

RELATION ESTABLISHED COMPLEX BASED ON ESTABLISHING TRI AZA MACRO CYCLIC LIGANDS

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ABSTRACT

A few novel chelators dependent on 1-hydroxy-2(1H)- pyridinone organizing bunches improving a triaza macrocyclic spine framework were orchestrated as potential amazing Fe³⁺ + chelators equipped for contending with bacterial siderophores. Specifically, a novel chloromethyl subsidiary of 1-hydroxy-2(1H)- pyridinoneA misusing a novel defensive gathering for this group of planning bunches was created. These are the primary instances of hexadentate chelators dependent on 1-hydroxy-2(1H)- pyridinone to be appeared to have a biostatic movement against a scope of pathogenic microscopic organisms. Their adequacy as biostatic specialists was evaluated uncovering that minor varieties in the design of the chelator can influence viability significantly. The insignificant inhibitory groupings of our best tried novel chelators approach or are practically identical to those for 1,4,7-tris(3-hydroxy-6-methyl-2-pyridylmethyl)- 1,4,7-triazacyclononane, the best Fe³⁺ + chelator known to date. The impeding impact these chelators have on microbial development proposes that they could have an expected application as a co-dynamic close by anti-microbials in the battle against contaminations.

Introduction

Bacterial protection from once compelling anti-toxins has arisen as a significant wellbeing danger of the 21st century [1], [2]. There is accordingly a pressing need to grow new systems to battle the spread of multi-drug safe contaminations. One of the potential alternatives as of now being contemplated is the utilization of biostatic specialists (for example inhibitors of bacterial development) that could work synergistically with existing anti-toxins and lift their viability [3], [4], [5], [6], [7]. Metal chelators can be utilized with that impact as their biostatic action upon microorganisms has for quite some time been known. Their method of activity is believed to be the inconvenience of metal starvation on the microorganisms [8], [9], [10].

The Fe³⁺/Fe²⁺ + redox couple can catalyze an expansive scope of natural reactions,[11] accordingly iron is a fundamental component universal to essentially all organic entities, making it an attractive objective for the

counteraction of microbial development by chelation [12], [13], [14], [15]. On account of contaminations, the microorganism's wellspring of iron is known to be simply the hosts [16], [17], [18]. Albeit iron is abundant, from the microbial viewpoint there is restricted bioavailability; pathogenic microorganisms utilize proficient iron securing systems, regularly dependent on little particles called siderophores [19]. Siderophores are prevalently hexadentate ferric chelators; their Fe^{3+} affinities/restricting qualities can be high and along these lines they can secure in any case inaccessible iron from sequestered host sources [20], [21], [22], [23], [24]. Accomplishing a biostatic impact by iron starvation in this way seems to rely on a misleadingly straightforward thermodynamic and motor rivalry in the authoritative of Fe^{3+} cations by the bacterial siderophores and the additional chelator. Close by the troublesome plan of chelators that can contend viably with siderophores, incredible consideration should likewise be taken to maintain a strategic distance from harmful demetallation of host metalloenzymes, making improvement of this innovation a non-trifling issue.

To rival solid siderophores, the correct decision of planning bunches is urgent. While considering just the thermodynamic rivalry between bacterial siderophores and an additional chelator, one should essentially consider the particular pFe^{3+} values (characterized as $-\log[\text{Fe}^{3+}]_{\text{free}}$, typically determined at pH 7.4, with $[\text{Chelator}]_{\text{total}} = 10 \mu\text{M}$ and $[\text{Fe}^{3+}]_{\text{total}} = 1 \mu\text{M}$ and referred to thus in these states) of the two chelators to appraise which is destined to be powerful in complexing the metal [25]. No doubt three isomers of the hydroxypyridinone (HOPO) family (Fig. 1) have the correct blend of pK_a and $\log\beta(\text{Fe}^{3+})$ to give high pFe^{3+} values and in this manner thermodynamically contend with siderophores (that are ordinarily founded on planning gatherings, for example, α -hydroxycarboxylic acids, hydroxamic acids and catechols) [24], [26].

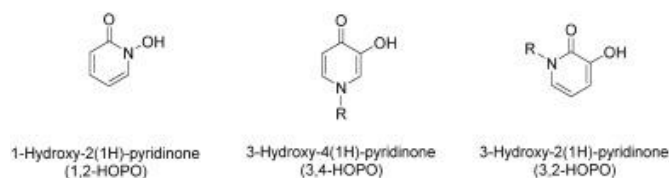


Fig. 1. Three relevant [isomers](#) of the HOPO family.

These hydroxypyridinones likewise offer alluring extra possibilities. Numerous microorganisms can utilize siderophores expounded by those of another species [18], [27]; to be powerful biostatic specialists, manufactured chelators should not experience the ill effects of this "siderophore robbery" and really advance bacterial development. Since not many HOPO are found in nature and just one has been portrayed as a siderophore ligand (1-hydroxy-5-methoxy-6-methyl-2(1H)-pyridinone likewise called cepabactin), the probability of the metallated chelators being perceived by bacterial receptors and utilized as a wellspring of Fe^{3+} is required to be little. Also, tainted human hosts are known to utilize different Fe^{3+} retaining methodologies to restrict bacterial development. One of these depends on the action of siderocalin, a protein that basically goes about as a snare for some siderophores [28], [29], [30], [31]. A remedial chelator should not cooperate with siderocalin and restrains its defensive activity. It is realized that some 1-hydroxy-2(1H)-pyridinone (1,2-HOPO) based chelators don't tie

unequivocally to siderocalin, recommending that this class of planning gatherings would supplement as opposed to overpower this protection technique [32].

