-1-nitro-5-(tetrahydropyran-2-ylsulfanyl)pentan-2-one (7). To a suspension of potassium *tert*-butoxide (0.69 g, 6.6 mmol) in DMSO (5.0 mL) was added nitromethane (0.36 mL, 6.6 mmol) at <20 °C. The mixture was stirred for 1 h at the same temperature followed by dropwise addition of a solution of **6** (0.90 g, 2.2 mmol) in DMSO (2.0 mL). The reaction mixture was stirred overnight and then poured into ice−water (15 mL). The solution was acidified with concentrated HCl to pH 1 and extracted with EtOAc. The extract was dried and concentrated. Chromatography with EtOAc and hexanes (1:1) on silica gel column provided **7** as an oil (0.65 g, 79%), which was a mixture of a pair of diastereomers and their corresponding tautomers: ^{1}H NMR δ 8.23−8.19 (m, 1H), 7.48−7.44 (m, 1H), 7.31−7.28 (m, 1H), 6.66 and 6.64 (s, 0.18H), 5.54 (dd, 0.91H, J = 6.2, 14.9

Hz), 5.04 (dd, 0.91H, J = 5.1, 14.9 Hz), 4.83−4.68 (m, 1H), 4.02−3.96 (m, 1H), 3.53−3.45 (m, 1H), 3.27−2.52 (m, 5H), 2.22−2.03 (m, 1H), 1.97−1.58 (m, 7H). Anal. Calcd for $C_{16}H_{21}ClN_2O_4S$: C, 51.54; H, 5.68; N, 7.51; S, 8.60. Found: C, 51.29; H, 5.82; N, 7.36; S, 8.31.

3-(6-Chloropyridin-3-yl)methyl-2-nitromethylenetetrahydrothiophene (2). Compound **7** (0.40 g, 1.1 mmol) was mixed with concentrated HCl (6.0 mL), kept at rt for 30 min, and then diluted with water (40 mL). The aqueous solution was extracted with EtOAc, dried with Na_2SO_4, and concentrated. The residue was subjected to silica gel chromatography with EtOAc and hexanes (1:1) to give **2** as a pale yellow solid (0.25 g, 86%), which was identified as the *Z*-isomer by NOESY experiment: mp 103−104 °C; ^{1}H NMR δ 8.24 (d, 1H, J = 2.6 Hz), 7.48 (dd, 1H, J = 2.6, 8.2 Hz), 7.32 (d, 1H, J = 8.2 Hz), 7.26 (d, 1H, J = 1.0 Hz), 3.33−3.25 (m, 1H), 3.21−3.06 (m, 2H), 2.96 (dd, 1H, J = 6.2, 13.9 Hz), 2.75 (dd, 1H, J = 8.7, 13.9 Hz), 2.23−2.12 (m, 1H), 2.05−1.95 (m, 1H); ^{13}C NMR δ 167.4, 150.5, 149.9, 139.2, 132.3, 128.4, 124.4, 50.1, 35.8, 33.4, 32.8; EIMS m/z 270 (17, M^+). Anal. Calcd for $C_{11}H_{11}ClN_2O_2S$: C, 48.80; H, 4.10; N, 10.35. Found: C, 48.93; H, 4.14; N, 10.15.

