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Design and synthesis of an isopenicillin N synthase mimic

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Abstract—Towards the aim of creating a functional mimic of isopenicillin N synthase, a small molecule designed to coordinate around iron(II) and model the enzyme active site has been prepared in nine synthetic steps from 2,6-bis(hydroxymethyl)pyridine, (*S*)-(+)-mandelic acid and pivaldehyde. One aspartate, two histidines and a water ligand in the natural enzyme are replaced by an α-hydroxy acid, pyridine and aniline in the model compound. Additionally, a free thiol designed to simulate the enzyme substrate, δ-(L-α-aminoadipoyl)-L-cysteinyl-D-valine, is linked to the ligand by a three carbon chain. We postulate that in the presence of molecular oxygen, the complex formed between this synthetic ligand and iron(II) will display oxidative chemistry similar to that observed in the active site of isopenicillin N synthase.

1. Introduction

Isopenicillin N synthase (IPNS) is a non-heme iron(II)-dependent oxidase that catalyzes the conversion of δ-(L-α-aminoadipoyl)-L-cysteinyl-D-valine (ACV) **1** to isopenicillin N (IPN) **2**, the precursor of all penicillin and cephalosporin antibiotics (Fig. 1).[1,2] IPNS uses the full oxidizing power of molecular oxygen to perform this remarkable bicyclization, producing 2 equiv of water over the course of the reaction. Comprehensive spectroscopic,[3] crystallographic,[4–6] and substrate-analogue turnover studies[7,8] have led to the proposal of a detailed mechanism for the enzymatic transformation (Fig. 2A).

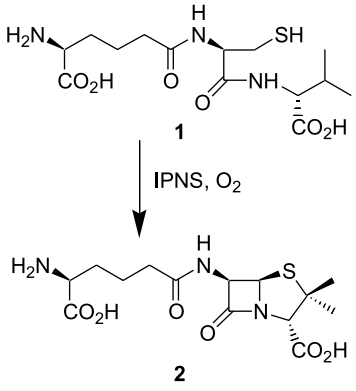

Figure 1. ACV **1** is converted to IPN **2** by IPNS in the presence of molecular oxygen.

Despite the development of a broad understanding of the IPNS active site, efforts to synthesize an iron-centered complex that mimics the activity of the enzyme have been unsuccessful.[9] Functional enzyme mimics can be important tools for advancing mechanistic knowledge as well as for testing existing hypotheses. Such mimics have recently been developed for several non-heme iron(II) oxidases including methane monooxygenase,[10] the extradiol-cleaving catechol dioxygenases[11,12] and the α-keto acid-dependent enzymes.[13] A detailed discussion of this topic appears in a recent review of the mechanisms and mimics of dioxygenase enzymes.[14]

The failure to create an equivalent IPNS mimic can be attributed primarily to the complexity of the reaction that this enzyme catalyzes. A major energetic hurdle must be overcome to hold an ACV-based substrate in the conformation required for the formation of such a strained system. When ACV is bound in the IPNS active site, multiple contacts between enzyme and substrate stabilize this conformation and promote formation of the bicyclic β-lactam product.[5] These stabilizing interactions cannot be easily replicated in a small model system, and efforts to mimic the entire reaction cycle of IPNS have consequently met with little success.[9]

A more achievable goal is the creation of a model system that mimics the early steps of the IPNS oxidation without requiring the system to duplicate the high-energy ring-closures. Recent studies with IPNS and the modified

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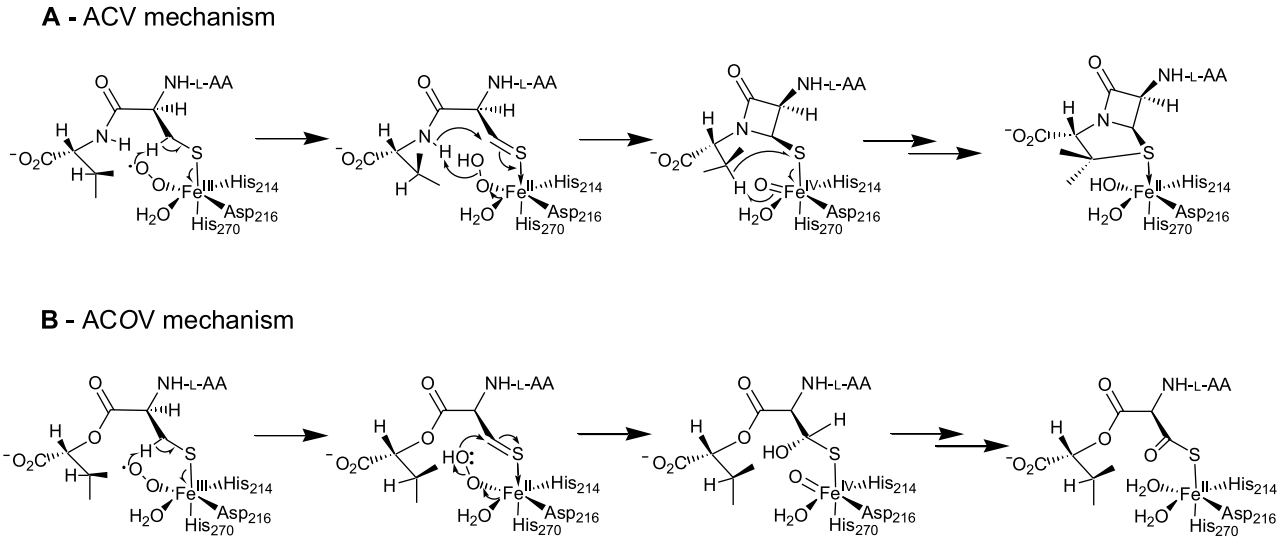


Figure 2. The reaction cycle of IPNS with ACV (A) and AC*O*V (B). With both substrates, it is proposed that initial hydrogen abstraction by an iron-bound superoxide results in the formation of a thioaldehyde bound to iron through the sulfur atom. With ACV, the valinyl amide nitrogen atom then attacks the thioaldehyde to form a β-lactam. In the reaction with AC*O*V, which lacks this internal nucleophile, the hydroperoxide formed after initial hydrogen abstraction itself attacks the thioaldehyde, and the resulting hydroxyl group is ultimately oxidized to give a thiocarboxylate.

substrate δ-(L-α-aminoadipoyl)-L-cysteine D-α-hydroxyisovaleryl ester (AC*O*V) provide support for a mechanism involving initial hydrogen abstraction by an iron(III)-bound superoxide species to give a thioaldehyde and an iron(II)-hydroperoxide (Fig. 2B).[15] In the absence of the substrate-derived nucleophile that is generated in the reaction of ACV, the hydroperoxide itself attacks the thioaldehyde, and the cysteine residue is ultimately oxidized to a thiocarboxylate.

