One-Pot, Regiospecific Assembly of (E)-Benzamidines From δ- and γ-Amino Acids Via an Intramolecular Aminoquinazolinone Rearrangement

Journal:	*Organic & Biomolecular Chemistry*
Manuscript ID	OB-ART-11-2015-002378.R1
Article Type:	Paper
Date Submitted by the Author:	04-Mar-2016
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Journal Name

ARTICLE

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Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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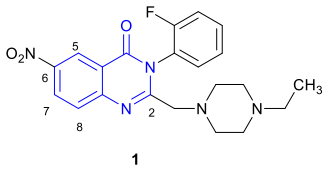
The efficient generation of novel, *N*-linked benzamidines resulting from a regiospecific rearrangement of quinazolinones is described. This methodology study explored reaction parameters including the effect of changing solvent and temperature, as well as varying electronic substituents on the structural core. The transformation was extensively optimized in terms of reaction conditions and scope, resulting in a protocol that consistently affords diversely functionalized amidines in high yield. The process permits regional structural derivatization that was previously inaccessible, and the multistep process was also reduced to a telescoped, five-step sequence that efficiently affords pharmacologically unique (*E*)-benzamidoamidines from *N*-BOC protected γ- and δ-amino acids.

Introduction

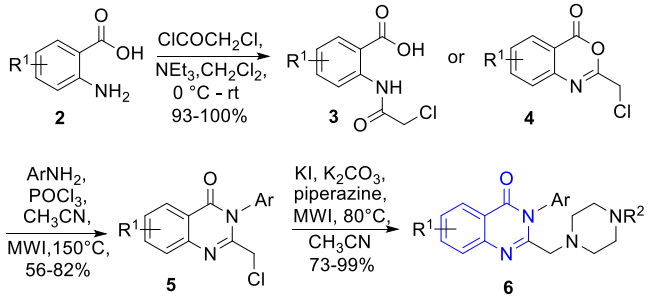
The 4-quinazolinone core has been extensively derivatized chemically and shown to possess diverse pharmacological activity. With a spectrum of activity that includes antimicrobial,[1] antiinflammatory,[2] antiproliferative, sedative/hypnotic, and anticonvulsant effects,[3] this structural framework is considered a privileged structure.[3, 4] In the pursuit of our own anti-infective program, we discovered a 4-quinazolinone-derived hit scaffold **1** that was worthy of additional structure-activity relationship (SAR) studies (Fig. 1).[5, 6] As such, we sought to explore the pharmacophoric elements of this chemotype for our project through synthetic manipulation.



1

Fig. 1. Highlighted 4-quinazolinone core of hit scaffold **1**

The synthesis of 2-alkylquinazolinones had been previously described, so our preliminary efforts focused on modifying components accessible within the scope of published protocols.[7-10] A three step procedure[11] was employed to prepare piperazine-containing analogs of **1** (Scheme 1). Substituted anthranilic acids **2** were treated with chloroacetyl chloride in the presence of triethylamine to generally afford 2-(2-chloroacetamido)benzoic acids **3**. While reported protocols[11, 12] alternatively describe the intermediacy of 2-(chloromethyl)-4*H*-benzo[*d*][1,3]oxazin-4-ones **4**, these were rarely obtained in our hands. Nonetheless, dehydrative amidation of **3** or **4** with selected anilines and $POCl_3$ led to chloromethylquinazolinones **5** which were subsequently aminated with *N*-alkylpiperazines to give substituted quinazolinones **6**.



Scheme 1. Synthetic route to quinazolinone analogs of **1**

While this stage of the medicinal chemistry effort proceeded as expected, we were intrigued by a series of failed chemistry experiments when our focus shifted to compounds bearing acyclic diamine variants of the piperazine moiety of **1**. Our initial attempts to generate these analogs involved treatment of 6-nitro-2-chloromethylquinazolinone **7a**[6, 13] with mono-*N*-BOC protected 1,2-ethanediamine or mono-*N*-BOC protected 1,3-propanediamine to afford the corresponding quinazolinones **8** or **9** with the incorporated BOC-protected alkylamine appendage (Scheme 2). [6] Intermediates **8** and **9** were stable and characterized by NMR and