4-(*tert*-Butyldimethylsilanyloxy)butyric Acid Phenyl Ester (9). To a stirred solution of 4-(*tert*-butyldimethylsilanyloxy)butyric acid (**8**) (prepared from sodium 4-hydroxybutyrate according to Renton's method,[11,12] 5.25 g, 23.6 mmol), DCC (5.40 g, 26.0 mmol), and DMAP (0.58 g, 4.7 mmol) in CH_2Cl_2 (50 mL) was added PhOH (2.70 g, 28.4 mmol). The mixture was stirred overnight, concentrated, dissolved in EtOAc, and filtered. The filtrate was concentrated and subjected to silica gel column chromatography with EtOAc and hexanes (1:30) to give **9** as an oil (5.60 g, 80%): ^{1}H NMR δ 7.42−7.08 (m, 5H), 3.74 (t, 2H, J = 6.0 Hz), 2.67 (t, 2H, J = 7.2 Hz), 2.01−1.92 (m, 2H), 0.93 (s, 9H), 0.09 (s, 6H); ^{13}C NMR δ 172.1, 150.7, 129.3, 128.3, 125.6, 121.5, 61.8, 30.7, 27.8, 25.9, 18.3, −5.4. Anal. Calcd for $C_{16}H_{26}O_3Si$: C, 65.26; H, 8.90. Found: C, 65.05; H, 8.86.

4-(*tert*-Butyldimethylsilanyloxy)-2-(6-chloropyridin-3-ylmethyl)butyric Acid Phenyl Ester (10). To a stirred solution of LDA (2.0 M, 6.0 mL, 12 mmol) in THF (50 mL) was added dropwise a solution of **9** (2.94 g, 10 mmol) in THF (10 mL) at −78 °C, and the mixture was stirred for 30 min at the same temperature. CIMP (3.04 g, 12 mmol) and HMPA (2.1 mL, 12 mmol) were added. After the mixture was stirred at rt overnight, the reaction was quenched with saturated NH_4Cl solution and extracted with EtOAc. The extract was washed with water and brine, dried, and concentrated. Silica gel column chromatography of the residue with CH_2Cl_2 and hexanes (1:1) provided **10** as an oil (1.2 g, 30%): ^{1}H NMR δ 8.31 (d, 1H, J = 2.6 Hz), 7.57 (dd, 1H, J = 2.6, 8.2 Hz), 7.38−7.19 (m, 4H), 6.90 (dd, 2H, J = 1.0, 8.7 Hz), 3.82−3.73 (m, 2H), 3.16−2.92 (m, 3H), 2.14−2.03 (m, 1H), 1.91−1.80 (m, 1H), 0.91 (s, 9H), 0.07 (s, 6H); ^{13}C NMR δ 173.1, 150.4, 150.1, 149.8, 139.3, 133.4, 129.4, 125.9, 124.0, 121.3, 60.4, 43.6, 34.8, 34.5, 25.9, 18.3, −5.4. Anal. Calcd for $C_{22}H_{30}ClNO_3Si$: C, 62.91; H, 7.20; N, 3.33. Found: C, 63.13; H, 7.31; N, 3.19.

5-(*tert*-Butyldimethylsilanyloxy)-3-(6-chloropyridin-3-ylmethyl)-1-nitropentan-2-one (11). To a suspension of potassium *tert*-butoxide (0.50 g, 4.8 mmol) in DMSO (4.0 mL) was added nitromethane (0.26 mL, 4.8 mmol) at <20 °C. The mixture was stirred for 1 h at the same temperature followed by dropwise addition of a solution of **10** (0.68 g, 1.6 mmol) in DMSO (2.0 mL). The reaction mixture was stirred overnight and then poured into ice−water (10 mL). The solution was then acidified with concentrated HCl to pH 3−4 and extracted with EtOAc. The extract was dried and concentrated. Chromatography with EtOAc and hexanes (1:3) on a silica gel column provided **11** as an oil (0.28 g, 44%). NMR indicated **11** contains ∼5% of the enol form: ^{1}H NMR δ 8.21 (d, 1H, J = 2.6 Hz), 7.45 (dd, 1H, J = 2.6, 8.2 Hz), 7.28 (d, 1H, J = 8.2 Hz), 5.43 (d, 1H, J = 14.9 Hz), 5.11 (d, 1H, J = 14.9 Hz), 3.72−3.61 (m, 2H), 3.07−2.73 (m, 3H), 2.04−1.73 (m, 2H), 0.88 (s, 9H), 0.06 (s, 6H); ^{13}C NMR δ 198.6, 150.1, 149.9, 139.3, 132.7, 124.3, 83.2, 60.4, 49.0, 34.5, 33.4, 25.9, 18.4, −5.6. Anal. Calcd for $C_{17}H_{27}ClN_2O_4Si$: C, 52.77; H, 7.03; N, 7.24. Found: C, 52.39; H, 7.14; N, 6.93.

