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Thioarylation of 6-Amino-2,3,6-trideoxy-D-*manno*-oct-2-ulosonic Acid (IminoKdo): Access to 3,6-Disubstituted Picolinates and Mechanistic Insights

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In this work, we present a metal-free coupling protocol for the regio- and stereoselective C3-thioarylation of 6-amino-2,3,6-trideoxy-D-*manno*-oct-2-ulosonic acid (iminoKdo). The developed procedure enables the coupling of electron-rich, electron-deficient, and hindered arylthiols, providing a series of C3-modified iminoKdo derivatives in moderate to good yields. Elucidation of active species through controlled experimental studies and time-lapse ^{31}P NMR analysis provides insights into the reaction mechanism. We demonstrate that, following a tandem Staudinger/aza-Wittig reaction of an azido-containing keto ester, an inseparable equimolar mixture of imine/enamine is formed. The enamine then undergoes a Stork-like nucleophilic attack with the *in situ*-formed disulfide reagent, resulting in the formation of the coupling products. Additionally, we describe a rarely reported acid-promoted aromatization of the C3-thioarylated iminoKdo skeleton into 3,6-disubstituted picolinates, which are reminiscent of dichotomines.

Introduction

Iminosugars represent an attractive class of organic compounds that mimic carbohydrates or their hydrolysis transition states.[1] Unlike carbohydrates, iminosugars feature a basic nitrogen atom in their ring system instead of oxygen. Being polar, low molecular weight compounds, they share efficient uptake pathways with carbohydrates.[2] Importantly, their chemical and biological stability surpasses that of natural sugars, rendering them resistant to carbohydrate-processing enzymes.[3] Iminosugars serve as potent inhibitors of glycosidases and glycosyltransferases,[4] enzymes responsible for cleaving or forming glycosidic bonds in poly/oligosaccharides and glycoconjugates. Since the discovery of naturally occurring nojirimycin[5] from *Streptomyces roseochromogenes* and 1-deoxynojirimycin[6] (Figure 1A) from *Morus alba*, the field of iminosugars has experienced rapid growth. This expansion results from both the isolation and synthesis of new derivatives, aiming to enhance their selectivity and efficiency against carbohydrate-processing enzymes. These research endeavors have culminated in the clinical approval of three iminosugars, currently available in the drug market.[3]

3-Deoxy-D-*manno*-oct-2-ulosonic acid (Kdo, Figure 1A) plays a crucial role as an eight-carbon sugar in Gram-negative bacteria (GNB).[7] In its α-configuration, Kdo is a fundamental component of the inner core of the lipopolysaccharide (LPS) found in virtually all GNB.[8] The formation of lipid A-Kdo_2, where lipid A is connected to two Kdo units, is essential for maintaining the integrity of the GNB outer membrane and ensuring cell viability.[9] Additionally, Kdo residues, in their β-configuration, act as a "glycolinker", helping to anchor the capsular polysaccharides of various pathogenic GNB to the outer membrane.[10] Notably, β-Kdo units have been identified

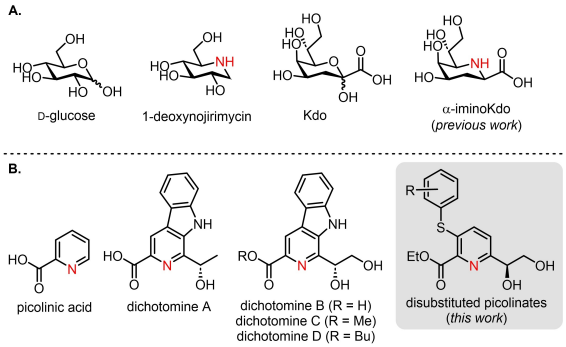

Figure 1. (**A**) Structures of D-glucose and Kdo with the corresponding iminosugars 1-deoxynojirimycin and iminoKdo, respectively. (**B**) Structures of picolinic acid, picolinate-containing natural products dichotomines A–D, and targeted 3,6-disubstituted picolinates.