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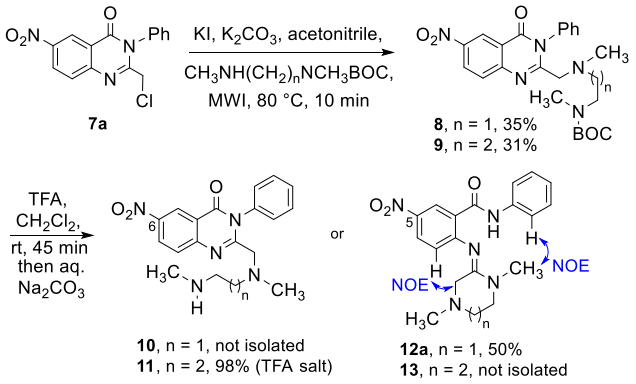
Electronic Supplementary Information (ESI) available: General methods, synthetic procedures and characterization for compounds **12a-m**, **18a-b**, **19a**, and **24a-d** as well as ^1H and ^{13}C NMR for final compounds. See DOI: 10.1039/x0xx00000x

LCMS/UV. Notably, upon treatment with TFA to remove the BOC group and subsequent basic workup, the open chain 1,2-ethanediamine product **10** could not be isolated. In cases employing a mono-*N*-BOC protected 1,3-propanediamine, the desired open chain analog **11** was obtained in 98% yield as the TFA salt. Analysis of the reaction employing **8** revealed the formation of a rearranged (*E*)-amidine **12a** in 50% yield that was structurally confirmed by ^{1}H and ^{13}C NMR, NOESY, and 2D NMR spectroscopy (Scheme 2). A diagnostic benzamide *N*-H signal was observed at 11.0 ppm in the proton spectrum, and clear nuclear Overhauser effects (NOEs) were observed between (a) the aryl 3-CH proton and the methylene protons of the newly formed aminoamidine ring, as well as between (b) the amidine *N*-methyl protons and the benzamide *ortho*-CH protons (*see* Scheme 2, **12a**). A crystalline, halogenated analog was later assessed by x-ray crystallography, thus confirming the initial NMR assignments (data not shown).



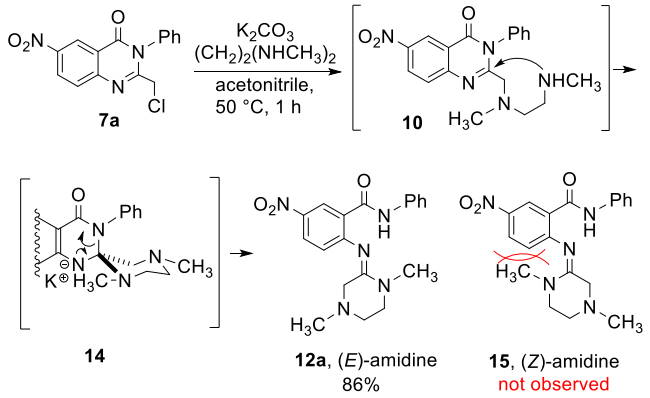
Scheme 2. Synthetic exploration of open chain analogs

The rearrangement of nitroquinazolinones to nitrobenzamidines was unprecedented, and fortuitously afforded compounds of significant pharmacological value that were inaccessible by other methods. Nonetheless, several considerable chemistry issues remained unresolved. Reaction conditions used in the rearrangement were un-optimized and exploited reagents that were deemed unnecessary and suboptimal. Notably, it was also not understood if the rearrangement was productive once TFA was introduced to remove the BOC-protecting group, or if the "work-up" with aqueous base was significant. Additionally, it was biologically advantageous for our amidines to bear a C-5 nitro group on the core; however, it was unclear if the presence of a strongly electron withdrawing group was required for the rearrangement to work. Perhaps most importantly, the rearrangement also featured a noteworthy limitation in that modification of the cyclic aminoamidine portion of the scaffold was not feasible based on the functional need of a diamine component as the reacting partner. Therefore, diversification of this structural region was impossible, thus severely restricting the level of pharmacological optimization that our team could pursue. Given these questions and our need to pursue our antiviral program, we launched a methodology study around this rearrangement in order to better understand and expand its efficiency and scope.

Results and discussion

With the structure and configuration of the rearranged product established, attention was shifted to optimizing the yield of the process and mechanistically accounting for amidine formation. Amidine **12a** was the only product isolated from the reaction with N^1,N^2-dimethylethane-1,2-diamine, and the two step process from **7a** modestly yielded 18% overall (*c.f*, Scheme 2). Our studies revealed that potassium iodide was not required and that thermal conditions afforded better yields. It was also determined that the BOC-protecting group was unnecessary for those reactions utilizing a symmetrical diamine and that the rearrangement was successful under basic conditions used to install the diamine linker onto the quinazolinone core. Thus, a one-step conversion from **7a** to **12a** was achieved with an improved 86% overall yield (Scheme 3).