Individuals from the 3-hydroxy-4(1H)-pyridinone (3.4-HOPO) sub-family have been broadly concentrated in chelation treatments [33]. A critical number of reports have likewise portrayed development restraint of a scope of pathogenic microorganisms by bidentate or hexadentate chelators dependent on 3.4-HOPO [34], [35], [36], [37], [38], [39], [40], [41], [42], [43]. To accomplish high pFe^{3+} , hexadentate chelators are wanted to bidentate ones, particularly since their method of activity as biostatic specialists is relied upon to be extracellular and hence don't experience the ill effects of size limitation to enter the organisms. An extremely modest number of mixes having a place with the 1.2-HOPO sub-family were depicted as antimicrobial specialists [44], [45] yet these are for the most part bidentate chelators. No hexadentate individual from that 1.2-HOPO sub-family has been portrayed as antimicrobial specialist. This nonattendance of reports is amazing looking at that as a couple hexadentate chelators dependent on 1.2-HOPO were depicted (for complexation of non-organically important metals) whose primary highlights and chelation properties make them alluring contender for this reason (for instance exacerbates 1 and 2, Fig. 2) [46], [47], [48], [49]. Albeit marginally more vulnerable planning bunches than their 3-hydroxy-2(1H)-pyridinone (3.2-HOPO) and 3.4-HOPO isomers, 1.2-HOPO organizing bunches have another favorable position that make them more alluring than their isomers. Their lower pK_a esteems (commonly 6 versus 8.5–10 relying upon replacement [26]) make them charged atoms at physiological pH and consequently less inclined to infiltrate cells of the host. This could have helpful security benefits in treating foundational contaminations.

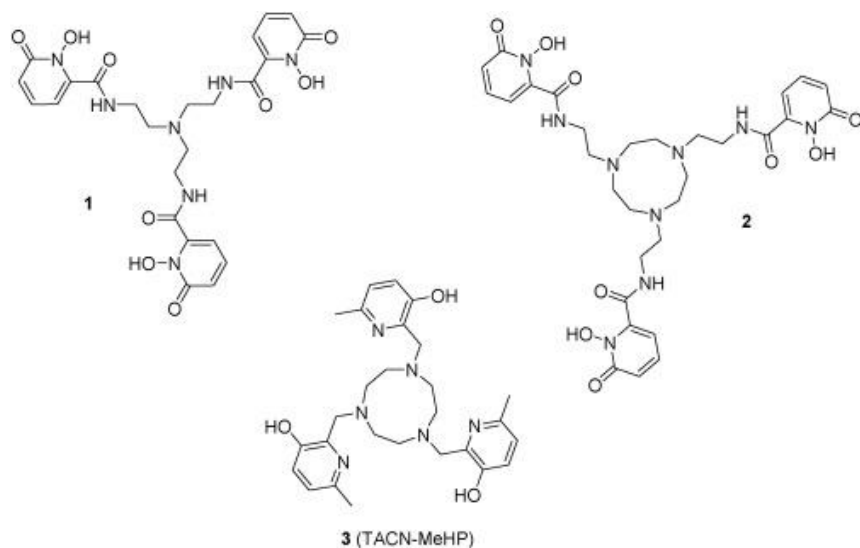


Fig. 2. Examples of known hexadentate chelators based on 1.2-HOPO (1, 3) and 1,4,7-tris(3-hydroxy-6-methyl-2-pyridylmethyl)-1,4,7-triazacyclononane (TACN-MeHP) (3).

Albeit not founded on HOPO, compound 3 has the most elevated known estimation of pFe_3 + (see beneath) and is subsequently expected to have the option to contend well with siderophores for ferric cations at low focus and along these lines show high biostatic viability [50]. Regardless of its high pFe_3 + esteem, to the most amazing aspect our insight this compound has never been depicted as a biostatic specialist. It was along these lines thought about important to blend, screen and utilize compound 3 as a benchmark.

Our premium in growing ground-breaking Fe_3 +-upgraded chelators as biostatic specialists incited us to explore a scope of hexadentate chelators dependent on 1.2-HOPO and a triaza macrocyclic spine platform to attempt to distinguish novel microbiostatic chelators. Not many polyaza macrocycles bearing HOPO planning bunches have been depicted [48], [51], [52], [53], [54]. Macrocycles offer the possibility to adjust the metal chelating properties by differing the cycle's size and flexibility. Our point was to join key underlying components of siderophores, HOPO and 1,4,7-tris(3-hydroxy-6-methyl-2-pyridylmethyl)-1,4,7-triazacyclononane (TACN-MeHP, 3) into a novel arrangement of chelators. Specifically by relationship with enterobactin (Fig. 3, compound 4), it is theorized that the impact of the macrocyclic spine framework could be helpful to the adequacy of metal authoritative and accordingly to the base inhibitory focus (MIC) of the chelator. Besides, no past amalgamation of hexadentate chelators where 1.2-HOPO moieties were connected to the sub-atomic platform by means of a methylene connect were ever detailed, the emphasis having been on a carbonyl linker. The effect of that linker on metal chelation viability can be emotional and along these lines merits top to bottom examination. Detailed in this is the amalgamation of novel triaza macrocyclic chelators bearing 1.2-HOPO moieties, connected through methylene or carbonyl gatherings and the investigation of their biostatic impact on a scope of microorganisms, incorporating an examination with that of known mixes 1–3.

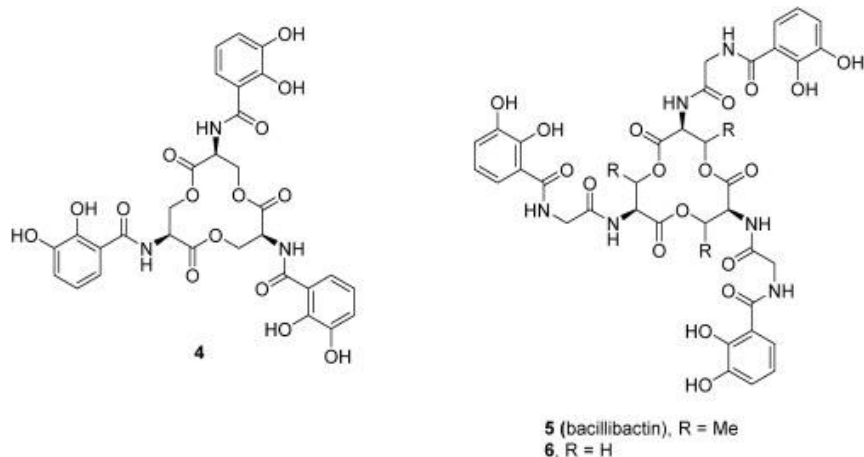


Fig. 3. Structure of [siderophores enterobactin](#) (4), bacillibactin (5) and synthetic analogue 6.

