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Silver(I)-Promoted Conversion of Thioamides to Amidines: Divergent Synthesis of a Key Series of Vancomycin Aglycon Residue 4 Amidines that Clarify Binding Behavior to Model Ligands

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Abstract

Development of a general Ag(I)-promoted reaction for the direct conversion of thioamides to amidines is disclosed. This reaction was employed to prepare a key series of vancomycin aglycon residue 4 substituted amidines that were used to clarify their interaction with model ligands of peptidoglycan precursors and explore their resulting impact on antimicrobial properties.

The glycopeptide antibiotics are the most important class of drugs used in the treatment of resistant bacterial infections, including those caused by methicillin-resistant *Staphylococcus aureus* (MRSA).[1] After more than 50 years of clinical use, the emergence of resistant Gram-positive pathogens including vancomycin-resistant Enterococci (VRE) and vancomycin-resistant *Staphylococcus aureus* (VRSA) presents a serious public health problem at a time few new antibiotics are being developed.[2] This has led to renewed interest in the search for additional effective treatments for resistant pathogens that display the durability of vancomycin, including the development of new derivatives of the glycopeptide antibiotics.[3,4] Discovered at Eli Lilly, vancomycin (**1**, Figure 1) was disclosed in 1956[5] and introduced into the clinic in 1958 although its structure was not established until nearly 30 years later.[6] With the emergence of MRSA, it has become the drug of last resort for the treatment of such resistant bacterial infections.[1]

The glycopeptide antibiotics inhibit bacterial cell wall synthesis by binding the precursor peptidoglycan peptide terminus D-Ala-D-Ala.[7,8] In the two most prominent resistant phenotypes (VanA and VanB), this precursor is remodeled to D-Ala-D-Lac, incorporating an ester in place of the amide in the natural ligand.[9] Synthesis of lipid intermediate I and II, containing the D-Ala-D-Ala termini, continues but vancomycin-resistant bacteria sense the antibiotic challenge[10] and initiate a late stage remodeling from D-Ala-D-Ala to D-Ala-D-Lac to avoid the antibiotic action. The binding affinity of vancomycin for the altered ligand is reduced (1000-fold), resulting in a corresponding loss in antimicrobial activity (1000-fold). Thus, efforts to redesign the vancomycin binding pocket for its use against vancomycin-resistant bacteria must target compounds that not only bind D-Ala-D-Lac, but that also maintain binding to D-Ala-D-Ala.

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Supporting Information Available: Experimental details are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

Following an initial success with [Ψ[CH_2NH]Tpg^4]vancomycin aglycon (**3**)[11] to achieve this dual binding by the removal of the lone pair repulsion between the vancomycin residue 4 carbonyl and D-Ala-D-Lac ester oxygens,[12] we reported [Ψ[C(=NH)NH]-Tpg^4]vancomycin aglycon (**4**)[13] in a search for improved dual binding affinities and antimicrobial activities (Figure 2). Amidine **4** displayed effective, balanced binding affinity for both model ligands at a level that is within 2- to 3-fold that exhibited by vancomycin aglycon for D-Ala-D-Ala. Accurately reflecting these binding properties, **4** exhibited potent antimicrobial activity (MIC = 0.31 μg/mL, VanA *E. faecalis*) against VRE, being equipotent to the activity that vancomycin displays against sensitive bacterial strains. Although this represents a single atom exchange in the antibiotic (O→NH) to counter a corresponding single atom exchange in the cell wall precursors of resistant bacteria (NH→O), the modified antibiotic also maintains vancomycin's ability to bind the unaltered peptidoglycan D-Ala-D-Ala by virtue of its apparent ability to serve as either a H-bond donor (for D-Ala-D-Lac) or H-bond acceptor (for D-Ala-D-Ala). Whereas the former entails binding of the expectedly protonated amidine (pK_a = 12.5), the latter requires binding of the unprotonated amidine.