Reaction of 11 with Concentrated HCl. A solution of **11** (100 mg, 0.26 mmol) and concentrated HCl (1.0 mL) was allowed to react at rt for 1 h, diluted with water, and extracted with EtOAc. The extract was dried and concentrated. The residue was subjected to chromatography on silica gel with EtOAc and hexanes (1:2). The major product was lactone **12** (43 mg, 80%): ^{1}H NMR δ 8.22 (d, 1H, J = 2.6 Hz), 7.56 (dd, 1H, J = 2.6, 8.2 Hz), 7.29 (d, 1H, J = 8.2 Hz), 4.34−4.13 (m, 2H), 3.23−3.14 (m, 1H), 2.91−2.78 (m, 2H), 2.35−2.25 (m, 1H), 2.04−1.90 (m, 1H); ^{13}C NMR δ 177.7, 150.2, 149.9, 139.4, 132.6, 124.3, 66.4, 40.5, 32.3, 27.8. Anal. Calcd for $C_{10}H_{10}ClNO_2$: C, 56.75; H, 4.76; N, 6.62. Found: C, 56.90; H, 4.70; N, 6.47.

Reaction of 11 with TBAF. Compound **11** (140 mg, 0.36 mmol) was mixed with a solution of TBAF in THF (1.0 M, 2.0 mL, 2.0 mmol) and stirred at rt for 4 h. The solution was then diluted with water and extracted with EtOAc. The extract was dried and concentrated. The residue was subjected to chromatography with EtOAc and hexanes (1:2). The major product was lactone **12** (75 mg, 98%).

4-Methylsulfanylbutyric Acid Phenyl Ester (14). To a stirred solution of 4-methylsulfanylbutyric acid (**13**) (0.91 g, 6.8 mmol, prepared by reaction of γ-butyrolactone and sodium thiomethoxide in HMPA[13]), DCC (1.55 g, 7.5 mmol), and DMAP (0.17 g, 1.4 mmol) in CH_2Cl_2 (14 mL) was added a solution of PhOH (0.77 g, 8.2 mmol) in CH_2Cl_2 (4.0 mL). The mixture was stirred overnight at rt, concentrated, dissolved in EtOAc, and filtered. The filtrate was concentrated and subjected to silica gel column chromatography with EtOAc and hexanes (1:10) to give **14** as an oil (1.30 g, 91%): ^{1}H NMR δ 7.42−7.08 (m, 5H), 2.72 (t, 2H, J = 7.2 Hz), 2.63 (t, 2H, J = 7.2 Hz), 2.14 (t, 3H), 2.06 (quintet, 2H, J = 7.2 Hz); ^{13}C NMR δ 171.6, 150.6, 129.3, 125.7, 121.4, 33.4, 32.9, 23.9, 15.3. Anal. Calcd for $C_{11}H_{14}O_2S$: C, 62.83; H, 6.71. Found: C, 62.96; H, 6.90.