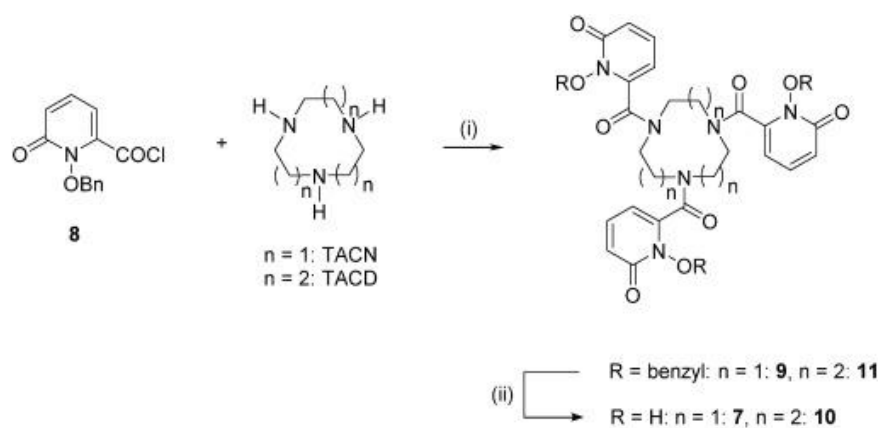
2. Results and discussion

2.1. Natural union

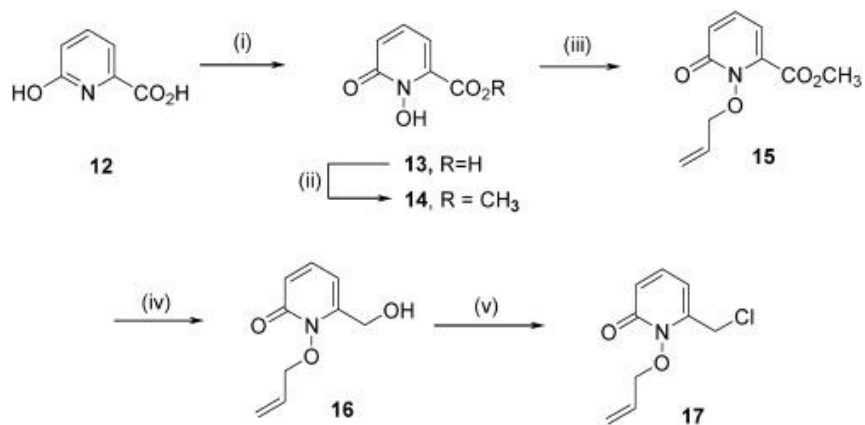
Our chelator configuration has zeroed in on thermodynamic (pFe^{3+}) as opposed to active contemplations. Quite possibly the most amazing bacterial siderophore is enterobactin (4), whose platform is made out of a triserine macrocycle (Fig. 3), and has been widely contemplated inferable from its exceptionally ground-breaking Fe^{3+} chelation ($\log\beta_{110} = 49$ and $pFe^{3+} = 34.3$, where β_{mlh} is characterized as the balance steady for the response $L + M + h H^+ \rightleftharpoons LIMmHh[25]$) [55]. The starting point of its proficient restricting has been explained and demonstrated to be affected by the pre-association of the planning gatherings, itself to a great extent impacted by the cyclic construction of its framework and intra-sub-atomic hydrogen holding [55], [56], [57], [58], [59]. Curiously, bacillibactin (5), a siderophore simple of enterobactin with glycine spacers connected to a tris-threonine macrocyclic framework likewise shows enormous $\log\beta_{110}$ and pFe^{3+} values (47.6, 33.1 individually) [55]. Nonetheless, engineered simple 6, likewise made out of a glycine spacer yet of a tris-serine platform has less fortunate $\log\beta_{110}$ and pFe^{3+} values (44.1 and 29.6 separately). No doubt the idea of the spacer and the idea of the platform profoundly affect pFe^{3+} that we estimate as impacting development hindrance.

Compound 3 has a Fe^{3+} coordination climate in edifices that is altogether different to that of enterobactin or HOPO-based hexadentate chelators. As opposed to the O6 giver set of 1, 2, 4, the coordination of 3 on Fe^{3+} happens through a N3O3 contributor set including the three nitrogen iotas of the 1,4,7-triazacyclononane (TACN) ring. As referenced before, 3 has the most noteworthy known $\log\beta_{110}$ and the most elevated pFe^{3+} estimations of any known ligand ($\log\beta_{110} = 49.98$, $pFe^{3+} = 39.4$) and subsequently is likewise deserving of thought as an expected layout to improve metal chelating capacity of the atoms [50]. Despite the fact that Fe^{3+} coordination concentrates with hexadentate analogs with a bigger triaza macrocyclic centers were not completely depicted, no doubt the three nitrogen molecules are reasonably at the correct distance to productively tie the metal iota.

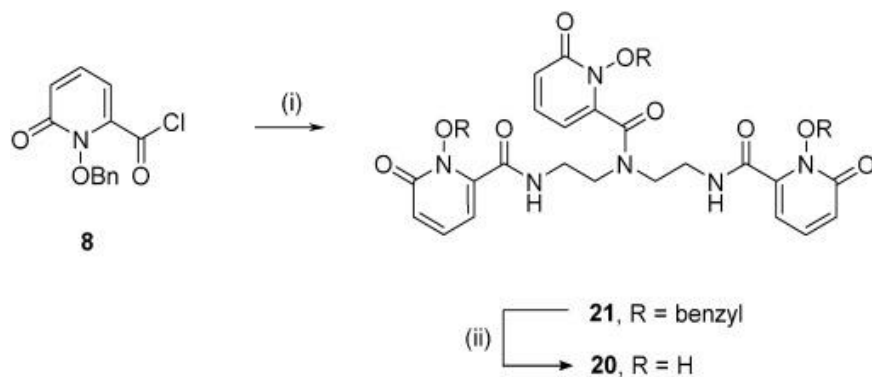
Enlivened by these perceptions, and needing to contemplate a scope of chelators dependent on 1.2-HOPO and bearing underlying likenesses to aggravates 1–3 we originally integrated compound 7 from TACN as portrayed in Scheme 1.