Herein, we report the synthesis of a key series of substituted amidines designed to clarify their protonation state when bound to model ligands and explore additional questions on the potential behavior of such derivatives (Figure 3). Since selective modification of vancomycin at the residue 4 site is not yet possible, a divergent[14] total synthesis based on our efforts targeting the naturally occurring aglycons[15–19] was designed that proceeds through an intermediate capable of late-stage diversification. The approach incorporated a residue 4 thioamide, which could be selectively modified at the final stage of the divergent synthesis. In these studies, we found that the thioamide **5** could be selectively converted to the amidine **4** in a single step using a previously unexamined AgOAc-promoted reaction with NH_3 in MeOH. Importantly, this reaction was successful (50–85%) on a fully functionalized and deprotected vancomycin aglycon.[13]

Because of the magnitude of the effort involved, the survey herein was conducted on the advanced synthetic intermediate **9** bearing the residue 4 thioamide, but a C-terminus hydroxymethyl group in place of the carboxylic acid. This intermediate is available in 22 versus 26 steps and its derivatives, including the amidine **10**, exhibit binding and in vitro antimicrobial properties indistinguishable from the corresponding vancomycin aglycon derivatives.[13b]

The first of the substituted amidines that we were especially interested in targeting was the *N*-methylamidine **11**. Unexpectedly, efforts to convert thioamide **9** to **11** using AgOAc and $MeNH_2$–MeOH under the reaction conditions used to prepare **4** and **10** were not successful. As a result, the various parameters of this reaction were examined first using the simpler substrate **15** (Figure 4).[20,21]

Like the reaction with **9**, attempts to convert **15** to **17** using $MeNH_2$ (2 M in MeOH) and AgOAc (2–10 equiv) in MeOH were not especially successful. More surprisingly, we also found that AgOAc (3 equiv) in NH_3–MeOH was not as effective in converting **15** to the parent amidine **16** although **15** is rapidly consumed.[22] This led to an examination of a series of alternative Ag(I) salts. These studies revealed that the more reactive Ag(I) salts including $AgBF_4$ and $AgOCOCF_3$ were effective at promoting the conversion of **15** to the parent amidine **16** (83%), the *N*-methylamidine **17** (93%, 1:1 *E:Z*), or the *N,N*-dimethylamidine **18** (82%) in good yields in MeOH at room temperature (Figure 4). Moreover, these conditions were successful in converting the residue 4 thioamide in **9** to the *N*-methylamidine **11** as an inseparable or equilibrating 1:1 mixture of *E:Z* isomers (5 equiv $AgBF_4$, 2 M $MeNH_2$ in MeOH, 25 °C, 30 min), Figure 3.

Extension of the methodology to the preparation of the *N*-hydroxyamidine (amidoxime) **19** upon reaction of **15** with hydroxylamine is summarized in Figure 5. AgOAc proved modestly effective at promoting formation of **19** in MeOH, whereas the more reactive Ag(I) salts resulted in further reaction of the product amidoxime **19**, leading to liberation of the *N*-hydroxyamidoxime and thioamide cleavage. This cleavage reaction of thioamide **15** was suppressed by running the reaction in less polar and aprotic solvents where **19** was isolated in excellent yields. Generation of **12**, requiring the use of a protic solvent (MeOH), provided the easily handled residue 4 amidoxime as a single *E*-isomer.

Similar observations were made in the preparation of the Boc protected *N*-aminoamidine (amidrazone) **20** upon reaction of **15** with $BocNHNH_2$ (Figure 5). Due to the high nucleophilicity of $BocNHNH_2$, most Ag(I)-promoted reactions led to double addition and cleavage of the thioamide. Short reaction times (5 min) with $AgBF_4$ (5 equiv) and limiting the amount of $BocNHNH_2$ (2 equiv, MeOH, 73%) or the use of aprotic, nonpolar solvents suppressed the overreaction and provided **20** in good yields. Such problems were less significant with **9**, where the residue 4 thioamide is sterically hindered. The well behaved Boc protected precursor to the amidrazone **13** was isolated in good yield as a single isomer.