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in the exopolysaccharide of *Burkholderia pseudomallei*, a potential bioterrorist threat causing melioidosis.[11] Despite the biological significance of Kdo, particularly in the context of GNB polysaccharides, the enzymes involved in Kdo processing, primarily glycosyltransferases, have been underexplored as drug targets.[12] Our hypothesis posits that inhibiting Kdo-processing enzymes could pave the way for an "antivirulence" strategy[13] by compromising the outer membrane integrity, rendering GNB more susceptible to immune system targeting and clearance. Taking a first step toward this goal, we recently reported the total synthesis of 6-amino-2,3,6-trideoxy-α-D-*manno*-oct-2-ulosonic acid (iminoKdo, Figure 1A)[14] as a possible inhibitor of Kdo-processing enzymes. IminoKdo, an amino acid mimic, also holds the potential to be incorporated into the GNB cytoplasm through active transport,[15] marking a promising avenue for further exploration.

The pyridine ring, which can be seen as the aromatized version of the six-membered iminosugars, also stands out as an appealing scaffold in drug discovery.[16] It remains the most frequently encountered nitrogen-containing aromatic ring among US FDA-approved pharmaceuticals.[17] Drugs containing pyridine rings showcase various medicinal properties, including anticancer, antimicrobial, anti-inflammatory, and analgesic activities.[18] Due to the medicinal significance of the pyridine scaffold, substantial efforts have been dedicated to developing methodologies for synthesizing functionalized pyridines. Such approaches encompass the preparation of pyridines through multicomponent synthesis,[19] cycloaddition reactions,[20] and cross-coupling chemistry.[21] However, limits in chiral resolution,[22] low reactivity,[23] and poor regioselectivity[24] still make the functionalization of the pyridine ring a strategic synthetic challenge.

In 2004, Yoshikawa and co-workers[25] reported the isolation of naturally occurring picolinate-containing dichotomines A–D (Figure 1B) from the roots of *Stellaria dichotoma* ("Yin Chai Hu"), a plant used in Chinese traditional medicine for treating fevers. Dichotomine C exhibits antiallergic effects associated with the inhibition of β-hexosaminidase release in RBL-2H3 cells. Following their discovery, the total syntheses of dichotomines A–D confirmed the structures of these β-carboline-type alkaloids.[26] Notably, the picolinate ring of dichotomines B–D features a 1,2-dihydroxyethyl chain at position C6, reminiscent of the iminoKdo scaffold.

Building upon our established approach for iminoKdo synthesis, we present here a simple, two-step iterative protocol to functionalize regio- and stereoselectively the iminoKdo scaffold with diverse thioaryl groups at the C3 position. An acid-promoted aromatization sequence to transform the iminoKdo scaffold into dichotomine-like 3,6-disubstituted picolinates (Figure 1B) was also developed. Elucidation of active species through experimental studies and ^{31}P NMR analysis provided crucial insights into the plausible reaction mechanism.

Results and Discussion

Synthesis of C3-Thioarylated IminoKdo Derivatives

In our previous work,[14] we successfully synthesized iminoKdo through a 15-step sequence starting from D-mannose (Figure 2A). A Horner-Wadsworth-Emmons (HWE)[27] two-carbon chain homologation of a protected C4 azido-containing mannose derivative initiated the process. Cyclization of keto ester **1** through a 6-*exo*-*trig* Pd-catalyzed reduction resulted in the formation of the α-iminoKdo scaffold. Alternatively, we achieved the same transformation through a tandem Staudinger/aza-Wittig (SaW)[28] reaction, followed by the stereoselective reduction of the resulting α-iminoester. In our current investigation (Figure 2B), we planned to leverage the SaW reaction to generate an imine intermediate capable of coupling with various nucleophiles, such as thioaryls. The resulting C3-functionalized iminoKdo derivatives, once obtained, could undergo aromatization, yielding chiral 3,6-disubstituted picolinates reminiscent of the southern section of natural dichotomines. It is noteworthy that the synthesis of thio-containing picolinates is scarce in the literature[29] and typically commences from commercially available picolinic acid derivatives. Notably, some of these thio-containing compounds have been shown to exhibit hypoglycemic activity by activating glucokinases.[29]

First, the SaW reaction underwent reexamination to gain further insights into the precise nature of the intermediates formed during the process (Figure 3A). Keto ester **1** was therefore submitted to SaW in the presence of PPh_3 in a THF/H_2O mixture at 85 °C for 24 h. TLC analysis revealed two distinct spots linked together by a drag (Figure S1). Despite our efforts, all attempts to separate these two compounds through silica