We now report the synthesis of a pyridine-based ligand **3** designed to form a pentadentate complex **4** with iron(II) (Fig. 3). We postulate that in the presence of iron(II), the complex formed should display oxidative chemistry similar to that seen in the early stages of the IPNS reaction cycle (Scheme 1).

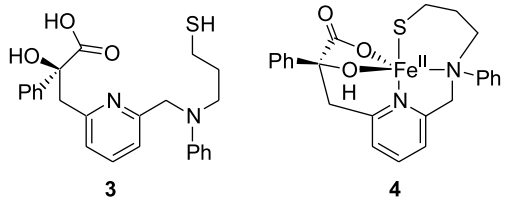
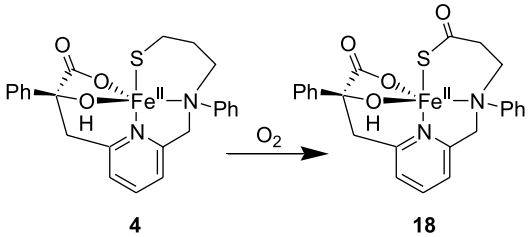


Figure 3. Ligand **3** and its complex **4** with iron(II), a proposed mimic for the IPNS active site.



Scheme 1. The proposed biomimetic oxidation of the IPNS enzyme mimic **4** upon exposure to molecular oxygen. We propose that the carbon adjacent to the iron-bound sulfur will be oxidized to a thiocarboxylate in the resulting complex **18**.

2. Results and discussion

Towards the goal of constructing an IPNS mimic, we designed a complex small molecule **3** to simulate the iron-coordinating ligands in the active site of the natural enzyme. The iron center in ACV-bound IPNS is coordinated by one aspartate (Asp_{216}) and two histidine (His_{214} and His_{270}) residues from the protein in a facial triad arrangement,[4] a motif that is conserved throughout the non-heme mononuclear iron(II) oxidases (Fig. 4).[16] A well-ordered water molecule is bound to iron opposite His_{214}, and ACV binds via the cysteinyl sulfur *trans* to His_{270}, giving a square pyramidal geometry around the metal center. Dioxygen is believed to initiate catalysis upon binding to iron in the site opposite Asp_{216}, creating an octahedral geometry that is maintained throughout the reaction cycle.[4,5]

Based on the requirements of this system, we designed the ligand **3** to form a pentadentate complex **4** with iron(II) while leaving a coordination site vacant for dioxygen binding (Fig. 3). The framework of this structure is based on a cobalt complex designed by Chin et al.[17] to stereospecifically bind amino acids. In our complex, pyridine and aniline ligands take the place of the histidine residues of IPNS, while an α-hydroxy acid moiety models both the

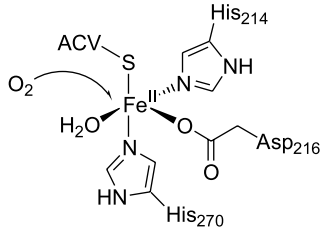
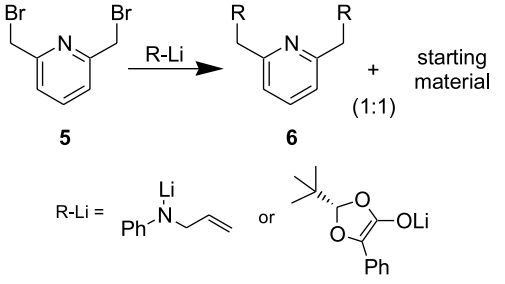


Figure 4. The active site of IPNS. The iron(II) center is coordinated by three endogenous protein ligands (His_{214}, Asp_{216}, and His_{270}), a water molecule, and the thiol sulfur of ACV in a square pyramidal arrangement. Molecular oxygen binds opposite Asp_{216}, giving the iron center an octahedral geometry.

carboxylate and water ligands. The design includes a thiol attached to the aniline nitrogen by a three-carbon linker. The sulfur atom was included to simulate binding of ACV, and it is at the adjacent carbon that we hope ultimately to achieve oxidation (Scheme 1).

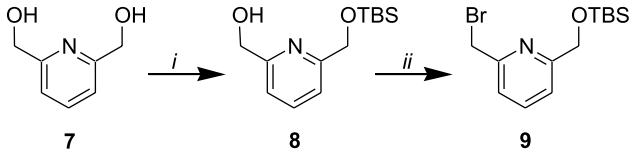
An important factor to be considered in the design of a ligand of this nature is the potential for intermolecular side reactions of the iron complex. Of particular concern is the propensity of free thiols and ferrous iron to undergo competing oxidation reactions to give disulfides and Fe(III)–O–Fe(III) species respectively, in the presence of atmospheric oxygen. Two phenyl groups are therefore included in the structure in order to increase steric bulk and inhibit intermolecular interactions that might render the complex inactive.

Initial efforts to synthesize the ligand were based on the approach taken by Chin et al., who reacted 2,6-bis(bromomethyl)pyridine **5** with the enolate of 2-(*N*-acetylamino)-diethylmalonate followed by dimethylamine to give a 2,6-hetero-disubstituted pyridine.[17] However, this approach did not prove effective in our hands, and treatment of 2,6-bis(bromomethyl)pyridine **5** with 1 equiv of an appropriate nucleophile resulted in a 1:1 mixture of homo-disubstituted product **6** and starting material (Scheme 2). This is not surprising as the mono-brominated species is highly reactive. 2-Bromomethylpyridine was found to be stable only as an HBr salt, forming polymers at pH 7 (data not shown). Conversely, 2,6-bis(bromomethyl)pyridine **5** is stable at neutral pH and can be stored indefinitely in its deprotonated form.



Scheme 2. Reaction of 2,6-bis(bromomethyl)pyridine **5** with 1 equiv of nucleophile to give a 1:1 ratio of the 2,6-homo-disubstituted pyridine **6** and the starting material.