The amine anticipated to be most challenging was cyanamide, due to its lower nucleophilicity (Figure 6). Remarkably, use of AgOAc (5 equiv) in MeOH led to rapid conversion of **15** to *N*-cyanoamidine **21** (30 equiv H_2NCN, 10 min, 85%). Extending this reaction to the preparation of the vancomycin aglycon *N*-cyanoamidine using AgOAc (5 equiv) provided **14** as a single isomer whose properties were consistent with the Z-configuration or equilibration to (*Z*)-**14** under the assay conditions. The conversion of the thioamide **15** to the *N*-cycanoamidine **21** could also be conducted in aprotic solvents (THF > CH_3CN > DMF). The further inclusion of Et_3N (10 equiv) gave rise to a reaction that was complete in minutes and provided superb yields of **21** (91–94%).

The results of the examination of the amidines **11**–**14** are summarized in Figure 3. *N*-Methylamidine **11** proved to be 30 to 50 times less effective than the parent amidine **10** at binding[23] the model D-Ala-D-Ala and D-Ala-D-Lac ligands **6** and **7**, respectively, but **11** bound both with near equal affinities. Accordingly, it was found to be active against VanA VRE (MIC = 20 μg/mL), albeit being 60-fold less potent than **10** precisely in line with its relative binding characteristics. Although the assessment was conducted with a sample composed of either an inseparable or equilibrating 1:1 mixture of *E:Z* isomers, the results still indicate that the substitution of the amidine with a small methyl group is sufficient to significantly diminish its binding and antimicrobial properties. Whereas it is difficult to infer details about the protonation state of an amidine when binding D-Ala-D-Ala, the comparison of **11** with **10** support expectations that it must be the protonated amidine that binds D-Ala-D-Lac. Unlike **10**, the unprotonated state of **11** would be incapable of H-bonding to the ligand and suffers a further destabilizing lone pair/lone pair interaction, Figure 7.

The behavior of *N*-cyanoamidine **14**, which cannot be protonated ($pK_a = 1$), proved even more interesting. Although its affinities and activity were reduced relative to the amide **8**, the relative behavior of **8** and **14** was identical and distinct from those of the amidines **10** and **11** (Figure 3). Like the amide **8**, *N*-cyanoamidine **14** bound D-Ala-D-Ala much more effectively than D-Ala-D-Lac, which it failed to bind (≥120-fold). Accordingly, **14** lacked antimicrobial activity against VanA VRE (MIC > 40 μg/mL), but remained active against vancomycin-sensitive *S. aureus* (MIC = 10 μg/mL) at a level consistent with its affinity for D-Ala-D-Ala. Moreover, this affinity for D-Ala-D-Ala was found to be roughly equivalent to that of *N*-methylamidine **11**, albeit 20-fold less than the parent amide **8** or amidine **10**. The inability of the unprotonated amidine **14** to bind D-Ala-D-Lac confirms that the effective D-Ala-D-Lac binding of the parent amidine **10** and *N*-methylamidine **11** must

entail binding of the protonated amidines, replacing the destabilizing lone pair repulsion with a stabilizing electrostatic interaction and weak reverse H-bond. Similarly, the comparable binding affinities of the unprotonated cyanoamidine **14** and the *N*-methylamidine **11** with D-Ala-D-Ala indicate both bind in their unprotonated state, accepting a H-bond from the linking amide in the bound ligand (Figure 8).

The amidoxime **12** and amidrazone **13** were important to examine for an additional reason. Both possess the potential of covalent attachment to bound D-Ala-D-Lac. Unlike the well-behaved physical properties of its *N*-Boc precursor, the amidrazone **13** obtained upon *N*-Boc deprotection (TFA, 25 °C, 12 h) proved unmanageable to work with. It was found to be insoluble in both protic (buffer, H_2O and MeOH) and polar aprotic solvents (DMSO), preventing its true assessment in binding or antimicrobial assays where it proved ineffective (Figure 3). Even prolonged incubation of suspensions of **13** with D-Ala-D-Lac in the binding assay buffer (>4 months) failed to provide evidence of either reaction with the ligand (ester amidation) or ligand hydrolysis. In contrast, the amidoxime **12** was well behaved and easy to characterize. It was isolated as a single isomer, which we assigned as the *E*-isomer because of a potential stabilizing H-bond from the amide NH linking residues 3 and 4. Consistent with this assignment, both its binding and antimicrobial activity are reduced ≥200-fold relative to the parent amidine **10** (Figure 3). Prolonged incubation of **12** with D-Ala-D-Lac in the binding assay buffer (>6 months) also failed to provide evidence of either reaction with the ligand (transesterification)[24] or ligand hydrolysis. Despite the lower activity of the amidoxime **12**, it still represents a derivative class that merits future consideration as an effective in vivo antimicrobial agent. Its well behaved physical properties as an unprotonated amidine derivative (pK_a = 6 vs 12.5), facilitating its absorption and permeability, as well as its likely rapid in vivo reduction to the active amidine suggest such amidoximes should continue to be examined in work going forward.[25]