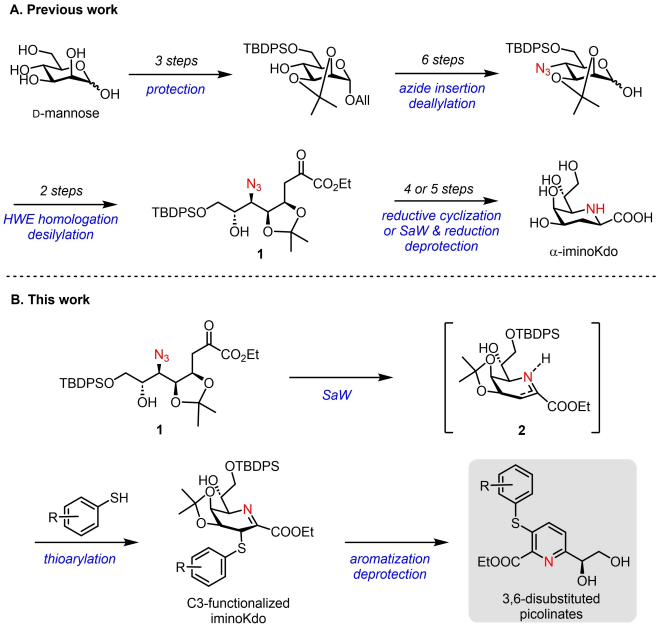


Figure 2. (**A**) Key steps in the total synthesis of α-iminoKdo (previous work).[14] (**B**) Synthesis of C3-thioarylated iminoKdo derivatives and subsequent aromatization toward 3,6-disubstituted picolinates (this work). HWE: Horner-Wadsworth-Emmons. SaW: tandem Staudinger/aza-Wittig reaction.

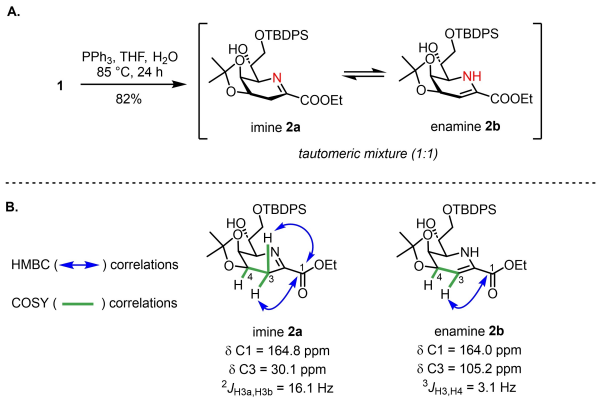
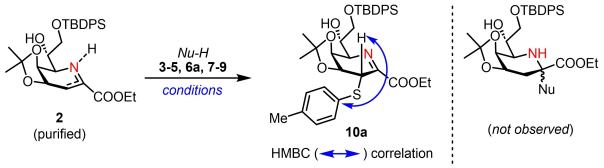


Figure 3. (**A**) Synthesis of a tautomeric mixture (1 : 1) of imine **2 a** and enamine **2 b** through tandem Staudinger/aza-Wittig reaction. (**B**) 1D and 2D NMR characterization of the compounds **2 a** and **2 b**.

gel chromatography proved unsuccessful as they were co-eluting. Extensive 1D and 2D NMR analysis with the purified compounds identified a tautomeric mixture (1 : 1) of imine **2 a** and enamine **2 b** (see Figure 3B and Tables S1 and S2). The two protons H3 (δ 1.78 and 3.36 ppm) exhibited cross-peak correlations with carbon C3 (δ 30.1 ppm) and carbon C1 (δ 164.8 ppm) in the HSQC and HMBC spectra, respectively, characteristic of imine **2 a**. Additionally, proton H3 (δ 5.56 ppm) displayed a distinctive cross-peak correlation with carbons C3 (δ 105.2 ppm) and C1 (δ 164.0 ppm) in the HSQC and HMBC spectra, respectively, providing evidence for the coexistence of enamine **2 b**. COSY correlations between protons H3 and H4, as depicted in Figure 3B, and HRMS data were also in agreement with the two proposed structures.