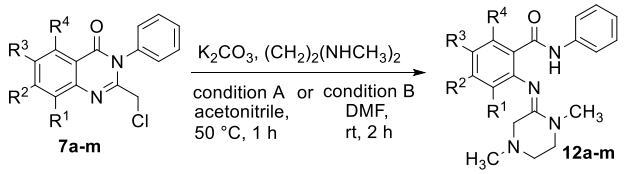
Isolation and characterization of the BOC-protected intermediates **8** and **9** indicated that the rearrangement did not occur until after the terminal amine was exposed. Interestingly, amidine **12a** was generated when chloromethylquinolinone **7a** was treated with a two-carbon linked diamine, but a three-carbon tethered diamine produced the expected quinazolinone **11**. It was reasoned that amidine formation was favored when the reaction proceeded through a six-membered, spirocyclic intermediate such as **14**, resulting from an intramolecular attack of the terminal amine, followed by carbon-nitrogen bond cleavage. Exclusive isolation of the (*E*)-configured double bond amidine was presumed to result from a more favorable transition state that did not result in a steric clash between the *N*-methylamidine moiety and the nitrophenyl component of the scaffold (Scheme 3).



Scheme 3. Modified reaction conditions and mechanistic considerations for (*E*)-amidine formation

It was unknown if the presence of the quinazolinone C6-nitro group of **7a** was essential for the rearrangement to occur or if the electronic nature of the substituent had any effect on the reaction. Consequently, variants were prepared that surveyed the effect of quinazolinone core substitution on the efficiency and scope of the reaction (Table 1). The reaction could be carried out efficiently in acetonitrile at 50 °C or at room temperature in DMF, though in the former case we observed in nearly half of the examples the formation of dimers that were otherwise absent when DMF was used at a milder temperature. Under either set of conditions, the (*Z*)-amidine was not observed for any of the alkyl chlorides we examined. In the case of reactions using **7b**, the desired (*E*)-amidine **12b** was isolated along with an unidentified, minor, and unstable compound with the same mass; however, it was clearly not the (*Z*)-isomer by ^{1}H NMR analysis.

Table 1. Effect of reaction conditions and chloromethylquinazolinone substituents on product yield

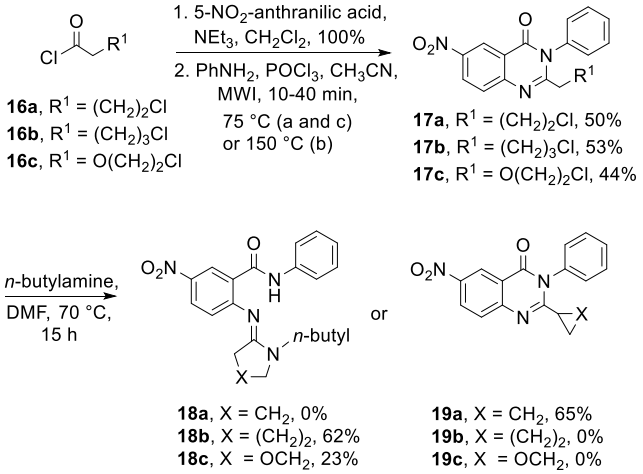


entry	sm	prdt	R^1	R^2	R^3	R^4	condition A, yield (%)	condition B, yield (%)
1	**7a**	**12a**	H	H	NO_2	H	86	77
2	**7b**	**12b**	NO_2	H	H	H	71[a]	97[a]
3	**7c**	**12c**	H	NO_2	H	H	94	85
4	**7d**	**12d**	H	H	H	NO_2	74	82
5	**7e**	**12e**	H	H	H	H	58[b]	83
6	**7f**	**12f**	OCH_3	H	H	H	13[b]	34
7	**7g**	**12g**	H	OCH_3	H	H	55[b]	85
8	**7h**	**12h**	H	H	OCH_3	H	16[b]	NA
9	**7i**	**12i**	H	H	H	OCH_3	44[b]	98
10	**7j**	**12j**	H	F	H	H	64[b]	72
11	**7k**	**12k**	H	H	F	H	80	65
12	**7l**	**12l**	H	H	CN	H	72	71
13	**7m**	**12m**	H	F	F	H	94	89

[a]Overall yield of a mixture containing **12b** and an unidentified, unstable minor isomer, ratio = 1.0:0.3-0.5; [b]Dimeric product was also isolated and characterized (yields not shown); NA = not available due to decomposition of product.