In order to overcome this problem, the synthesis was modified to begin with 2,6-bis(hydroxymethyl)pyridine **7** (Scheme 3). The diol **7** was treated with 1 equiv of sodium hydride, and the resulting mono-anion reacted with 1 equiv of *tert*-butyldimethylsilyl chloride (TBS-Cl) to give the singly-protected diol **8** in moderate yield.[18] Mono-bromination of the protected diol proceeded smoothly with

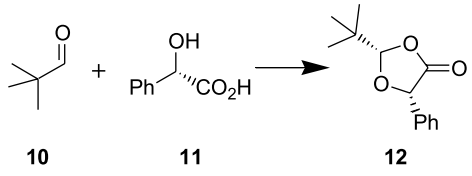


Scheme 3. Conditions: i. NaH, then TBS-Cl, DCM, RT, 5 h, 54%; ii. CBr_4, PPh_3, Et_2O, RT, 1.5 h, 81%.

triphenylphosphine and carbon tetrabromide, giving the bromide **9** in 81% yield.

At this stage, the α-hydroxy acid moiety was introduced stereoselectively in the masked form of a [1,3]-dioxolanone. Seebach and co-workers have shown that *cis*-2-*tert*-butyl-5-substituted-[1,3]-dioxolanones can be stereospecifically alkylated at the 5-position.[19,20] They have also demonstrated that these *cis*-2,5-disubstituted dioxolanones can be synthesized from pivaldehyde and enantiopure α-substituted-α-hydroxy acids in high diastereomeric purity. Hydrolysis under either acidic or basic conditions cleaves the pivaldehyde moiety, leaving an α-disubstituted-α-hydroxy acid as a single enantiomer.

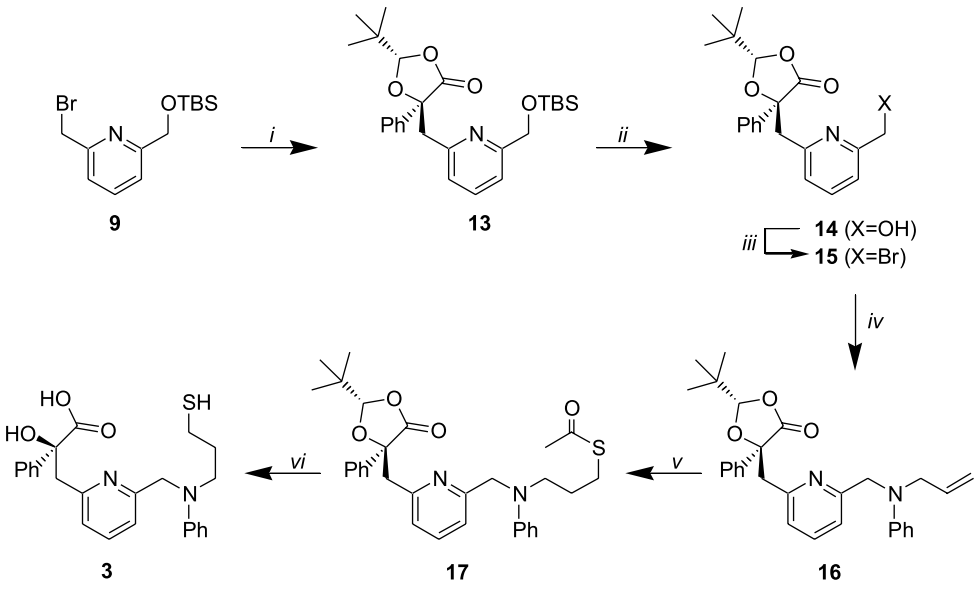
In order to introduce the phenyl group required to bring the desired steric bulk to the exterior of the mimic, a *cis*-2,5-disubstituted dioxolanone was synthesized from pivaldehyde **10** and (*S*)-(+)-mandelic acid **11** (Scheme 4). In the presence of catalytic triflic acid, these two reagents were refluxed in pentane with azeotropic removal of water to give the desired dioxolanone **12**,[21] which was isolated in diastereomerically pure form and 81% yield after a single recrystallization. Deprotonation of the dioxolanone **12** with LDA followed by addition to the mono-protected bromide **9** gave the 2,5,5-trisubstituted dioxolanone **13** in 84% yield (Scheme 5). The TBS group was then removed with tetra-*n*-butylammonium fluoride in high yield (93%), and the resulting alcohol **14** was brominated with triphenylphosphine and carbon tetrabromide to give the brominated dioxolanone **15** as a crystalline solid. The stereochemistry of all dioxolanone products was determined by nOe spectroscopy, and the solution of an X-ray crystal structure for the brominated dioxolanone **15** further confirmed that the (2*S*,5*S*)-dioxolanone system had been formed as anticipated (data not shown).



Scheme 4. Conditions: triflic acid (cat), pentane, reflux, 5 h, 81%.

Having prepared the bromide **15**, the aniline and thiol functionalities could be introduced. Deprotonation of *N*-allylaniline with *n*-butyllithium in the presence of *N*, *N*′-dimethyl-*N*, *N*′-propylene urea (DMPU), followed by addition to the bromide **15**, led to formation of the unsaturated amine **16** in 94% yield (Scheme 5). The sulfur functionality was then incorporated by radical addition of thiolacetic acid to the alkene **16**. To this end, a solution of the unsaturated compound **16** and thiolacetic acid in toluene was irradiated at 254 nm, and conversion to the thioester **17** proceeded in 56% yield.

The thioester **17** represents a protected form of the target molecule **3**, as unmasking of the thiol and the α-hydroxy acid by hydrolysis leads to the pentadentate ligand **3**. While it was originally thought that acid-catalyzed hydrolysis would be more effective in minimizing disulfide formation,



Scheme 5. Conditions: i. LDA-**12** (premixed), THF, −78 °C, 6 h, 84%; ii. TBAF, THF, 0 °C→RT; 1 h, 93%; iii. CBr_4, PPh_3, DCM, 0 °C→RT, 1.5 h, 81%; iv. *n*BuLi-*N*-allylaniline (premixed), THF, DMPU, −78 °C, 4 h, 90%; v. AcSH, toluene, *hv*, 4 h, 56%; vi. LiOH, H_2O–THF, reflux, 14 h.

the deprotection proceeded more smoothly when run under basic conditions using dilute lithium hydroxide in tetrahydrofuran-water. Mass spectrometric analysis of the crude product provides strong evidence that the pentadentate ligand **3** has been formed (HRMS: *m*/*z* found=423.1737, required for **3**=423.1742). However, separation of ligand **3** from reaction by-products was problematic, primarily due to poor solubility properties of the product. As a result, the NMR spectrum of the crude material is complicated by residual impurities (identified by HRMS as the starting material **17** and a partially deprotected product in which only the thioester has been hydrolyzed), precluding further characterization of the product by this method. Nonetheless, it is apparent from the HRMS data that the putative iron ligand **3** has indeed been formed.