Our next task involved screening various nucleophiles to assess the reactivity of the purified mixture of imine **2 a** and enamine **2 b** (Table 1). We hypothesized two possible regioselectivities, either at the C2 or C3 position. Initially, a reaction with 2-adamantanol (**3**) in the presence of catalytic amounts of trimethylsilyl trifluoromethanesulfonate (TMSOTf) yielded no products under these conditions (entry 1). Attempts with the Grignard reagents phenyl- and ethylmagnesium bromide [PhMgBr (**4**) and EtMgBr (**5**)], with and without the presence of a promoter ($ZnCl_2$), also failed to yield a condensed product, likely due to decomposition under strongly basic conditions (entries 2 and 3). Subsequent testing with 4-methylbenzenethiol (HSTol, **6 a**) as a nucleophile in the presence of sodium methoxide (NaOMe) proved once again unsuccessful (entry 4). Inspired by a recent study involving the coupling of cysteine residues with 2*H*-azirines,[30] we switched the solvent from MeOH to an equimolar ratio of EtOH and PBS buffer (pH = 7.2). However, the tautomeric mixture was recovered (entry 5). Encouragingly, the addition of triphenylphosphine oxide ($O{=}PPh_3$), which is formed following the SaW reaction, resulted in the complete consumption of the starting material, forming a single spot according to TLC (entry 6). Isolation of this product and subsequent characterization by 1D and 2D NMR and HRMS confirmed the formation of compound **10 a** in 54% yield, featuring the thioaryl functionality at the C3 position, as

Table 1. Screening of reaction conditions.



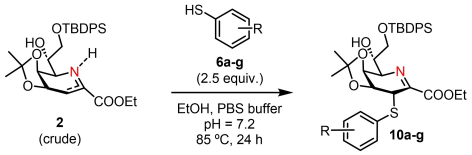
entry	nucleophile	promoter	solvent	yield[a](%)
1[b]	2-adamantanol (**3**)	TMSOTf	DCE	nd[c]
2[d]	PhMgBr (**4**)	–	THF	nd[c]
3[e]	EtMgBr (**5**)	$ZnCl_2$	THF	nd[c]
4[f]	HSTol (**6 a**)	NaOMe	MeOH	nd[c]
5	HSTol (**6 a**)	–	EtOH, PBS buffer	nd[g]
6[h]	HSTol (**6 a**)	$O{=}PPh_3$	EtOH, PBS buffer	54[i]
7[h]	TolSSTol (**7**)	$O{=}PPh_3$	EtOH, PBS buffer	62[i]
8[h]	HSePh (**8**)	$O{=}PPh_3$	EtOH, PBS buffer	nd[g]
9[h]	HOPh (**9**)	$O{=}PPh_3$	EtOH, PBS buffer	nd[g]

[a] Isolated yield. [b] 2-Adamantanol (2.0 equiv.), TMSOTf (10 mol%), 0–25 °C, 0.5 h. [c] Decomposition of starting material. [d] PhMgBr (2.0 equiv. 1 M in THF), 0–25 °C, 0.5 h. [e] EtMgBr (2.0 equiv.), $ZnCl_2$ (0.1 equiv.), 0–25 °C, 6 h. [f] HSTol (2.0 equiv.), NaOMe (2.2 equiv.). [g] Recovery of starting material. [h] $O{=}PPh_3$ (2.2 equiv.), PBS buffer (pH = 7.2), EtOH : PBS buffer (1 : 1), 85 °C, 24 h. [i] Isolated yield for compound **10 a**. nd: not determined.

evidenced by the HMBC cross-peak correlation between proton H3 and carbon C1′ on the aromatic ring. Repeating the reaction with the corresponding disulfide **7** provided the same compound **10 a** with a slightly improved yield of 62% (entry 7). Structural analogs of thiol **6 a**, *i.e.*, benzeneselenol (**8**) and phenol (**9**), were also screened but failed to yield any products (entries 8 and 9).