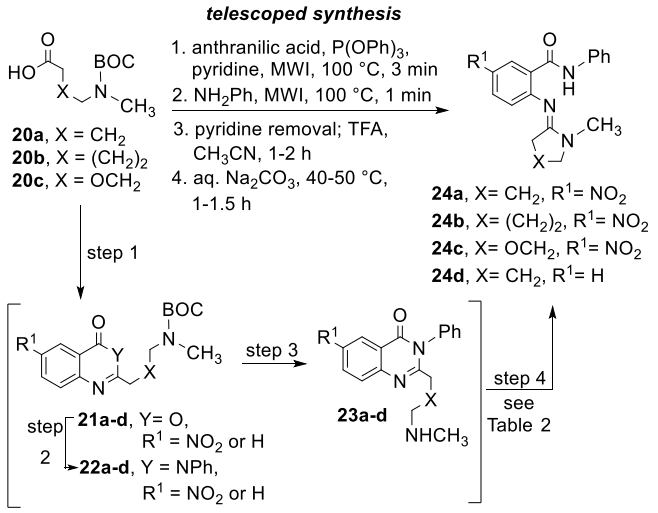
Overall, yields of the amidine products were reasonably high except in a few select cases. Migration of the nitro group to any of the available quinazolinone C5-, C7-, or C8-positions afforded amidines **12b-d** in high yields (entries 2-4, Table 1). The presence of a strong electron-withdrawing group was not required for the rearrangement to occur, as replacement of the nitro group with a hydrogen atom afforded amidine **12e** in 83% when DMF was employed (entry 5). The effect of installing an electron-donating methoxy group on the core was also examined. Yields were especially poor for those substrates bearing methoxy groups at the R^1 or R^3 positions (entries 6 and 8, respectively), while R^2 and R^4 substitution was tolerated when the reaction was performed in DMF (entries 7 and 9, respectively). This effect was likely due to the increased electron density contributed at the reacting alkyl chloride via resonance of methoxy groups at the R^1 or R^3 positions. Installation of alternative electron withdrawing substituents such as fluorine and nitrile groups was found to produce the corresponding amidines analogously to the nitro group bearing substrates.

Within the scope of our medicinal chemistry program, it was desirable to also modulate the character of the cycloamino-amidine moiety itself; however, we were initially unable to explore any changes in this structural space using this or several other methods. Therefore, focus shifted to exploiting the rearrangement to expand our structural interrogation, specifically permitting the generation of carbon- and oxygen-containing cycloamidines. It was initially anticipated that analogs with structural permutations of the amidine portion of the scaffold could be prepared by incorporating alternative chloroalkyl appendages off of the quinazolinone core. To this end, chloroacylalkylchlorides **16a-b** were used to construct chloroalkylquinzalinones **17a-c** (Scheme 5). Subsequent treatment of **17a-c** with an amine was expected to generate appended amines *in situ* and trigger the rearrangement to afford the corresponding amidines **18a-c**. Feasibility of this approach was explored using *n*-butylamine due to its lower volatility at higher temperatures versus methylamine.



Scheme 5. Structural diversity from chloroalkylquinazolinones

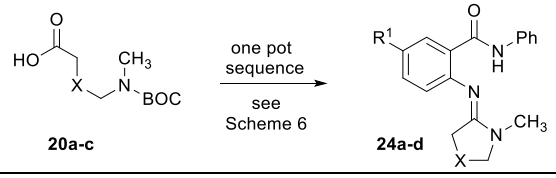
The successful conversion of chloroalkyl intermediates **17a-c** to the desired amidines **18a-c** was determined to hinge on multiple factors including substitution pattern of the quinazolinone core, nucleophilic amine, solvent, temperature, and duration of the reaction (data not shown). For example, preliminary conditions scouted with **17b** encouragingly afforded the desired amidine **18b** exclusively in a reasonable 62% yield; however, the same conditions employed with chloroalkylquinzalinone **17a** afforded only novel cyclopropylquinazolinone **19a** (65% yield), likely formed from intramolecular displacement of the chloride after deprotonation of the allylic-like carbon of the alkyl appendage. While interesting in their own right, the formation of these and other by-products, along with the variability in amidine yield as a function of multiple parameters, ultimately motivated us to investigate a more efficient route to modified amidines. Based on these results, we reasoned that the conversion of an anthranilic acid to the desired amidine could be achieved in a one-pot, multicomponent assembly. To study this approach, requisite carboxylic acids **20a-c** were prepared bearing a BOC protected amine (Scheme 6).