3. Conclusion

We report the synthesis of a pentadentate ligand **3** to be used in the construction of a functional mimic of the key biosynthetic enzyme IPNS. Compound **3** has been prepared in nine steps from 2,6-bis(hydroxymethyl)pyridine, (*S*)-(+)-mandelic acid and pivaldehyde. We propose that the ligand **3** will bind to iron(II) and that in the presence of oxygen, the resulting complex may function as the first small molecule mimic of IPNS (Scheme 1). Further work is required to test this hypothesis.

4. Experimental

4.1. General

Reactions were carried out under an atmosphere of argon. All reagents and solvents were used as received from manufacturers unless otherwise specified. Trimethylacetaldehyde (pivaldehyde) and *N*-allylaniline were purified by Kugelröhr distillation at reduced pressure. Triphenylphosphine was recrystallized from heptane. Anhydrous dichloromethane (DCM) was obtained directly before use by refluxing over calcium hydride under an atmosphere of nitrogen, followed by distillation under those conditions. Anhydrous tetrahydrofuran (THF) and diethyl ether were similarly obtained by distillation from sodium/benzophenone ketyl. Hexane and *N*, *N*′-dimethyl-*N*, *N*′-propylene urea (DMPU) were dried with calcium hydride, fractionally distilled, and stored over 4.0 Å molecular sieves. Diisopropyl amine was similarly dried and distilled, and was stored over potassium hydroxide pellets. 'Petroleum ether' refers to the fraction of light petroleum boiling between 30 and 40 °C and was distilled prior to use. Concentrations of lithium diisopropylamide and *n*-butyllithium were determined by titration against 1,3-diphenylacetone-*p*-tosyl-hydrazone.

Melting points (mp) were measured using a Cambridge Instruments Gallen™ III Melting Point Microscope. Optical rotations ($[\alpha]_D$) were recorded on a Perkin–Elmer 241 polarimeter at 589 nm. Low-resolution mass spectra were recorded using a Micromass Platform spectrometer, and high-resolution mass spectra (HRMS) were recorded on Micromass Autospec, GCT, and LCT spectrometers. Infrared spectra were recorded on a Perkin–Elmer Paragon 1000 Fourier Transform spectrometer. Nuclear magnetic resonance spectra were recorded on Brüker DPX200 and Brüker DPX400 spectrometers, and nOe spectroscopy was performed on a Brüker DRX500 spectrometer. Elemental analysis was performed by Elemental Microanalysis Limited (Okehampton). Single-crystal X-ray diffraction data were measured using an Enraf-Nonius KappaCCD diffractometer (graphite-monochromated Mo K_α radiation, λ=0.71073 Å) and processed using the DENZO-SMN package.[22]

4.1.1. 2-Hydroxymethyl-6-(*tert*-butyldimethylsilyloxymethyl)pyridine (8).[18] Sodium hydride (880 mg of a 60% dispersion in oil, 21.9 mmol) was washed with petroleum ether (3×25 mL), and residual solvent was removed under

reduced pressure. The resulting powder was added to a slurry of 2,6-bis(hydroxymethyl)pyridine **7** (3.01 g, 21.6 mmol) in DCM (25 mL), and this mixture was stirred at room temperature. After 45 min, a solution of *tert*-butyldimethylsilyl chloride (3.30 g, 21.9 mmol) in DCM (5 mL) was added to the reaction mixture, which was stirred at room temperature for 5 h. The mixture was diluted with DCM (250 mL) to dissolve all the solid material, and this solution was washed with saturated aqueous sodium bicarbonate (2×125 mL) and saturated brine (125 mL), and dried with magnesium sulfate. The solvent was evaporated under reduced pressure to give the crude product as an orange oil which was purified by column chromatography (petroleum ether/diethyl ether, 1:1) to give the mono-protected diol **8** as a yellow oil (2.96 g, 54%); R_f 0.25 (petroleum ether/diethyl ether, 1:1); ν_{max} (thin film): 3276 (m, br O–H str), 2955, 2932, 2886, 2857 (s, aliphatic C–H str), 1598, 1580, 1462 (m, ring str), 1257 (s), 1118, 838 (s, Si–O str); δ_H (400 MHz, $CDCl_3$): 0.14 (6H, s, $Si(CH_3)_2$), 0.97 (9H, s, $SiC(CH_3)_3$), 3.82 (1H, t, J=5.0 Hz, CH_2OH), 4.74 (2H, d, J=5.0 Hz, CH_2OH), 4.84 (2H, s, CH_2OSi), 7.10 (1H, d, J=7.5 Hz, one of $CH(Py)_{3,5}$), 7.42 (1H, d, J=7.5 Hz, one of $CH(Py)_{3,5}$), 7.71 (1H, t, J=7.5 Hz, $CH(Py)_4$); δ_C (100.6 MHz, $CDCl_3$): −5.37 ($Si(CH_3)_2$), 18.35 ($SiC(CH_3)_3$), 25.89 ($SiC(CH_3)_3$), 63.81 (CH_2OH), 65.90 (CH_2OSi), 118.40, 118.53 (2×$CH(Py)_{3,5}$), 137.27 ($CH(Py)_4$), 157.60, 160.30 (2×$C(Py)_{2,6}$); *m/z* (AP+): 254 (100%, $[MH]^+$); HRMS: found $[MH]^+$=254.1577, $C_{13}H_{24}NO_2Si$ requires 254.1576.