With our optimized reaction conditions in place, we then proceeded to screen a series of thiols featuring various electron-donating, electron-withdrawing, or hindered functionalities on the aromatic ring (Table 2). In this instance, we chose to conduct the reactions using the crude tautomeric mixture of imine **2 a** and enamine **2 b** obtained through the SaW of keto ester **1**, as the residual presence of $O{=}PPh_3$ could serve as an asset to achieve our target compounds. Gratifyingly, our hypothesis proved successful, resulting in an improved yield of 63% for compound **10 a** over two steps from keto ester **1** using the same thiol **6 a** (entry 1). Further screening of thiols without any substituents on the aromatic ring (**6 b**, entry 2) or with electron-donating substituents (**6 c** and **6 d**, entries 3 and 4) cleanly underwent the thioarylation reaction to furnish products **10 b**, **10 c**, and **10 d** in 53, 67, and 61% yield, respectively. Sterically hindered thiol **6 e** also furnished product **10 e** in 55% yield (entry 5). However, thiols with electron-withdrawing substituents **6 f** and **6 g** only yielded moderate amounts of

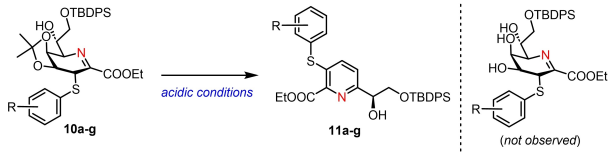
Table 2. Scope of the thioarylation reaction.



entry	arylthiol (R)	product	yield[a] (%)
1	**6a** (4-Me)	**10a**	63
2	**6b** (H)	**10b**	53
3	**6c** (4-*tert*-Bu)	**10c**	67
4	**6d** (4-OMe)	**10d**	61
5	**6e** (2,6-di-Me)	**10e**	55
6	**6f** (4-Cl)[b]	**10f**	46
7	**6g** (2-CF_3)[b]	**10g**	39

[a] Isolated yield over two steps from compound **1**. [b] 4.0 and 6.0 equiv. of arylthiol (**6f** and **6g**) were used, respectively.

Table 3. Acid-promoted aromatization of iminoKdo to picolinate scaffold.



entry	substrate, conditions	product, yield (%)
1	**10a**, AcOH (80% aq.), 80 °C, 16 h	**11a**, 0[a]
2	**10a**, TFA (170 equiv.), THF:H_2O (2:1), rt, 4 h	**11a**, 40[b]
3	**10a**, TFA (1.7 equiv.), DCM, rt, 48 h	**11a**, 72
4	**10a**, $BF_3 \cdot Et_2O$ (1.2 equiv.), CH_3CN, rt, 24 h	**11a**, nd[c]
5	**10a**, AcCl (2.0 equiv.), MeOH, rt, 1 h	**11a**, nd[c]
6	**10a**, *p*-TsOH (3.0 equiv.), MeOH, rt, 1 h	**11a**, nd[c]
7	**10a**, PPTS (1.7 equiv.), MeOH, rt, 4 h	**11a**, nd[c]
8	**10a**, TFA (7.5 equiv.), DCM, rt, 3 h	**11a**, 73
9	**10b**[d]	**11b**, 75
10	**10c**[d]	**11c**, 82
11	**10d**[d]	**11d**, 68
12	**10e**[d]	**11e**, 73
13	**10f**[d]	**11f**, 78
14	**10g**[d]	**11g**, 77

[a] Recovered starting material; [b] TBDPS group was cleaved; [c] nd: not detected. Decomposition of starting material; [d] TFA (7.5 equiv.), DCM, rt, 3 h. TFA: trifluoroacetic acid; *p*-TsOH: *p*-toluenesulfonic acid; PPTS: pyridinium *p*-toluenesulfonate.

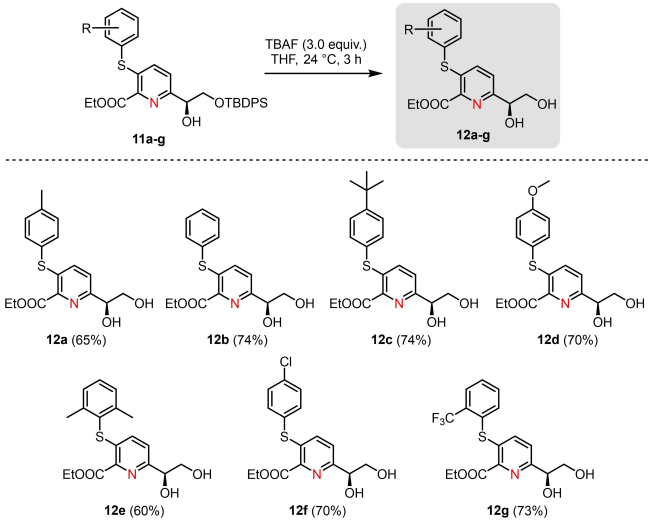
coupling products (46 and 39%, respectively), even when used in excess (entries 6 and 7).