Scheme 6. One-pot synthesis of benzamidoamidines from *N*-BOC protected δ- or γ-amino acids

Use of the stepwise protocol in hand with **20a** led to amidine **24a** in 18% yield while the same conditions applied to **20b** led to significant decomposition. While the initial yields were not ideal, we were encouraged that the multi-step sequence delivered moderate quantities of desired products on the first pass and that the individual steps could be optimized further to accommodate new substrates. Moreover, scrutiny of each individual reaction step revealed that insightful modifications of the reaction conditions would likely permit an efficient, one-pot assembly. Thus, treatment of acids **20a-c** with an anthranilic acid in the presence of pyridine and triphenylphosphite[9] (not $POCl_3$) generated benzoxazinones **21a-d** which were then transformed to the corresponding quinazolinones **22a-d** by the addition of aniline under microwave irradiation. Removal of the pyridine *in vacuo*, followed by the addition of TFA revealed the appended amines **23a-d** *in situ*. The addition of aqueous base to quench the TFA and adjust the pH to 10 at elevated temperature generally afforded the desired (*E*)-benzamidoamidines **24a-d** in good yield (Table 2).

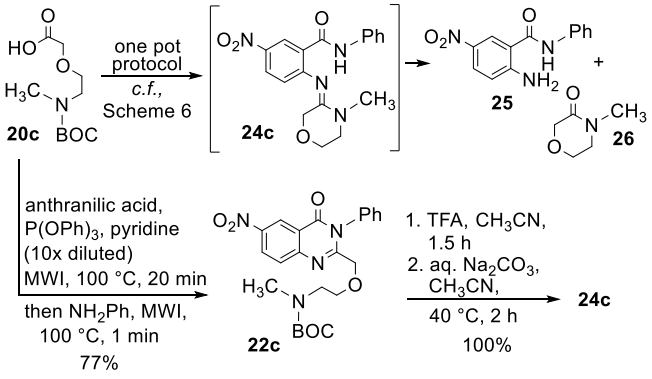
Table 2. Yields of (*E*)-benzamidoamidines from BOC-protected amino acids



entry	*N*-BOC-amino acid	amidine product	X	R^1	amidine yield (%)
1	**20a**	**24a**	CH_2	NO_2	80
2	**20b**	**24b**	$(CH_2)_2$	NO_2	62
3	**20c**	**24c**	OCH_2	NO_2	24 (77%)[a]
4	**20a**	**24d**	CH_2	H	89

[a]77% yield obtained when a two-pot sequence was employed.

The reaction was unaffected by the presence or absence of a core nitro group substituent, as both anthranilic acid substrates, when treated with *N*-BOC-amino acid **20a,** led to five-membered, carbocyclic amidine ring products **24a** and **24d** in high yields (80-89%). The six-membered, carbocyclic amidine **24b** was prepared in good yield as well. Introduction of an oxygen atom in the amine linker led to a suboptimal yield of amidine **20c** (24%). A stepwise analysis of the reaction sequence for **20c** revealed that desired amidine was generated efficiently; however, the product was unstable under one-pot conditions, as determined from the isolation and characterization of cleavage products **25** and **26** (Scheme 7). Thus, we found that the penultimate *N*-BOC aminoalkoxy quinazolinone **22c** was isolated in 77% overall yield from a telescoped sequence that started with *N*-BOC protected amino acid **20c**. Isolation and purification of **22c**, followed by submission to the TFA and base-catalyzed rearrangement conditions afforded amidine **24c** quantitatively.