4.1.2. 2-Bromomethyl-6-(*tert*-butyldimethylsilyloxymethyl)pyridine (9). Carbon tetrabromide (6.70 g, 20.2 mmol) was added as a solid to a solution of the mono-protected diol **8** (4.70 g, 18.6 mmol) in diethyl ether (20 mL) and the resulting mixture was stirred at room temperature. A solution of triphenylphosphine (5.25 g, 20.0 mmol) in diethyl ether (10 mL) was added, and the reaction mixture was stirred for a further 90 min at room temperature, during which time a white precipitate formed. The precipitate (triphenylphosphine oxide) was removed by vacuum filtration, and the filtrate was concentrated under reduced pressure to give a crude orange oil. The product was purified by column chromatography (petroleum ether/diethyl ether, 20:1) to give the bromide **9** as a pale yellow oil (4.75 g, 81%); R_f 0.20 (petroleum ether/diethyl ether, 19:1); ν_{max} (thin film): 3063 (w, aromatic C–H str), 2955, 2929, 2886, 2856 (m-s, aliphatic C–H str), 1592, 1578, 1460, 1431 (m, ring str),1257 (s), 1123, 838 (s, Si–O str); δ_H (400 MHz, $CDCl_3$): 0.13 (6H, s, $Si(CH_3)_2$), 0.97 (9H, s, $SiC(CH_3)_3$), 4.52 (2H, s, CH_2Br), 4.84 (2H, s, CH_2OSi), 7.31 (1H, d, J=7.5 Hz, one of $CH(Py)_{3,5}$), 7.46 (1H, d, J=7.5 Hz, one of $CH(Py)_{3,5}$), 7.72 (1H, d, J=7.5 Hz, $CH(Py)_4$); δ_C (100.6 MHz, $CDCl_3$): −5.39 ($Si(CH_3)_2$), 18.33 ($SiC(CH_3)_3$), 25.89 ($SiC(CH_3)_3$), 33.85 (CH_2Br), 65.87 (CH_2OSi), 119.25, 121.50 (2×$CH(Py)_{3,5}$), 137.64 ($CH(Py)_4$), 155.56, 161.50 (2×$C(Py)_{2,6}$); *m/z* (AP+): 318 (80%, $[MH]^+$ for ^{81}Br), 316 (100%, $[MH]^+$ for ^{79}Br), 268 (82%), 237 (21%, $[MH–Br]^+$), 202 (41%, $[MH–SiC(CH_3)_3]^+$ for ^{79}Br), 154 (42%); HRMS: found $[MH]^+$=316.0729, $C_{13}H_{23}^{79}BrNOSi$ requires 316.0732.

4.1.3. (2*S*,5*S*)-2-*tert*-Butyl-5-phenyl-[1,3]-dioxolan-4-one (12).[21] Pivaldehyde **10** (2.65 g, 30.8 mmol) was added to a slurry of (*S*)-(+) mandelic acid **11** (3.92 g, 25.8 mmol) in pentane (45 mL). The mixture was stirred at room temperature while trifluoromethanesulfonic acid (100 μL, 1.1 mmol) was added dropwise, and then heated to reflux for 5 h under Dean-Stark conditions. The reaction mixture was cooled to room temperature and neutralized with aqueous sodium bicarbonate (8%, *w/v*) and then with solid sodium bicarbonate. During this process, a white precipitate formed from what had become an orange solution. The pentane was removed under reduced pressure, and the white solid was isolated from the aqueous suspension by vacuum filtration. Two recrystallizations of the solid (ethyl acetate–heptane) gave the dioxolanone **12** as large white needles (4.54 g, 81%); mp 138–140 °C; R_f 0.20 (petroleum ether/diethyl ether, 20:1); $[\alpha]_D^{25}$ +90.0 (chloroform, c=0.1; lit. $[\alpha]_D^{25}$ +88.7,[19] chloroform, c=1.2); ν_{max} (KBr disk): 3070, 3039 (w, aromatic C–H str), 2980, 2960, 2922, 2873 (m, aliphatic C–H str), 1799 (s, C=O str), 1483, 1457 (m, ring, str), 1206 (s), 1183 (s, C–O–C str); δ_H (400 MHz, $CDCl_3$): 1.10 (9H, s, $C(CH_3)_3$), 5.25 (1H, d, J=1.5 Hz, PhC*H*), 5.34 (1H, d, J=1.5 Hz, $(CH_3)_3CCH$), 7.38–7.49 (5H, m, 2×C*H* $(Ph)_o$, 2×$CH(Ph)_m$, $CH(Ph)_p$); δ_C (100.6 MHz, $CDCl_3$): 23.63 ($C(CH_3)_3$), 34.48 ($C(CH_3)_3$), 77.02 (PhCHC=O), 109.32 ($(CH_3)_3CCH$), 127.06, 128.74 (2×$CH(Ph)_o$, 2×$CH(Ph)_m$), 129.19 ($CH(Ph)_p$) 133.51 ($C(Ph)_i$), 171.86 (C=O); *m/z* (TOF+): 238 (100%, $[M+NH_4]^+$), 221 (20%, $[MH]^+$), 176 (18%), 152 (25%); HRMS: found $[M+NH_4]^+$=238.1444, $C_{13}H_{20}NO_3$ requires 238.1443; found C 70.67%, H 7.34%, $C_{13}H_{16}O_3$ requires C 70.89%, H 7.32%.

4.1.4. (2*S*,5*S*)-2-*tert*-Butyl-5-[6-(*tert*-butyldimethylsilyloxymethyl)pyridin-2-ylmethyl]-5-phenyl-[1,3]-dioxolan-4-one (13). A solution of the dioxolanone **12** (1.11 g, 5.0 mmol) in THF (25 mL) was cooled to −78 °C and a solution of lithium diisopropylamide (1.63 M solution in THF, 3.10 mL, 5.0 mmol) was added. The solution was stirred at −78 °C for 50 min, then added dropwise via cannula to a solution of 2-bromomethyl-6-(*tert*-butyldimethylsilyloxymethyl)pyridine **9** (1.59 g, 5.0 mmol) in THF (25 mL) at −78 °C. The reaction mixture was stirred at −78 °C for an additional 6 h. It was then poured into half-saturated aqueous ammonium chloride (40 mL), and the resulting solution was extracted with diethyl ether (4× 50 mL). The organic phase was dried with magnesium sulfate, and the solvent was removed under reduced pressure to give a peach-colored oil. The crude material was purified by column chromatography (petroleum ether/diethyl ether, 15:2) to yield the trisubstituted dioxolanone **13** as an off-white crystalline solid (1.93 g, 84%); mp 65–67 °C; R_f 0.15 (petroleum ether/diethyl ether, 8:1); $[\alpha]_D^{25}$ −37.3 (chloroform, c=0.9); ν_{max} (KBr disc): 3054, 3037 (w, aromatic C–H str), 2955, 2902, 2855 (m, aliphatic C–H str), 1787 (s, C=O str), 1578, 1549, 1483, 1460 (m, ring str), 1257 (m), 1192 (s, C–O–C str); δ_H (400 MHz, $CDCl_3$): 0.13 (6H, s, $Si(CH_3)_2$), 0.86 (9H, s, $HCC(CH_3)_3$), 0.97 (9H, s, $SiC(CH_3)_3$), 3.33 (1H, d, J=14.5 Hz, one of $PhCCH_2Py$), 3.65 (1H, d, J=14.5 Hz, one of $PhCCH_2Py$), 4.69 (1H, s, $HCC(CH_3)_3$), 4.81 (1H, d, J=15.5 Hz, one of $PyCH_2OSi$), 4.84 (1H, d, J=15.5 Hz, one of $PyCH_2OSi$), 6.99 (1H, d, J=7.5 Hz, one of $CH(Py)_{3,5}$), 7.30–7.35 (1H, m, $CH(Ph)_p$), 7.36–7.45 (3H, m, 2×$CH(Ph)_o$, one of $CH(Py)_{3,5}$), 7.63 (1H, t, J=7.5 Hz, $CH(Py)_4$), 7.74–7.78 (2H, m, 2×$CH(Ph)_m$); δ_C (100.6 MHz, $CDCl_3$): −5.37 ($Si(CH_3)_2$),