Synthesis of 3,6-Disubstituted Picolinates

We next focused our attention on the cleavage of the isopropylidene protecting group to reveal the C4-C5 diol. We initiated this challenge by optimizing conditions using compound **10a**, screening various Lewis and Brönsted acids (Table 3). Initially, we treated compound **10a** with 80% aqueous acetic acid (AcOH) at 80 °C. However, the isopropylidene group remained intact even after 16 hours (entry 1). Subsequently, we turned to the stronger trifluoroacetic acid (TFA) in a THF/H_2O mixture. To our surprise, this acid-promoted transformation not only cleaved the isopropylidene and TBDPS groups but also induced the aromatization of the Kdo scaffold, providing a 3,6-disubstituted picolinate in 40% yield (entry 2). We explored milder acidic conditions, commonly used for acetonide deprotection, including $BF_3 \cdot OEt_2$, AcCl in MeOH, *p*-TsOH, and PPTS, in polar solvents (CH_3CN or MeOH), but all failed to catalyze the transformation (entries 4 to 7). Inspired by the outcome of entry 2, we conducted a reaction using 100-fold less TFA in an aprotic solvent (DCM). Although this slowed down the reaction rate, these conditions cleanly provided picolinate **11a** in an improved 72% yield while preserving the acid-labile TBDPS group (entry 3). A similar yield was achieved by increasing the number of TFA equivalents to 7.5, allowing the reaction to complete within three hours (entry 8). Applying these optimized conditions to the series of C3-functionalized iminoKdo derivatives **10b**–**10g** cleanly furnished picolinate derivatives **11b**–**11g** in excellent yields (entries 9 to 14). This unprecedented acid-driven transformation of the iminoKdo ring into the picolinate scaffold could tentatively be explained through a Ferrier-type rearrangement[31] upon protonation of the endocyclic nitrogen atom and subsequent aromatization (see Figure S2 for a plausible mechanism). It is worth noting that attempts to reduce the imine functionality prior to the isopropylidene cleavage were unsuccessful, as the starting material remained unreacted with various reducing agents, including $NaCNBH_3$, $NaBH_4$, $LiAlH_4$, and Pd-catalyzed hydrogenolysis (data not shown).

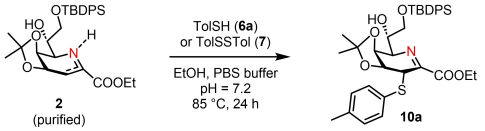
The final step of our synthetic journey involved the removal of the TBDPS group. Pleasingly, fluoride-assisted desilylation using TBAF (3.0 equiv.) in THF cleanly produced the 3,6-disubstituted picolinates **12a**–**12g** in good to excellent yields without any formation of by-products (Scheme 1). The final compounds underwent purification through silica gel normal phase flash chromatography and C_{18} reversed-phase chromatography. NMR (Figure S96–S137) and HPLC-UV traces (Figure S138–S144) indicated that the compounds were obtained in pure forms with estimated purities $>96\%$ (HPLC).

IminoKdo and the corresponding picolinates **12a**–**12g** were assessed for their inhibitory activities against various glycosidases and one tyrosinase. Unfortunately, the results showed only weak inhibitions at the maximum tested concentrations (see Table S4). Interestingly, compound **11f**, featuring a *para*-chloro substituent on the thiophenol ring, exhibited selective inhibition of mushroom tyrosinase with an IC_{50} of 724 μM.



Scheme 1. Desilylation of disubstituted picolinates.