Complementary to the studies detailed herein, the parent amidines **4** and **10** were shown to display identical dipeptide ligand binding selectivities and affinities as the corresponding amides **2** and **8**, confirming that they (1) bind such ligands in the same manner, and (2) are subject to the same structural recognition features that dominate the vancomycin interaction with D-Ala-D-Ala and related ligands. This eliminated the possibility that the amidines may be interacting with the ligands in a unique manner.[13] With the development of a general single step Ag(I)-promoted reaction applicable to amines with a wide range of nucleophilicities, the late-stage divergent synthesis of a key series of substituted amidines from a common residue 4 thioamide was conducted. The resulting amidine derivatives were used to define additional details of their interaction with model ligands, indicating that it requires the unprotonated amidine to bind D-Ala-D-Ala and the protonated amidine to bind D-Ala-D-Lac.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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22. In MeOH and in the absence of a reacting amine, treatment of 15 with AgOAc (3 equiv) rapidly provides the corresponding *O*-methylimidate (>90%). However, subsequent treatment of the in situ generated *O*-methylimidate with NH_3 (7 M in MeOH) fails to provide 16, indicating that it is not an intermediate enroute to the amidine.

23. UV-difference titration assays were run as described: Nieto M, Perkins HR. Biochem J. 1971; 124:845. [PubMed: 4331859] Nieto M, Perkins HR. Biochem J. 1971; 123:773. [PubMed: 5124385] Nieto M, Perkins HR. Biochem J. 1971; 123:789. [PubMed: 5124386]

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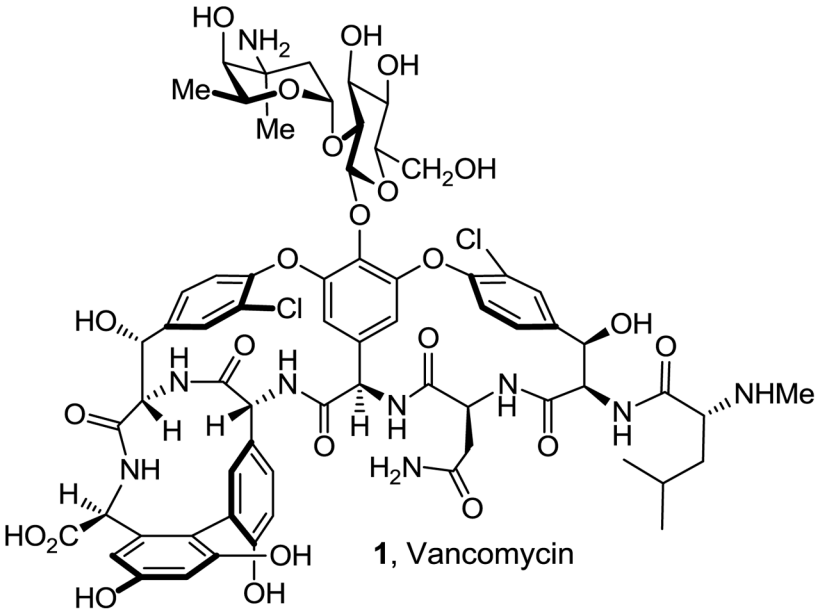
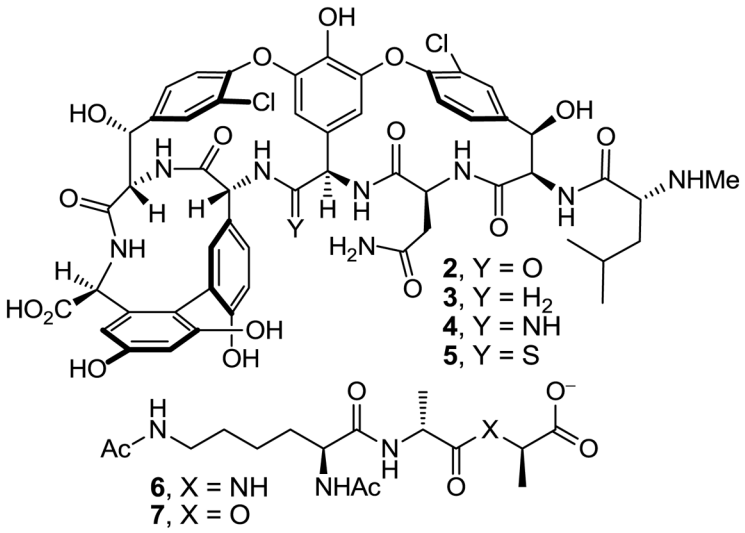