2-(6-Chloropyridin-3-ylmethyl)-4-methylsulfanylbutyric Acid Phenyl Ester (15). To a stirred solution of LDA (2.0 M, 4.8 mL, 9.6 mmol) in THF (30 mL) was added dropwise a solution of **14** (1.0 g, 4.8 mmol) in THF (10 mL) at −78 °C, and the mixture was stirred for 30 min at the same temperature. CIMP (2.42 g, 9.6 mmol) and HMPA (1.7 g, 9.6 mmol) were added. After the mixture was stirred at rt overnight, the reaction was quenched with saturated NH_4Cl solution and extracted with EtOAc. The extract was washed with water and brine, dried, and concentrated. Silica gel column chromatography of the residue with EtOAc and hexanes (1:5) provided **15** as an oil (1.2 g, 75%): ^{1}H NMR δ 8.32 (d, 1H, J = 2.5 Hz), 7.58 (dd, 1H, J = 2.5, 8.2 Hz), 7.39−7.19 (m, 4H), 6.93−6.89 (m, 2H), 3.11−3.03 (m, 2H), 2.96−2.90 (m, 1H), 2.67−2.62 (m, 2H), 2.24−2.14 (m, 1H), 2.11 (s, 3H), 1.98−1.88 (m, 1H); ^{13}C NMR δ 172.7, 150.3, 150.0, 139.3, 133.0, 129.4, 128.3, 126.0, 124.0, 121.2, 45.9, 34.5, 31.7, 31.2, 15.4. Anal. Calcd for $C_{17}H_{18}ClNO_2S$: C, 60.80; H, 5.40; N, 4.17. Found: C, 60.62; H, 5.60; N, 4.23.

3-(6-Chloropyridin-3-ylmethyl)-5-methylsulfanyl-1-nitropentan-2-one (16). To a suspension of potassium *tert*-butoxide (0.56 g, 5.4 mmol) in DMSO (3.0 mL) was added nitromethane (0.29 mL, 5.4 mmol) at <20 °C. The mixture was stirred for 1 h at the same temperature followed by dropwise addition of a solution of **15** (0.60 g, 1.8 mmol) in DMSO (1.0 mL). The reaction mixture was stirred overnight and then poured into ice−water (10 mL). The solution was acidified with concentrated HCl to pH 1 and extracted with EtOAc. The extract was dried and concentrated. Chromatography with EtOAc and hexanes (1:1) on a silica gel column provided **16** as an oil (0.47 g, 87%). NMR indicated that **16** was a mixture of tautomers: ^{1}H NMR δ 11.87 (br s, 0.18H), 8.20 (d, 1H, J = 2.1 Hz), 7.45 (dd, 1H, J = 2.1, 8.2 Hz), 7.29 (d, 1H, J = 8.2 Hz), 6.63 (s, 0.18H), 5.50 (d, 0.82H, J = 14.9 Hz), 5.06 (d, 0.82H, J = 14.9 Hz), 3.02−2.43 (m, 5H), 2.21−2.05 (m, 1H),

2.04 (s, 0.5 H), 1.98 (s, 2.5 H), 1.90−1.75 (m, 1H); ^{13}C NMR major tautomer (**16**) δ 198.3, 150.3, 149.9, 139.3, 132.2, 124.4, 83.7, 50.1, 34.0, 31.7, 30.2, 15.0; minor tautomer (enol form) δ 174.1, 150.1, 149.8, 139.0, 132.4, 124.2, 116.9, 44.2, 34.6, 31.4, 30.9, 15.3. Anal. Calcd for $C_{12}H_{15}ClN_2O_3S$: C, 47.60; H, 4.99; N, 9.25. Found: C, 47.54; H, 5.01; N, 9.01.