Table 4. Control experiments for elucidating the active species.



entry	thioreagent[a]	additive[b]	yield[b] (%)
1	**6a**	–	0[c]
2	**6a**	PPh_3	64[d]
3	**7**	PPh_3	65[d]
4	**7** + PPh_3[e]	–	0[c]
5	**6a**	$O{=}PPh_3$	65[d]
6	**7**	$O{=}PPh_3$	66[d]

[a] 2.2 equiv. of thioreagent was used. [b] 2.2 equiv. of additives was used. [c] Not observed. [d] NMR yields were calculated using ^{1}H NMR and 1,1,2,2-tetrabromoethane as an internal standard. [e] Synthesized as indicated in Table S3, entry 4 (premix of thiol **7** and PPh_3).

Elucidation of Thioarylation Mechanism

The observed regioselectivity of the imine/enamine tautomeric mixture (**2**) toward thioaryl coupling was found to be distinctive compared to that reported for iminoglycals.[32] Typically, nucleophilic attacks usually proceed at the pseudo-anomeric position for iminoglycals, with concomitant double bond migration through Ferrier rearrangement[31] to yield 2,3-unsaturated compounds. In our case, thioarylation at the C3 position of the iminoKdo scaffold, while preserving the imine functionality, appears to rule out the possibility of a nucleophilic addition reaction.[33] To shed light on this specific reaction mechanism, we conducted additional experimental studies and NMR analyses, as discussed below.

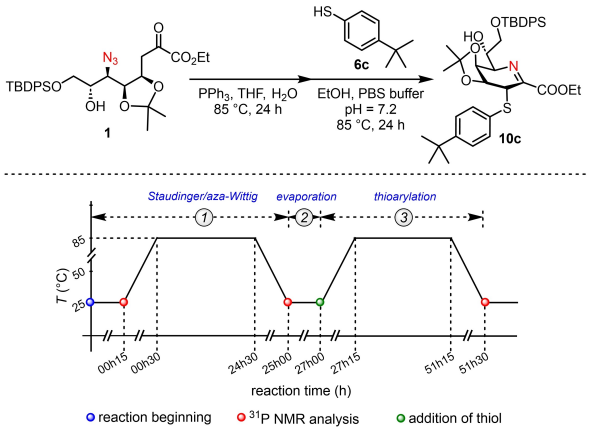
Given that the disulfide reagent **7** demonstrated similar potency to the corresponding thiol **6a** in enabling the thioarylation reaction (see Table 1, entry 7), we initially aimed to assess if it was formed throughout the reaction process (Table S3). Therefore, thiol **6a** was dissolved in an EtOH:PBS buffer mixture at 85 °C for 24 h. Under these standard conditions, disulfide **7** was formed in 77% yield, with no traces of unreacted thiol **6a** detected (entry S3), as confirmed by ^{1}H NMR analysis (Figure S3). Deviation from these reaction conditions, *i.e.*, without PBS buffer (entry S1) or without EtOH (entry S2), both resulted in a lower yield of disulfide **7** (5 and 52%, respectively) with the presence of unreacted thiol **6a**. The addition of PPh_3 under the standard conditions led to a complex mixture (entry S4). To eliminate the possibility of disulfide formation in the presence of air, the reaction was performed under oxygen-free conditions. In this case, the outcome was similar, resulting in the oxidation of thiol **6a** into disulfide **7** in 72% yield (entry S5). These control experimental studies revealed that, under the standard conditions, thiol **6** is transformed into disulfide **7**.

Next, the imine/enamine mixture (**2**) was screened to elucidate the probable reactive species (Table 4). Under the standard reaction conditions, where the purified tautomeric mixture **2** and thiol **6a** were allowed to react in the presence of PPh_3, the coupling product **10a** was obtained in a 64% yield (entry 2). Switching thiol **6a** to disulfide **7** provided similar results (65%, entry 3). However, in the absence of PPh_3, no traces of product **10a** were formed (entry 1). Further screening with the complex mixture (as shown in Table S3, entry S4) did not afford any product (entry 4). The addition of $O{=}PPh_3$ instead of PPh_3, either in the presence of thiol **6a** (entry 5) or disulfide **7** (entry 6), also gave conclusive results with the formation of target compound **10a** in similar yields. Taken together, these experiments have revealed the crucial importance of the presence of either PPh_3 or $O{=}PPh_3$ for the thioarylation reaction to proceed.