Figure 1.
Structure of vancomycin.



compound	ligand, K_a (M^{-1}) **6**, X = NH	**7**, X = O	K_a(**6**/**7**)	VanA[a] MIC, μg/mL
2, Y = O	1.7 x 10^5	1.2 x 10^2	1400	640
3, Y = H_2	4.8 x 10^3	5.2 x 10^3	0.9	31
4, Y = NH	7.3 x 10^4	6.9 x 10^4	1.05	0.31
5, Y = S	1.7 x 10^2	1.1 x 10^1	–	> 640

[a]Minimum inhibitory conc., *E. faecalis* (BM4166, VanA VRE).

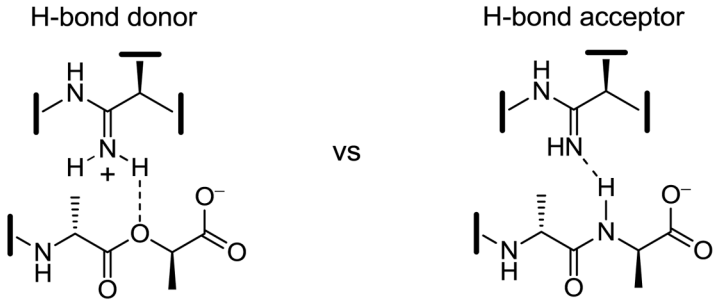
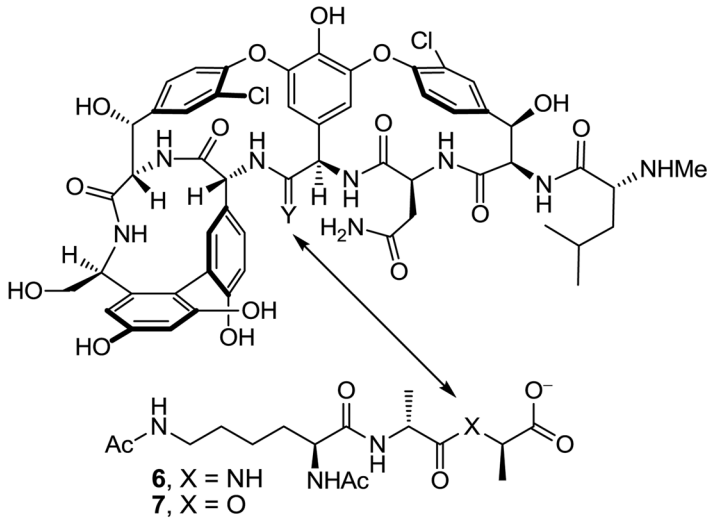


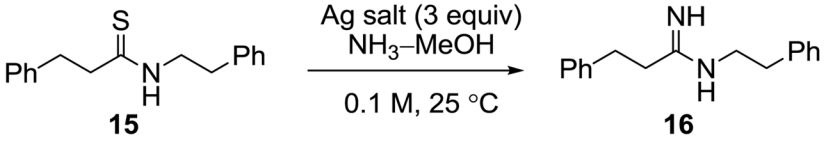
Figure 2.
Vancomycin aglycon residue 4 modifications and proposed dual binding behavior of the amidine **4**.