Scheme 7. Two-step modified procedure for oxygen-containing cycloamidine 24c

Conclusions

This effort was driven by a necessity to efficiently synthesize structural architecture that was otherwise inaccessible and to address multiple limitations and unknowns associated with our discovery of a new quinazolinone rearrangement. Inessential reagents were excluded, the most favorable temperature and solvent conditions were defined leading to optimal yields for a range of substrates of various electronic character, and we determined that the key reaction was triggered under basic conditions. Importantly, the process has been modified to permit augmentation of the cyclic amidine, thereby improving the overall scope and use of the transformation. As a result, we have now developed divergent methodology that permits the efficient, regiospecific synthesis of structurally and pharmacologically novel (*E*)-amidines from either chloroalkylquinazolinones or *N*-BOC-protected δ- and γ-amino acids. This unprecedented intramolecular rearrangement tolerates a good range of substitution on the benzamide core as well as changes in the amidine-forming linker. Furthermore, the transformation was optimized to generate the benzamidoamidine framework in a one-pot assembly that comprises at least 5 distinct chemical operations. This methodology is currently being leveraged to examine amidine-related structure-activity relationships in our virology program; however, we are also studying the use of this reaction in combination with other cascade

reactions to generate unique, structurally diverse species as templates for further pharmacological exploration and new chemical methodology development.

Experimental

General Procedure for the one pot synthesis of (*E*)-benzamidines:

(*E*)-2-((1-Methylpyrrolidin-2-ylidene)amino)-5-nitro-*N*-phenylbenzamide (24a). 5-Nitroanthranilic acid (91 mg, 0.50 mmol) and 4-((*tert*-butoxycarbonyl)(methyl)amino)butanoic acid (**20a**, 217 mg, 1.00 mmol) were dissolved in dry pyridine (1.0 mL). Triphenyl phosphite (0.40 mL, 1.5 mmol) was added, and the resulting mixture was heated at 100 °C under MWI for 1 min and slowly allowed to cool down to rt over a period of 10 min. This MWI heating process (100 °C for 1 min. , followed by slow cool-down to rt over 10 min) was repeated two more times. TLC analysis (50% EtOAc/hexanes) showed the disappearance of starting material (5-nitroanthranilic acid) at R_f = 0.2 and the presence of the corresponding benzoxazinone at R_f = 0.5. Aniline (0.18 mL, 2.0 mmol) was added to the mixture and the reaction mixture was heated at 100 °C with MWI for 1 min and slowly allowed to cool down to rt over a period of 10 min. TLC analysis showed the disappearance of the benzoxazinone (R_f = 0.5) and the presence of the corresponding quinazolinone (R_f = 0.4). Pyridine was removed *in vacuo* and the resulting clear, yellow oil was dissolved in dry CH_3CN (9 mL) and TFA (5.8 mL). The mixture was stirred at rt for 1-2 hours until the BOC-protected quinazolinone (R_f = 0.4) was consumed. The mixture was diluted with CH_3CN (30 mL) and slowly quenched to pH 10 with saturated aq. Na_2CO_3 (50 mL). The reaction mixture was stirred at 50 °C for 1 hour. The mixture was allowed to reach rt and water (30 mL) was added. The product was extracted with CH_2Cl_2 (3 x 100 mL), with minimal shaking in order to avoid an emulsion. The separated, organic extracts were combined and concentrated *in vacuo* to give a clear, yellow oil, which was purified by reverse-phase chromatography (10-100% CH_3CN/water) yielding **24a** (135 mg, 80%) as a yellow solid, mp 180-182 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.62 (s, 1H), 9.17 (d, J = 2.8 Hz, 1H), 8.16 (dd, J = 8.8, 2.9 Hz, 1H), 7.69–7.64 (m, 2H), 7.38–7.32 (m, 2H), 7.15–7.09 (m, 1H), 6.85 (d, J = 8.8 Hz, 1H), 3.56 (t, J = 7.0 Hz, 2H), 3.22 (s, 3H), 2.64 (t, J = 7.8 Hz, 2H), 2.10 (p, J = 7.4 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.3, 162.8, 155.8, 142.7, 138.5, 129.1, 127.5, 126.4, 126.3, 124.1, 123.2, 120.2, 51.7, 31.9, 28.8, 20.0. HRMS (*m*/*z*): calcd for $C_{18}H_{19}N_4O_3$ $(M + H)^+$ 339.1452; found 339.1449.

Acknowledgements

This work was funded by the National Institutes of Health (J.Aubé, U54HG005031 and J. Golden, 1R01AI118814-01). We thank Dr. Victor Day for X-ray crystallography assistance and acknowledge the following support for KU NMR instrumentation: NIH S10RR024664 and NSF 0320648. J. Golden thanks postdoctoral research associate Dr. Xufeng Cao at UW-Madison for the resynthesis and characterization of **17a** and **22c**.

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