Scheme 1. Reagents and conditions: $n = 1$: (i) TACN, NEt_3 , THF, $60\text{ }^\circ\text{C}$; 25% (ii) HCl: AcOH; 97%; $n = 2$: (i) TACD, NEt_3 , DMF, $60\text{ }^\circ\text{C}$; 49% (ii) HCl: AcOH; 71%.



Scheme 3. Reagents and conditions: (i) TACN, K_2CO_3 , CH_3CN , reflux; 93% (ii) BCl_3 , CH_2Cl_2 ; 87%.



Scheme 4. Reagents and conditions: (i) [diethylenetriamine](#), NEt₃, THF, 60 °C; 91% (ii) HCl: AcOH; 70%.

The initial phase in the arrangement of **7** was performed dependent on a current technique by responding known acyl chloride **8** with TACN in THF [60]. Secured chelator **9** was acquired in low yield (25%). Expulsion of the benzyl bunches from **9** utilizing a combination of concentrated hydrochloric and icy acidic corrosive yielded **7** in fantastic yield (97%). A simple of that compound with the bigger triaza macrocycle (for example 1,5,9-triazacyclododecane, TACD) was likewise blended to consider correlation of the impact of the ring size upon bacterial development hindrance (Scheme 1). Response of acyl chloride **8** with TACD in DMF considered the disconnection of the bigger center ensured atom **11** in 49% yield. A comparable corrosive deprotection gave **10** in great yield (71%).

It was foreseen that the amide linkage utilized in **7** and **10** would largely affect the conformational adaptability of the chelator [61] and furthermore perhaps an electronic impact on the 1,2-HOPO planning gatherings. That thusly could have a significant effect (either valuable or negative) on Fe³⁺ official yet the specific impact of these linkers is as yet unreported. Along these lines, we additionally researched the combination of a simple of **7**, supplanting the carbonyl gatherings by methylene units. An epic HOPO assurance technique away from the benzyloxy security customarily utilized for 1,2-HOPO would be required. Undoubtedly, it was anticipated that benzyl insurance would not be appropriate as the most widely recognized techniques utilized for its expulsion could likewise sever the linkage between our HOPO gathering and the sub-atomic spine. Of the potential defensive strategies recognized, assurance of the N-hydroxyl bunch as the allyloxy bunch was attempted. It was viewed as that deprotection can be managed under a scope of moderately mellow conditions by twofold security isomerisation and ensuing hydrolysis without bargaining different securities in the chelator [62]. The key allyl secured 6-hydroxymethyl transitional **17** was orchestrated in five stages from monetarily accessible **12** (Scheme 2) [63]. This hydroxymethyl subordinate could then be changed over to the novel chloromethyl subsidiary **17** by thionyl chloride for fuse onto ligand centers.

Oxidation to the costly 1.2-HOPO-6-carboxylate 12 was performed utilizing industrially accessible peroxyacetic corrosive, an adjustment to the current strategy of Xu et al. [60], bringing about a somewhat expanded yield (77%) without the utilization of likewise costly trifluoroacetic corrosive. Treatment of the free corrosive 13 with thionyl chloride in methanol, following the strategy for Burgada et al. [64], gave the methyl ester 14 in magnificent yield (96%). Ensuing assurance of the N-hydroxyl gathering, utilizing allyl bromide and potassium carbonate, yielded the novel methyl ester 15 (94%). Decrease of the ester 15 to the methyl liquor 16 was refined by moderate expansion of methanol to sodium borohydride in THF, astoundingly without decrease of the allyl bunch [65], [66]. Eminently, a large portion of these means offered satisfactory to superb yields. Workup of the response combinations were not difficult to perform and the items either required no refinement at all or were not difficult to sanitize.

Response of a stoichiometric amount of 17 with TACN within the sight of potassium carbonate delivered the allyl secured macrocyclic item 18 of every a yield of 93% (Scheme 3). The expulsion of the allyl defensive gathering was performed utilizing boron trichloride, without cleavage of the recently framed Csingle bondN bond, to give compound 19 as the said simple of 7. To the most awesome aspect our insight, compound 19 is the primary illustration of a 1.2-HOPO metal chelator secured to its platform by means of a methylene connect in position 6.

At last, to perceive any relationship of platform inflexibility and biostatic impact, the acyl chloride halfway 8 was likewise responded with diethylenetriamine, a straight simple of the cyclic TACN, to give chelator 20 after corrosive deprotection (Scheme 4).

In spite of the fact that chelators 1–3 were recently depicted in the writing they have not been assessed as biostatic specialists subsequently their blend was likewise embraced to evaluate their antimicrobial properties [48], [49], [67].

Chelator 19 was viewed as an intriguing compound with regards to a few regards. One of them concerns the manner by which it can chelate Fe^{3+} . The common method of coordination of 1.2-HOPO-put together hexadentate chelators with respect to trivalent metals, for example, Fe^{3+} and Ga^{3+} is through the two oxygen iotas (Fig. 4A), giving an O6 coordination climate [32]. By similarity with subordinates of TACN-HP [68], [69]but all the more significantly with a ligand firmly identified with 19, for example a TACN-based tris-hydroxypyranone ligand 1,4,7-tris(3-hydroxy-6-(hydroxymethyl)-4(1H)-pyranon-2-ylmethyl)-1,4,7-triazacyclononane (NOKA) [70], it is conceivable to think about another method of Fe^{3+} chelation, by means of a N₃O₃ coordination mode including the TACN spine and hydroxyl gatherings (Fig. 4B).

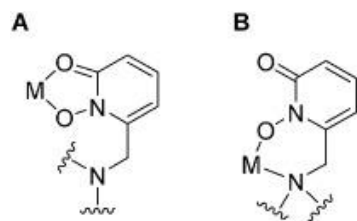


Fig. 4. Possible coordination modes for compound 19 with trivalent metal M (e.g. Fe^{3+} , Ga^{3+}).