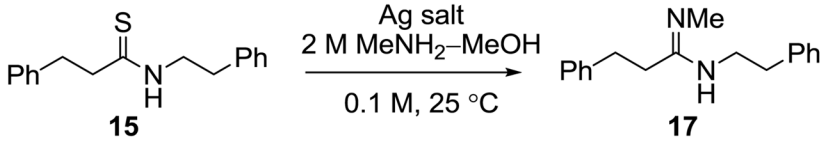
compound	ligand, K_a (M^{-1}) **6**, X = NH	**7**, X = O	K_a(**6**/**7**)	VanA[a] MIC (μg/mL)
11, Y = NMe	1.8 x 10^3	1.3 x 10^3	1.4	20
12, Y = NOH	2.7 x 10^2	1.8 x 10^2	1.5	>160[b]
13, Y = NNH_2	nd	nd	nd	> 40[b]
14, Y = NCN	5.7 x 10^3	4.9 x 10^1	≥120	> 40[b,c]
10, Y = NH	5.7 x 10^4	6.3 x 10^4	0.9	0.31
8, Y = O	1.4 x 10^5	1.3 x 10^2	1100	>320[b]
9, Y = S	6.2 x 10^2	3.1 x 10^1	–	>320[b]

[a]MIC = minimum inhibitory concentration required for complete growth inhibition. *E. faecalis* (BM4166, VanA VRE). [b]Highest conc. tested. [c]MIC = 10 μg/mL against sensitive *S. aureus*.

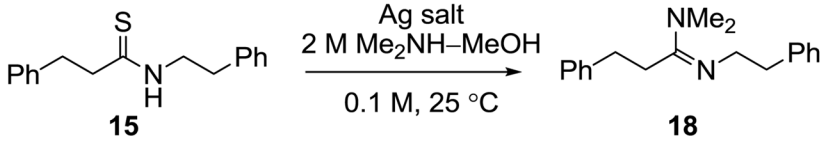
Figure 3.
Residue 4 substituted amidines.



Ag salt	time	% yield
AgOAc	1–24 h	30–32%
$AgNO_3$	10 min	0%
AgBr	24 h	0%
Ag_2CO_3	10 min	0%
AgO	2 h	0%
AgOTf	3 h	49%
$AgSbF_6$	5 min	65%
$AgOCOCF_3$	10 min	70%
$AgBF_4$	2 h	83%



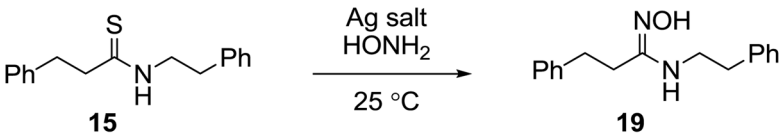
Ag salt	time	% yield
AgOAc (5 equiv)	24 h	<35% (1:1)
$AgBF_4$ (3 equiv)	3 h	93% (1:1)
$HgCl_2$	12 h (THF)	79% (1:1)



Ag salt	time	% yield
AgOAc (5 equiv)	24 h	16%
$AgBF_4$ (5 equiv)	3 h	44–57%
$AgBF_4$ (5 equiv)[a]	3 h	82%

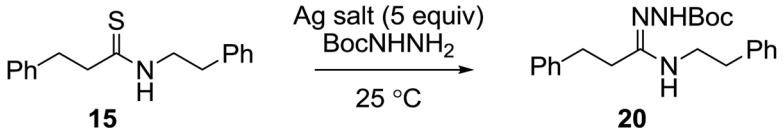
[a]With Et_3N (10 equiv)

Figure 4.
Amidine, *N*-methylamidine, and *N,N*-dimethylamidine formation.