18.36 ($SiC(CH_3)_3$), 23.52 ($HCC(CH_3)_3$), 25.92 ($SiC(CH_3)_3$), 34.86 ($HCC(CH_3)_3$), 48.49 (PyCH_2CPh), 65.82 (PyCH_2OSi), 82.20 (PhCCH_2Py), 109.57 ($HCC(CH_3)_3$), 118.20, 122.31 (2×$CH(Py)_{3,5}$), 124.91 (2×$CH(Ph)$-$_m$), 127.92 ($CH(Ph)_p$), 128.24 (2×$CH(Ph)_o$), 137.06 ($CH(Py)_4$), 139.18 ($C(Ph)_i$), 154.11, 161.17 (2×$C(Py)_{2,6}$), 173.28 (C=O); *m/z* (ES+): 456 (100%, $[MH]^+$); HRMS: found $[MH]^+$=456.2572, $C_{26}H_{38}NO_4Si$ requires 456.2570; found C 68.50%, H 8.23%, N 3.19%, $C_{26}H_{37}NO_4Si$ requires C 68.35%, H 8.18%, N 3.07%.

4.1.5. (2*S*,5*S*)-2-*tert*-Butyl-5-(6-hydroxymethylpyridin-2-ylmethyl)-5-phenyl-[1,3]-dioxolan-4-one (14). A solution of the protected dioxolanone **13** (397 mg, 0.87 mmol) in THF (8 mL) was cooled to 0 °C, and TBAF (1.0 M solution in THF, 1.75 mL, 1.75 mmol) was added. The solution was stirred at 0 °C for 5 min and at room temperature for 70 min. It was then poured into water (10 mL) and extracted with DCM (3×20 mL). The organic layer was washed with saturated brine (10 mL), dried with magnesium sulfate, and the solvent was removed under reduced pressure to give a runny yellow oil. The crude product was purified by column chromatography (petroleum ether/diethyl ether, 1:1) to give the alcohol **14** (275 mg, 93%) as a viscous pale yellow oil; R_f 0.20 (petroleum ether/diethyl ether, 1:1); $[\alpha]_D^{25}$ −57.8 (chloroform, c=0.8); ν_{max} (thin film): 3422 (s, br, O–H str), 3064, 3029 (m, aromatic C–H str), 2974, 2935, 2908, 2874 (s, aliphatic C–H str), 1790 (s, C=O str), 1594, 1578, 1483, 1459 (s, ring str),1178 (s, C–O–C str); δ_H (400 MHz, $CDCl_3$): 0.88 (9H, s, $HCC(CH_3)_3$), 3.39 (1H, d, J=14.5 Hz, one of PhCCH_2Py), 3.66–3.72 (2H, m, one of PhCCH_2Py and CH_2OH), 4.73 (2H, d, J=4.5 Hz, PyCH_2OH), 4.85 (1H, s, CHC$(CH_3)_3$), 7.01 (1H, d, J=7.5 Hz, one of C$H(Py)_{3,5}$), 7.11 (1H, d, J=7.5 Hz, one of C$H(Py)_{3,5}$), 7.31–7.36 (1H, m, C$H(Ph)_p$), 7.37–7.42 (2H, m, C$H(Ph)_m$), 7.59 (1H, t, J=7.5 Hz, C$H(Py)_4$), 7.71–7.75 (2H, m, C$H(Ph)_o$); δ_C (100.6 MHz, $CDCl_3$): 23.49 ($HCC(CH_3)_3$), 34.88 ($HCC(CH_3)_3$), 47.81 (PyCH_2CPh), 63.98 (PyCH_2OH), 81.91 (PhCCH_2Py), 109.44 ($HCC(CH_3)_3$), 118.81, 122.91 (2×$CH(Py)_{3,5}$), 124.94 (2×$CH(Ph)_o$), 128.03 ($CH(Ph)_p$), 128.26 (2×$CH(Ph)_m$), 137.10 ($CH(Py)_4$), 128.74 ($C(Ph)_i$), 154.28, 158.49 (2×$C(Py)_{2,6}$), 173.24 (C=O); *m/z* (ES+): 364 (25%, $[M+Na]^+$), 342 (100%, $[MH]^+$); HRMS: found $[MH]^+$=342.1696, $C_{20}H_{24}NO_4$ requires 342.1705.