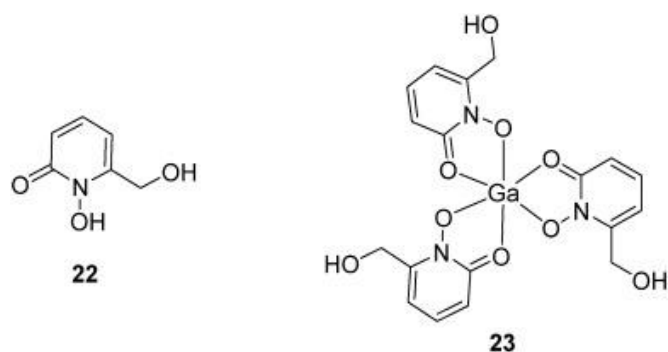


Fig. 5. 6-hydroxymethyl-1-hydroxy-2(1H)-pyridinone (**22**) and its expected 3:1 complex with Ga(III) (**23**).

To additional investigation the specific coordination method of ligand 19, specifically by NMR, Ga^{3+} was utilized as a proxy for Fe^{3+} [71]. No top to bottom investigation by MS or NMR of 1.2-HOPO based edifices of Ga(III) exist in the writing. For instance, just a single subsidiary of 1.2-HOPO complex (in light of the 1-oxo-2-hydroxy-isoquinoline-3-carboxylic corrosive) of Ga(III) has been described by NMR in CDCl_3 [72]. Basically, no investigation of the chelation by NMR or MS was acted in water, a dissolvable more pertinent to our development media and where protonation responses can dramatically affect the species framed [73]. To attempt to set up spectroscopic highlights that would permit us to recognize a N_3O_3 from an O_6 coordination mode, we had to initially explore by MS and NMR the complexation of Ga(III) on a model framework. Compound 22 that we depicted for another kind of utilization has been chosen as a particularly model of ligand 19 [63]

It was judged that compound 22 in the presence of Ga(III) would form a 3:1 complex via the six oxygen atoms of three 1.2-HOPO bidentate ligands (Fig. 5). Therefore, compound 23 would be a good model of the coordination environment around Ga(III) if ligand 19 coordinated Ga(III) in a O_6 mode (Fig. 4A) and by extension, would give us some information on the way Fe^{3+} is chelated by 19.

Proton and carbon NMR were first performed on solutions of **22** and **19** in D₂O. To these solutions, a stoichiometric amount of Ga(acac)₃ (acac = acetylacetonate) was then added and the solutions incubated at room temperature for 24 h. The ¹H and ¹³C NMR of the resulting complexes were then recorded. Also, these solutions, after dilution with methanol (non-deuterated), were analysed by MS.

Starting with MS, **22** + Ga(acac)₃ (3:1 molar ratio) gave major peaks that match (*m/z* values and isotopic distribution) the formula [(**22** - 3H)Ga + Na]⁺ at *m/z* 511.93 (expected 512.02), 512.93 (expected 513.02), 513.93 (expected 514.02), 515.00 (expected 515.02) and 516.00 (expected 516.02) (Fig. S1), confirming the displacement of the three acac ligands and strongly suggesting the formation of the 3:1 complex **23** as expected.

The MS of **19** + Ga(acac)₃ (1:1 molar proportion) gave significant pinnacles that coordinate (*m/z* esteems and isotopic circulation) the equation [(**19** - 3H)Ga + H]⁺ at *m/z* 565.13 (anticipated 565.13), 566.13 (anticipated 566.13), 567.13 (anticipated 567.13), 568.13 (anticipated 568.13) and 569.07 (anticipated 569.13) (Fig. S2). This outcome affirms that ligand **19** is fit for dislodging the three acac ligands and that development of a 1:1 complex happened and that these include the deprotonation of the three hydroxyl gatherings. This outcome can't permit us to separate between the two coordination modes referenced previously.

In NMR, we expected that coordination of the HOPO moiety to Ga(III) would bring about particular changes to the substance movements of key protons and additionally carbon molecules of the ligand and it was foreseen that these could be normal for the two coordination modes. The ¹H and ¹³C NMR spectra are given in Figs. S3 and S4 separately. The ¹H of ligand **22** in D₂O in the nonattendance or presence of 1/3 molar likeness Ga(acac)₃ showed that upon complexation, the three signs of the 1.2-HOPO bunch are moved downfield by close to 0.3 ppm. The ¹H NMR of **19** + Ga(acac)₃ additionally indicated a downfield move of the substance moves however by a bigger estimation of 0.6 ppm. These distinctions could propose an alternate coordination mode. The presence of just one bunch of somewhat expand signals for the 1.2-HOPO moiety on account of **19** + Ga(acac)₃ demonstrates that they are altogether to a great extent same and thusly all comparatively planning, as proposed by the mass range. The signs for the TACN platform are significantly more affected by the presence of the metal. Rather than all around characterized singlet in the ligand, different expansive signs are seen somewhere in the range of 3.1 and 4.1 ppm. Appearance of wide signals for In(III) edifices of NOKA has been seen at raised temperature (in opposition to the Ga(III)NOKA complex where the signs stayed also characterized multiplets even at 85 °C) [70]. This has been credited to fast trade between the Δ and Λ isomers. The wide signals saw in our complex recommend that this quick trade exist even at room temperature.

In the ¹³C range of **22** with and without Ga(acac)₃, the sign appointed to the carbon particle of the carbonyl is additionally moved, upfield this time by 3.3 ppm. Curiously, the most affected signs relate to the three C_{single} bondH gatherings. The ¹³C NMR of **19** + Ga(acac)₃ is definitely not the same as that of the parent ligand and that

of the similar to complex 23. Once more, zeroing in on the signs for the TACN's methylene gathering, a few signs are currently seen, affirming the misshaped idea of the framework. The signs related with the carbon molecules in the 1.2-HOPO bunches are likewise definitely moved upon complexation. For instance, one sign was found at 195.4 ppm relating to a quaternary carbon particle that didn't show up in the range of the free ligand. The contrasts between the ¹H and ¹³C NMR of 19 and 22 with and without Ga(III) recommend that the two ligands give an alternate coordination mode. Since 22 is relied upon to give an O6 coordination climate, we probably recommend that a N3O3 coordination mode exists for ligand 19 + Ga(III) and thusly likewise to 19 + Fe(III) however further work will be important to reach a firmer inference.