Ag salt (equiv)	[a]$HONH_2$ (equiv)	time	solvent	% yield
AgOAc (3)	10	12 h	MeOH	36% (61:1)
$AgBF_4$ (3)	10	2 h	MeOH	0%
$AgBF_4$ (5)	100	3 h	THF	78% (–)
$AgBF_4$ (3)	10	2 h	CH_3CN	87% (18:1)

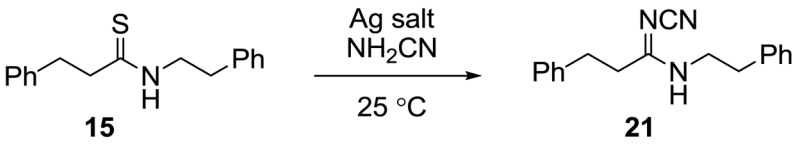
[a]NH_2OH (aq. 50% w/w)



Ag salt	$BocNHNH_2$ (equiv)	time	solvent	% yield
$AgBF_4$	30	5 min	MeOH	<30%
$AgBF_4$	30[a]	5 min	THF	<20%
$AgBF_4$	30[b]	5 min	THF	51% (ca. 1:1)
$AgBF_4$	2[b]	5 min	THF	55% (ca. 1:1)
$AgBF_4$	2[b]	5 min	MeOH	73% (ca. 1:1)

[a]With Et_3N (10 equiv). [b]With $NaHCO_3$ (10 equiv).

Figure 5.
Amidoxime and amidrazone formation.



Ag salt (equiv)	NH_2CN (equiv)	time	solvent	% yield
AgOAc (5)	30	10 min	MeOH	85% (3:1)
AgOAc (5)	30	10 min	MeOH	93%[a] (1:110)
AgOAc (5)	30	10 min	DMF	71%[a] (1:6)
$AgBF_4$ (5)	30	30 min	CH_3CN	91%[b] (1:40)
$AgBF_4$ (5)	30	10 min	THF	94%[b] (1:30)

[a]With $NaHCO_3$ (10 equiv). [b]With Et_3N (10 equiv).

Figure 6.
N-Cyanoamidine formation.

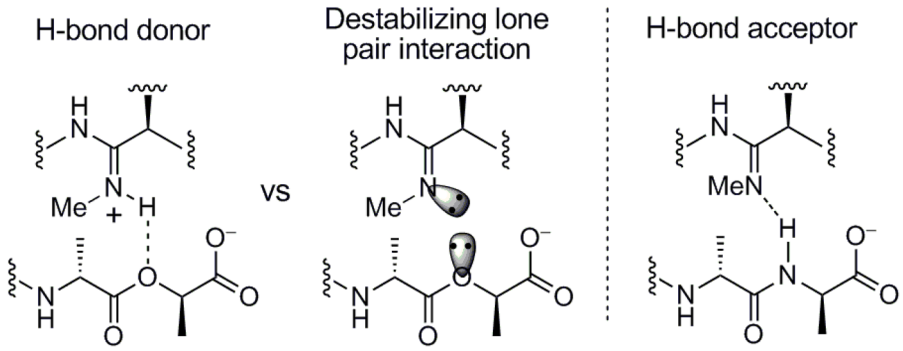


Figure 7.
Dual binding of *N*-methylamidine **11**. Effective binding to D-Ala-D-Lac must entail the protonated amidine.

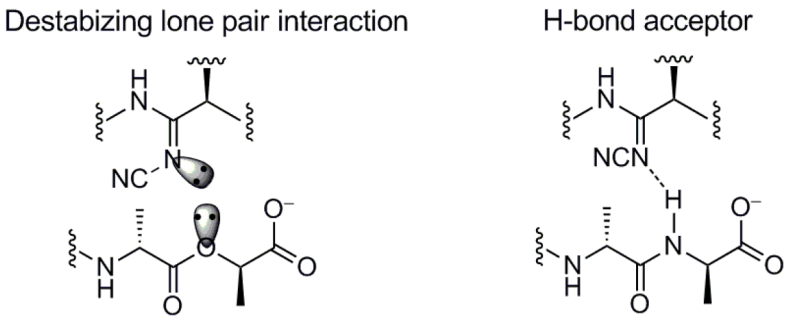


Figure 8.

N-Cyanoamidine **14** behavior paralleling that of amide **8**. D-Ala-D-Ala (and lack of D-Ala-D-Lac) binding represents unprotonated amidine binding.