4.1.6. (2*S*,5*S*)-5-(6-Bromomethylpyridin-2-ylmethyl)-2-*tert*-butyl-5-phenyl-[1,3]-dioxolan-4-one (15). A solution of the alcohol **14** (1.45 g, 4.2 mmol) in DCM (30 mL) was cooled to 0 °C, and a solution of carbon tetrabromide (1.54 g, 4.6 mmol) in DCM (5 mL) was added to it via cannula. A solution of triphenylphosphine (1.23 g, 4.7 mmol) in DCM was then added via cannula, and the resulting yellow solution was stirred at 0 °C for 5 min, and then at room temperature for 90 min. The reaction mixture was diluted with diethyl ether (300 mL), causing the precipitation of triphenylphosphine oxide. The white precipitate was removed by vacuum filtration, and the solvent was evaporated under reduced pressure to give a thick yellow oil. This crude material was purified by column chromatography (petroleum ether/diethyl ether, 9:2) to give the bromide **15** (1.39 g, 81%) as a white crystalline solid; mp 101–102 °C; R_f 0.20 (petroleum ether/diethyl ether, 4:1); $[\alpha]_D^{25}$ −58.4 (chloroform, c=0.9); ν_{max} (KBr disc): 3068 (w, aromatic C–H str), 2970, 2905, 2872 (m, aliphatic C–H str), 1794 (s, C=O str), 1597, 1574, 1482, 1459 (m, ring str),1210 (m), 1172 (m, C–O–C str); δ_H (400 MHz, $CDCl_3$): 0.89 (9H, s, $HCC(CH_3)_3$), 3.39 (1H, d, J=15.0 Hz, one of PyCH_2CPh), 3.66 (1H, d, J=15.0 Hz, one of PyCH_2CPh), 4.55 (2H, s, PyCH_2Br), 5.03 (1H, s, $HCC(CH_3)_3$), 7.04 (1H, d, J=7.5 Hz, one of C$H(Py)_{3,5}$), 7.31–7.36 (2H, m, $CH(Ph)_p$) and one of C$H(Py)_{3,5}$), 7.37–7.43 (2H, m, 2×C$H(Ph)_m$), 7.62 (1H, t, J=7.5 Hz, C$H(Py)_4$), 7.75–7.79 (2H, m, 2×C$H(Ph)_o$); δ_C (100.6 MHz, $CDCl_3$): 23.58 ($HCC(CH_3)_3$), 33.71 (PyCH_2Br), 34.96 ($HCC(CH_3)_3$), 48.41 (PyCH_2CPh), 81.95 (PyCH_2CPh), 109.72 ($HCC(CH_3)_3$), 121.97, 123.54 (2×$CH(Py)_{3,5}$), 124.91 (2×$CH(Ph)_o$), 127.98 ($CH(Ph)_p$), 128.29 (2×$CH(Ph)_m$), 137.55 ($CH(Py)_4$), 139.29 ($C(Ph)_i$), 155.48, 156.39 (2×$C(Py)_{2,6}$), 173.28 (C=O); *m/z* (ES+): 406 (100%, $[MH]^+$ for ^{81}Br), 404 (95%, $[MH]^+$ for ^{79}Br); HRMS: found $[M]^+$=403.0787, $C_{20}H_{23}^{79}BrNO_3$ requires 403.0783.

4.1.7. (2*S*,5*S*)-5-{6-[(*N*-Allyl-*N*-phenylamino)methyl]-pyridin-2-ylmethyl}-2-*tert*-butyl-5-phenyl-[1,3]-dioxolan-4-one (16). *N*-Allylaniline (442 mg, 3.32 mmol) in THF (25 mL) was cooled to 0 °C, and *n*BuLi (2.26 M in hexanes, 1.52 mL, 3.44 mmol) was added. The solution was stirred for 30 min, after which DMPU (445 mg, 3.47 mmol) was added. Stirring was continued at 0 °C for another 15 min, before the lithium amide solution thus formed was added dropwise via cannula to a solution of the bromide **15** (1.39 g, 3.4 mmol) in THF (25 mL) at −78 °C. The resulting solution was stirred at −78 °C for 4 h and then quenched by pouring into half-saturated ammonium chloride (40 mL). This mixture was extracted with diethyl ether (3×100 mL), and the organic phase was washed with brine (100 mL) and dried with magnesium sulfate. The solvent was removed under reduced pressure to give a yellow oil, which was purified by column chromatography (petroleum ether/diethyl ether, 6:1), giving the alkene **16** (1.43 g, 94%) as a viscous, colorless oil; R_f 0.10 (petroleum ether/diethyl ether, 8:1); $[\alpha]_D^{25}$ −44.5 (chloroform, c=1.0); ν_{max} (thin film): 3062, 3040 (w, aromatic C–H str), 2962, 2934, 2907, 2836 (m, aliphatic C–H str), 2361 (w, overtone of C–O–C str), 1792 (s, C=O str), 1643 (w, C=C str), 1599, 1576, 1506, 1482 (m-s, ring str), 1178 (s, br, C–O–C str); δ_H (400 MHz, $CDCl_3$): 0.90 (9H, s, $C(CH_3)_3$), 3.40 (1H, d, J=14.5 Hz, 1 of PyCH_2C), 3.72 (1H, d, J=14.5 Hz, 1 of PyCH_2C), 4.11 (2H, ddd, X of ABMX, J_{XM}=5.0 Hz, J_{XA}=1.5 Hz, J_{XB}=1.5 Hz, NCH_2CHCH_2), 4.63 (2H, s, PyCH_2N), 4.80 (1H, s, $(CH_3)_3CCH$), 5.22 (1H, ddt, B of ABMX, J_{BM}=10.0 Hz, J_{AB}=1.5 Hz, J_{BX}=1.5 Hz, H_{trans} of NCH_2-CHCH_2), 5.25 (1H, ddt, A of ABMX, J_{AM}=17.0 Hz, J_{AB}=1.5 Hz, J_{AX}=1.5 Hz, H_{cis} of NCH_2CHCH_2), 5.94 (1H, ddt, M of ABMX, J_{AM}=17.0 Hz, J_{BM}=10.0 Hz, J_{MX}=5.0 Hz, NCH_2CHCH_2), 6.65–6.75 (3H, m, 2×CH(Ph–N)$_o$, CH(Ph–N)$_p$), 7.01 (1H, d, J=7.5 Hz, one of C$H(Py)_{3,5}$), 7.14 (1H, d, J=7.5 Hz, one of C$H(Py)_{3,5}$), 7.16–7.23 (2H, m, 2×CH(Ph–N)$_m$), 7.32–7.38 (1H, m, CH(Ph–C)$_p$), 7.39–7.45 (2H, m, 2×CH(Ph–C)$_m$), 7.53 (1H, t, J=7.5 Hz, C$H(Py)_4$), 7.77–7.81 (2H, m, 2×CH(Ph–C)$_o$); δ_C (100.6 MHz, $CDCl_3$): 23.57 ($C(CH_3)_3$), 34.88 ($C(CH_3)_3$), 48.41 (PyCH_2C), 53.83 (NCH_2CHCH_2), 56.19 (PyCH_2N), 82.17 (PhCCH_2Py), 109.60 ($(CH_3)_3CCH$), 112.28 (2×CH(Ph–N)$_o$), 116.65 (NCH_2CHCH_2), 116.70 (CH(Ph–N)$_p$), 119.11,