2.2. Biostatic action

We chose a little board of assorted microorganisms to survey the viability of these chelators; these included microbes from both Gram negative and Gram positive heredities just as the pathogenic dimorphic organism *Candida albicans*. A few animal varieties were chosen for their suggestion in medical services related contaminations, where potential strains recently utilized in the evaluation of iron chelators or drugs were utilized. *Bacillus subtilis* strain DSM-23,778 comes up short on a key phosphopantetheinyl transferase movement [74] needed for siderophore biosynthesis and creates the bidentate itoic corrosive instead of the hexadentate bacillibactin [75]. Stock microdilution tests were embraced dependent on a standard system [76]. The outcomes got are appeared in Table 1. The economically accessible N,N',N'',N''-diethylenetriaminepentaacetic corrosive (DTPA) was likewise examined for examination purposes.

Table 1. [Minimum Inhibitory Concentrations](#) (MIC) expressed in μM (in mg/L between brackets) for the ligands tested upon a panel of [microorganisms](#). Results were determined in triplicate. The [molar mass](#) (g/mol) of each chelator is given between brackets in the first column.

Minimum Inhibitory Concentration/ μM (mg/L)

Chelator (molar mass/g/ mol)	<i>E. coli</i> DS M-18039	<i>K. pneumoniae</i> DSM-30104	<i>P. aeruginosa</i> D SM-19880	<i>A. baumannii</i> D SM-30007	<i>B. subtilis</i> D SM-23778	<i>S. aureus</i> D SM-1104	<i>C. albicans</i> D SM-1386
DTPA (393.35)	2542 (1000)	2542 (1000)	> 2542 (> 1000)	2542 (1000)	159 (62.5)	79 (31.1)	318 (125.1)
1 (557.52)	625	313 (174.5)	313 (174.5)	313 (174.5)	313 (174.5)	5 (2.8)	313 (174.5)

Minimum Inhibitory Concentration/ μ M (mg/L)

Chelator (molar mass/g/ mol)	<i>E. coli</i> DS M- 18039	<i>K. pneumoniae</i> DSM-30104	<i>P. aeruginosa</i> D SM-19880	<i>A. baumannii</i> D SM-30007	<i>B. subtilis</i> D SM-23778	<i>S. aureus</i> D SM-1104	<i>C. albicans</i> D SM-1386
	(348.5)						
2 (669.70)	5000 (3348)	5000 (3348)	5000 (3348)	1250 (837.1)	313 (209.6)	625 (418.6)	78 (52.2)
3 (492.63)	78 (38.4)	313 (154.2)	625 (307.9)	156 (76.9)	78 (38.4)	78 (38.4)	39 (19.2)
7 (537.60)	5000 (2688)	> 5000 (> 2688)	5000 (2688)	5000 (2688)	5000 (2688)	2500 (1344)	313 (168.3)
10 (576.66)	5000 (2883)	5000 (2883)	2500 (1442)	313 (180.5)	1250 (720.8)	625 (360.4)	39 (22.5)
19 (492.63)	625 (307.9)	1250 (615.8)	625 (307.9)	313 (154.2)	313 (154.2)	313 (154.2)	39 (19.2)
20 (511.56)	5000 (2558)	> 5000 (> 2558)	5000 (2558)	1250 (639.5)	313 (160.1)	313 (160.1)	313 (160.1)

Across the scope of microorganisms tried, doubtlessly aggravates 1 and 3 are methodically the best inhibitors of microbial development (strengthening material, Table S). Compound 19 additionally has all the earmarks of being one of the better chelators yet it neglected to approach the previous two. Unexpectedly, exacerbates 2, 7 and 20 seem, by all accounts, to be the most exceedingly awful performing chelators of this arrangement. Strangely, compound 10 performs ineffectively against *E. coli* however very well against *C. albicans*. On the off chance that the viability of the chelators was basically connected to the thermodynamics of their Fe³⁺ + chelation (for example pFe³⁺), one could hope to notice a similar position requesting across the microorganism board, the interspecies varieties in MIC being demonstrative of the limit of every organism to manage the limited serious test characterized by the chelator portion. The way that 1, 3, 19 seem, by all accounts, to be the best inhibitors and 2, 7 and 20 the least fortunate however that the adequacy of 10 seems variable proposes that strength of metal chelation, if predominant, isn't the lone factor that impacts the deliberate MIC esteems. The way that pFe³⁺ + just thinks about iron chelation (while microscopic organisms depend on other organically applicable metals, for example Cu²⁺, Zn²⁺ that can likewise be chelated pretty much effectively by the additional chelators) and does excludes energy impacts (for

example metallation of the chelator, siderophores and potential transmetallation responses) may clarify these perceptions.

The chelators' positioning doesn't seem, by all accounts, to be distinctive between Gram-positive and Gram-negative microorganisms in the screened set. Notwithstanding, no doubt the MIC esteems got on the four tried Gram-negative microbes are higher than on the tried Gram-positive microorganisms. No doubt our strains of *S. aureus* and *C. albicans* were more touchy to the presence of a chelator than different microorganisms, up to two significant degrees for *S. aureus* (strengthening materials, Table S2). On the opposite the strains of *P. aeruginosa*, *K. pneumonia* and *E. coli* give off an impression of being the most safe of the multitude of life forms tried to the presence of the chelators. Both *E. coli*[77], [78] and *K. pneumoniae*[79], [80] produce enterobactin (4), the most remarkable known siderophore and thusly they are normal dependent on thermodynamic contemplations to have a serious level of capacity to bear added chelators. Nonetheless, apparently the best opposition is displayed by *P. aeruginosa* which creates the less strong chelators, pyoverdins and pyochelin [81] instead of enterobactin. These siderophores are not expected to thermodynamically contend well with chelators, for example, 3. Except if later on it is discovered that *P. aeruginosa* as recommended shows "still-neglected take-up abilities" [12], or secretes amazing (yet unidentified) siderophores, this outcome would propose that there are different variables impacting everything than basic thermodynamically contending chelation responses.