3-(6-Chloropyridin-3-yl)methyl-2-nitromethylenetetrahydrofuran (3). Into a solution of **16** (0.13 g, 0.33 mmol) in methanol (1.3 mL) was added iodomethane (0.7 g, 4.9 mmol). The solution was kept at rt overnight, and the solvent was then removed. The residue was dissolved in DMF (1.5 mL), cooled to −20 °C, and then treated dropwise with a solution of potassium *tert*-butoxide (0.05 g, 0.48 mmol) in DMF (1.0 mL) followed by 18-crown-6 (0.13 g, 0.49 mmol). The reaction mixture was stirred at rt for 2 h and quenched by pouring into a solution of 1 N HCl. The aqueous solution was extracted with EtOAc. The extract was dried and concentrated. The residue was purified by silica gel column chromatography with EtOAc and hexanes (2:3 to 1:1) to give two isomers of **3** as oils. The more polar one corresponds to the *Z*-isomer (30 mg, 27%) as confirmed by a NOESY experiment: ^{1}H NMR δ 8.27 (d, 1H, J = 2.6 Hz), 7.52 (dd, 1H, J = 2.6, 8.2 Hz), 7.34 (d, 1H, J = 8.2 Hz), 6.60 (d, 1H, J = 1.1 Hz), 4.65−4.58 (m, 1H), 4.54−4.46 (m, 1H), 3.32−3.22 (m, 1H), 3.02 (dd, 1H, J = 5.1, 13.9 Hz), 2.76 (dd, 1H, J = 9.7, 13.9 Hz), 2.26−2.15 (m, 1H), 1.96−1.84 (m, 1H); ^{13}C NMR δ 168.8, 150.6, 149.8, 139.0, 131.8, 124.5, 114.1, 74.4, 44.8, 34.6, 28.7. Anal. Calcd for $C_{11}H_{11}ClN_2O_3$: C, 51.88; H, 4.35; N, 11.00. Found: C, 52.02; H, 4.40; N, 10.83. Accordingly, the less polar isomer (23 mg, 21%) was assigned as the (*E*)- isomer. ^{1}H NMR δ 8.30 (d, 1H, J = 2.5 Hz), 7.72 (dd, 1H, J = 2.5, 8.2 Hz), 7.35 (d, 1H, J = 8.2 Hz), 7.17 (s, 1H), 4.56−4.50 (m, 1H), 4.35−4.24 (m, 1H), 4.19−4.12 (m, 1H), 3.21−3.16 (m, 1H), 2.67−2.60 (m, 1H), 2.23−2.15 (m, 1H), 2.03−1.96 (m, 1H). ^{13}C NMR δ 178.2, 150.4, 149.8, 139.4, 132.9, 124.6, 118.8, 72.0, 44.9, 32.9, 27.5. Anal. Calcd for $C_{11}H_{11}ClN_2O_3$: C, 51.88; H, 4.35; N, 11.00. Found: C, 51.55; H, 4.54; N, 10.57. Another isolated product (15 mg, 14%) was 2-chloro-5-(2-nitromethyl-4,5-dihydrofuran-3-ylmethyl)pyridine (**17**): ^{1}H NMR δ 8.25 (d, 1H, J = 2.6 Hz), 7.54 (dd, 1H, J = 2.6, 8.2 Hz), 7.30 (d, 1H, J = 8.2 Hz), 5.08 (s, 2H), 4.36 (t, 2H, J = 9.2 Hz), 3.47 (s, 2H), 2.57 (t, 2H, J = 9.2 Hz); ^{13}C NMR δ 150.1, 149.5, 142.5, 138.8, 132.6, 124.4, 115.3, 70.2, 68.9, 32.8, 29.3; FAB-MS m/z 255 (100, $[M + H]^+$). Anal. Calcd for $C_{11}H_{11}ClN_2O_3$: C, 51.88; H, 4.35; N, 11.00. Found: C, 52.20; H, 4.57; N, 10.61.

Hydrolysis of 3. In the kinetic studies, the hydrolysis of **3** in 0.1 M pH 7.4 phosphate buffer (66 μM) was followed with UV. The absorption at 306 nm was recorded, and the reaction rate was calculated according to Kezdy−Swinbourne's method.[16] In the characterization studies, a solution of **3** (10 mg) in 0.1 M pH 7.4 phosphate buffer (5.0 mL) was stirred at rt for 2 h and then extracted with EtOAc. The organic layer was dried with Na_2SO_4 and concentrated. The residue was identified as **12** by ^{1}H and ^{13}C NMR.

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