122.50 (2×*C*H(Py)$_{3,5}$), 124.96 (2×*C*H(Ph–C)$_o$), 127.98 (*C*H(Ph–C)$_p$), 128.27 (2×*C*H(Ph–C)$_m$), 129.18 (2×*C*H(Ph–N)$_m$), 133.39 (NCH$_2$*C*HCH$_2$), 137.15 (*C*H(Py)$_4$), 139.13 (*C*(Ph–C)$_i$), 148.38 (*C*(Ph–N)$_i$), 155.22, 159.20 (2×*C*(Py)$_{2,6}$), 173.24 (*C*=O); *m/z* (AP+): 479 (15%, [M+Na]$^+$); 457 (100%, [MH]$^+$); HRMS: found [MH]$^+$=457.2497, $C_{29}H_{33}N_2O_3$ requires 457.2491.

4.1.8. Thiolacetic acid *S*-(3-{[6-((2*S*,4*S*)-2-*tert*-butyl-5-oxo-4-phenyl-[1,3]-dioxolan-4-ylmethyl)pyridin-2-ylmethyl]phenylamino}propyl) ester (17). To a solution of the alkene **16** (248 mg, 0.54 mmol) in toluene (1.25 mL) in a quartz flask was added thiolacetic acid (130 mg, 1.7 mmol). Argon was bubbled through the solution for 20 min to remove dissolved oxygen. The flask was then sealed and the solution was irradiated with 4×8 W bulbs at 254 nm for 4 h. The solvent was removed under reduced pressure to give a crude yellow oil which was purified by column chromatography (petroleum ether/diethyl ether, 2:1), giving the thioester **17** as a yellow oil (162 mg, 56%); R_f 0.30 (petroleum ether/diethyl ether, 2:1); $[\alpha]_D^{25}$ −48.4 (chloroform, c=0.5); ν_{max} (thin film): 3092, 3062, 3028 (w, aromatic C–H str), 2961, 2933, 2873 (aliphatic C–H str), 1790 (s, lactone C=O str), 1693 (s, thioester C=O str), 1599, 1576, 1505, 1456 (s, ring str), 1195 (s), 1177 (s, C–O–C str); δ_H (400 MHz, $CDCl_3$): 0.89 (9H, s, HCC(C*H*$_3$)$_3$), 1.98 (2H, quint, J=7.5 Hz, NCH$_2$C*H*$_2$CH$_2$S), 2.36 (3H, s, SC(=O)C*H*$_3$), 2.95 (2H, t, J=7.0 Hz, NCH$_2$-CH$_2$C*H*$_2$S), 3.38 (1H, d, J=14.5 Hz, one of PyC*H*$_2$CPh), 3.54 (2H, t, J=7.5 Hz, NC*H*$_2$CH$_2$CH$_2$S), 3.69 (1H, d, J=14.5 Hz, one of PyC*H*$_2$CPh), 4.59 (2H, s, PyC*H*$_2$N), 4.79 (1H, s, (CH$_3$)$_3$CC*H*), 6.61–6.65 (2H, m, 2×C*H*(Ph–N)$_o$), 6.67–6.72 (1H, m, C*H*(Ph–N)$_p$), 6.98 (1H, d, J=7.5 Hz, one of C*H*(Py)$_{3,5}$), 7.04 (1H, d, J=7.5 Hz, one of C*H*(Py)$_{3,5}$), 7.16–7.21 (2H, m, 2×C*H*(Ph–N)$_m$), 7.31–7.37 (1H, m, C*H*(Ph–C)$_p$), 7.38–7.44 (2H, m, 2×C*H*(Ph–C)$_m$), 7.49 (1H, t, J=7.5 Hz, C*H*(Py)$_4$), 7.74–7.79, (2H, m, 2×C*H*(Ph–C)$_o$); δ_C (100.6 MHz, $CDCl_3$): 23.57 (HCC(*C*H$_3$)$_3$), 26.56 (NCH$_2$CH$_2$*C*H$_2$S), 27.27 (NCH$_2$*C*H$_2$CH$_2$S), 30.63 (SC(=O)*C*H$_3$), 34.88 (HC*C*(CH$_3$)$_3$), 48.34 (Py*C*H$_2$CPh), 50.72 (N*C*H$_2$CH$_2$CH$_2$S), 56.73 (Py*C*H$_2$N), 82.15 (PyCH$_2$-*C*Ph), 109.53 (H*C*C(CH$_3$)$_3$), 112.24 (2×*C*H(Ph–N)$_o$), 116.67 (*C*H(Ph–N)$_p$), 119.12, 122.53 (2×*C*H(Py)$_{3,5}$), 124.96 (2×*C*H(Ph–C)$_o$), 127.97 (*C*H(Ph–C)$_p$), 128.25 (2×*C*H(Ph–C)$_m$), 129.25 (2×*C*H(Ph–N)$_m$), 137.11 (*C*H(Py)$_4$), 139.05 (*C*(Ph–C)$_i$), 147.76 (*C*(Ph–N)$_i$), 155.17, 158.92 (*C*(Py)$_{2,6}$), 173.15 (O–*C*=O), 195.47 (S–*C*=O); *m/z* (ES+): 533 (100%, [MH]$^+$); HRMS: found [MH]$^+$=533.2477, $C_{31}H_{37}N_2O_4S$ requires 533.2474.

4.1.9. (2*S*)-2-Hydroxy-3-(6-{[(3-mercaptopropyl)phenylamino]methyl}pyridin-2-yl)-2-phenylpropionic acid (3). Lithium hydroxide (15 mg, 0.63 mmol) was dissolved in water (2.5 mL) and a solution of the thioester **17** (106 mg, 0.20 mmol) in THF (2.5 mL) was added. The solution was then heated at reflux for 16 h and the solvent was removed under reduced pressure to give the deprotected molecule **3** as an off-white solid; *m/z* (ES+): 423 (100%, [MH]$^+$); HRMS: found [MH]$^+$=423.1737, $C_{24}H_{27}N_2O_3S$ requires 423.1742.

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