The more noteworthy vulnerability of Gram-positive microorganisms to added chelator than Gram-negative has just been noticed [42] and proposed to be because of the hindrance capacity of Gram-negative external layer introduced to atoms of this size (molar masses in Table 1). In any case, we contend here that the chelators' commitment to biostasis is likely extracellular, in chelating the metal in the development medium and thusly that the differential vulnerability of Gram-positive and Gram-negative microscopic organisms is because of different components. The helplessness of *B. subtilis* DSM-23,778 to these metal chelators is as opposed to the strength of two *Bacillus* species (*B. subtilis* and *B. thuringiensis*) to 3,4-HOPO-based hexadentate chelators [42]. Notwithstanding, these were wild separates that may have could create the hexadentate siderophore bacillibactin. It ought to be noticed that *B. subtilis* DSM-23,778 was picked here for its manufactured constraint to a bidentate siderophore [75] instead of its cell engineering, so the information introduced here ought not be considered in contentions encompassing adequacy and the ownership of an external layer. Besides, the size and arrangement of the microbial boards tried here and somewhere else [42] are restricted and such unthinking subtlety will require cautious plan of more broad tests than were suitable in this fundamental examination.

As the siderophores created by every one of these bacterial strains have not all been officially distinguished or potentially their Fe^{3+} restricting viability estimated, it isn't yet conceivable to decide with a significant level of certainty whether the microscopic organisms generally impervious to outer chelators are efficiently the ones that produce the most grounded siderophores (most elevated pFe^{3+}). Additionally, for each siderophore, its pFe^{3+} is

impacted by its absolute fixation and these can differ after some time and as a component of the capacity of the microorganism to discharge them in huge amount.

The helplessness of hexadentate chelators dependent on 1,2-HOPO depicted in the writing should be tended to if these are to be truly viewed as in remedial applications. Thought of the high adequacy of chelator 19, where the HOPO bunch is connected to the TACN center through a methylene gathering, and the helplessness of 7, where the linker is a carbonyl, recommends that advancement of the linker will be basic. Correlation of chelators 1, 7 and 19 recommends that the utilization of a more unbending macrocyclic platform contrasted with the tris(2-aminoethyl)amine center (to advance chelation through entropic impacts) was not fruitful in improving organic movement.

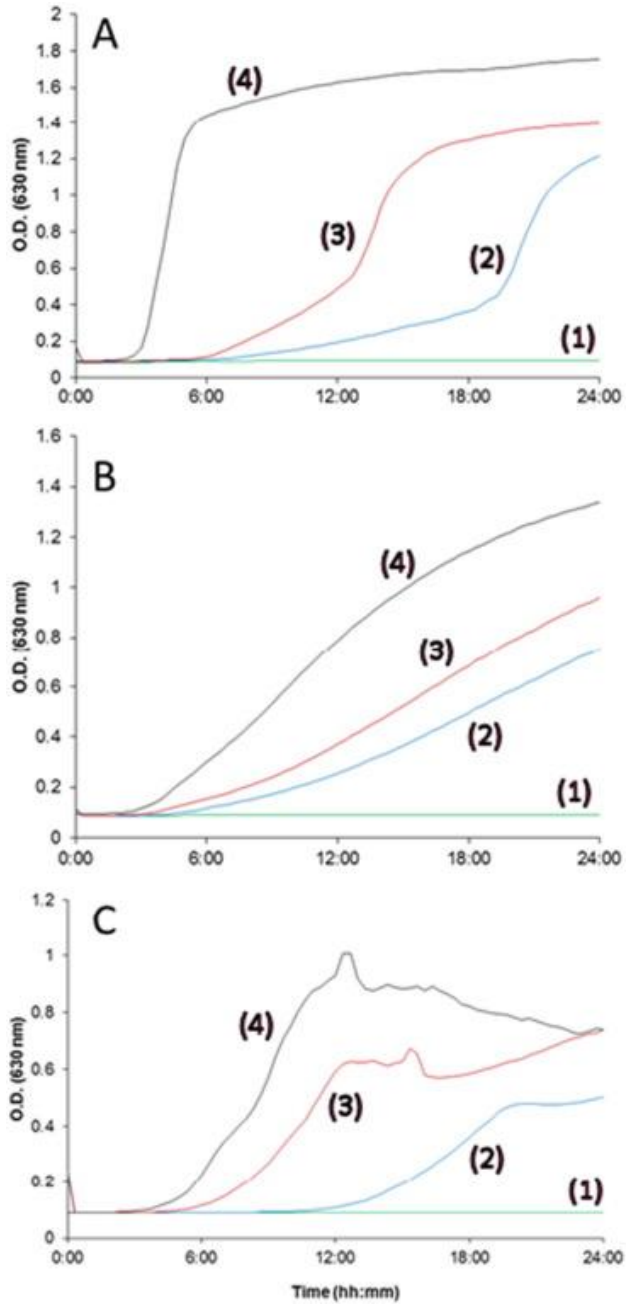
All the HOPO-based chelators tried in this were planned with the objective of upgrading Fe³⁺ chelation. This doesn't imply that catching of other fundamental metals isn't correspondingly accomplished that prompts the biostatic impact. The measure of key metals in the development medium was estimated and discovered to be 65.7 μM for iron, 31.5 μM for zinc, 13.7 μM for manganese, 8.3 μM for cobalt and 4.5 μM for copper. For all the microorganisms tried aside from *C. albicans*, the MIC is over the centralization of iron, as would be normal if our estimated instrument of activity is legitimate. In any case, the MIC saw on *C. albicans* of 39 μM for 3, 10 and 19 proposes either that halfway chelation of iron is sufficient to have a biostatic impact on that microorganism or that our chelator additionally targets different metals that have a significantly more sensational impact on its development or that it influences the life form in a way not identified with metal limit.

At last, the impact of added chelators on the accessibility of other organically significant metals should likewise be contemplated as examined previously. It is consequently rash to attempt to justify the information introduced thus dependent on relative pFe³⁺ values. Progress towards a comprehension of their method of activity anyway can be made by considering the siderophores that are known to be created by the tried living beings. Further work is in advancement to research top to bottom the instrument of activity of these chelators and to associate their movement to the thermodynamics and energy of their metal official.

True to form every microorganism without the additional chelator, shows a slack stage prior to entering outstanding development. Within the sight of the additional chelator, the slack stage shows up longer sometimes (for example *K. pneumoniae* + 3 at MIC/4) however isn't generously expanded in different cases tried, despite the fact that a reduction in development rate is apparent. In all the tried cases and by definition, the slack stage was more than 24 h when the chelator was utilized at the MIC esteem got from the past examines. What is evident for all tried blend is that the pace of development is fundamentally decreased with expanding convergences of chelators. These development profiles are reliable with the normal method of activity of the chelators. The microorganisms that experience the ill effects of the biostatic impact of a metal chelator wind up in iron hardship conditions because of the presence of the said chelator. They along these lines need to create a lot of siderophore to contend and just when the chelator's pFe³⁺ is overwhelmed by the siderophore's pFe³⁺ worth will any development be started. The higher

the measure of chelator, the more it takes the microorganisms to defeat the impact of the biostatic specialist. This is predictable with the theory the mixes work as an extracellular chelator.

The effect of sub-MIC fixations on the development of the microorganisms was trailed by optical thickness (Fig. 6).



3. Conclusion

We report the union of a scope of hexadentate chelators dependent on triaza macrocycles, including the principal report of the utilization of a methylene as a linker between a 1.2-HOPO organizing gathering and the atomic framework. The ligands have an obvious biostatic impact upon the development of a scope of microorganisms. It is proposed that metal chelation is the fundamental method of activity of these chelators yet that a straightforward thermodynamic rivalry between the chelator and bacterial siderophore for Fe^{3+} is too basic an image. Further work is in advancement to comprehend the method of activity of these chelators in detail and to orchestrate a more extensive scope of hexadentate chelators dependent on 1.2-HOPO to evaluate their viability as biostatic specialists. These mixes will likewise shape the reason for the investigation of the impact of the linker between the 1.2-HOPO moiety and the atomic spine on the thermodynamic adequacy of Fe^{3+} chelation through estimations of pK_a and β_{110} values.

4. Experimental

4.1. Arrangement of stock arrangements

China was flushed with a deionised fluid arrangement of EDTA (0.1 M) at that point washed altogether with deionised water (18 m Ω) before ligands were broken up in deionised water to the ideal focus (5 mM; 5 mL). The stock arrangement was then gone through a layer channel (0.22 μM) into a sterile bijoux tube (7 mL) and put away at 4 °C until required.

4.2. Bacterial strains

All strains were bought from DSMZ. *E. coli* DSM-18,039, *K. pneumoniae* DSM-30,104, *P. aeruginosa* DSM-19,880 and *S. aureus* DSM-1104 were refined onto cerebrum heart imbuement (BHI) agar and brooded at 37 °C for 24 h. Comparative techniques were directed for strains of *A. baumannii* DSM-30,007 and *C. albicans* DSM-1386 brooded at 30 °C and *B. subtilis* brooded at 25 °C, just for 48 h. The refined plates were then put away at 4 °C until required.

4.3. Antimicrobial test

The test led depended on a comparative writing methodology [76]. Stock arrangements of ligands (5000 μM) were added to the primary wells of a 96 well-microtitre plate (200 μL) and sterile BHI stock (100 μL) was added to the leftover wells in the line. Ligand arrangement from the primary well (100 μL) was added to the following great in

the column and blended. The method was then rehashed along the column from the weaken arrangements and disposed of after the penultimate well. Inoculum (105 CFU/mL; 100 µL) was then added to all wells and the plate brooded without unsettling at 37 °C. Readings were taken after for 24 and 48 h, contingent on the microorganism, and the MIC decided based on visual turbidity of the well. Measures were acted in three-fold.

4.4. Natural amalgamation

All solvents and reagents were bought from Sigma-Aldrich, Acros Organics or Alfa-Aeser and utilized minus any additional cleansing except if in any case determined. Responses were trailed by TLC utilizing silica gel with UV254 fluorescent pointer and section chromatography was led utilizing 0.060–0.20 mm silica gel (70–230 lattice), where computerized streak segment chromatography was directed utilizing a Biotage Isolera One ISO1SV. Hydrogenations performed utilizing a H-cube® persistent stream hydrogen generator was worked as determined.

4.5. Actual estimations

Dissolving focuses were taken on a SRS DigiMelt MPA161 computerized softening point mechanical assembly with tests arranged in SAMCO soft drink glass slender cylinders 100 mm. NMR spectra were recorded utilizing a Jeol JNM Ex270 instrument at 270 MHz and 68 MHz or a Jeol JNM-ECS400 instrument at 400 MHz and 100 MHz, as determined, for ¹H and ¹³C NMR separately, and are accounted for in ppm (δ). Infrared spectra were acquired utilizing Durascope jewel ATR framework on a Perkin Elmer RX1 FTIR spectrometer. Positive and negative electrospray ionization mass spectrometry (ESI-MS) was led utilizing a Thermo LCQ Advantage mass spectrometer by direct infusion. High goal mass spectrometry were gotten in a Finnigan MAT900XLT high-goal twofold focussing mass spectrometer utilizing nano-electrospray ionization (NESI) at the EPSRC National Mass Spectrometry Service (University of Swansea, Wales, UK). UV–Visible spectrophotometry was led utilizing a Varian Cary 50 UV–vis spectrophotometer (range 200–800 nm) utilizing a 1 cm quartz cell at room temperature (18–22 °C). Optical thickness readings were taken utilizing a Biotek HT Multi-mode Microplate peruser at the frequency indicated. Mixes 1[49], 2[48], 3[67] and 8[60] were incorporated as portrayed. TACN was orchestrated utilizing the Richman-Atkins strategy with slight adjustment [82], [83]. TACD was readied through alteration to the philosophy created by Alder et al. [84], [85].

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