To gain further insights into the nature of the active phosphine species enabling thioarylation, we monitored the reaction by ^{31}P NMR spectroscopy over a time scale of 51 hours (Table 5 and Figure S4). ^{31}P NMR analysis of the first aliquot, conducted 15 minutes after the initiation of the reaction at 25 °C, revealed the presence of PPh_3 (δ −5.32 ppm),[34] which indicated that the SaW reaction had not started yet (entry 1). The reaction was maintained at 85 °C for 24 hours and cooled down to room temperature thereafter. At this stage, a second aliquot was taken, exclusively showing the presence of $O{=}PPh_3$ (δ 29.0 ppm, entry 2).[35] The reaction mixture was then evaporated under reduced pressure and placed over high vacuum for one hour. The resulting crude mixture was then treated with thiol **6c** under the standard conditions for 24 hours at 85 °C. The third aliquot, taken after cooling down the reaction to room temperature, again revealed the presence of $O{=}PPh_3$ (δ 29.0 ppm, entry 3). This time-lapse ^{31}P NMR study revealed that $O{=}PPh_3$, and not PPh_3, is present in the reaction mixture once the SaW reaction is completed.

Taken together, these experimental data provide a framework for postulating a plausible mechanism for the thioarylation reaction. As depicted in Figure 4, following the tandem SaW reaction, keto ester **1** undergoes a clean conversion into

Table 5. ^{31}P NMR analysis of the C(sp^3)–S(sp^3) bond formation.



entry	condition	^{31}P NMR (time)	^{31}P NMR (ppm)
1	**1**, PPh_3	00 h15	−5.32
2	**1**, PPh_3	25 h00	29.0
3[a]	**1** + **6c**	51 h30	29.0

[a] After 24 h at 85 °C, the reaction mixture was evaporated under reduced pressure and successively treated with thiol **6c** and the reaction was monitored for the next 24 h. Chemical shifts (δ) are in ppm and referenced to 85 % aq. H_3PO_4 (used as an external standard).

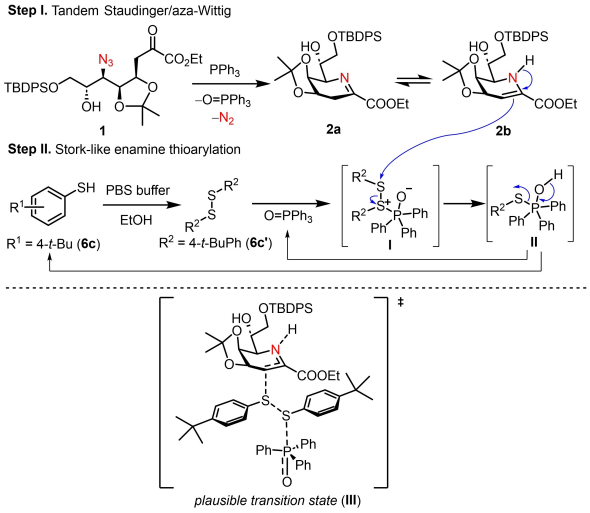


Figure 4. Plausible reaction mechanism for the thioarylation.

the imine/enamine mixture **2a**/**2b** together with the formation of O=PPh_3. Concurrently, thiol **6c** in the presence of the EtOH: PBS buffer mixture is converted into disulfide **6c**′. Subsequently, the coordination of disulfide **6c**′ toward the electrophilic phosphorus of O=PPh_3 generates a pentacoordinate species (**I**). Although attempts to isolate this species were unsuccessful, the formation of similar species is well-documented in the literature.[36] Following this, tautomer **2b** engages a Stork enamine-type attack[37] at the electrophilic sulfur atom of the partially weakened disulfide bond to yield the thioarylation product **10c**. It is noteworthy that imine **2a** acts as a reservoir for enamine **2b** and is completely consumed during the reaction process. After the coupling reaction, the unstable intermediate **II** collapses to form O=PPh_3 and disulfide **6c**′ through the intermediacy of thiol **6c**. This step is supported by the recovery of disulfide **6c**′ during purification of target compound **10c**. We postulate the formation of a single transition state (**III**), thereby providing a rationale for the observed stereoselectivity at the newly formed stereocenter at position C3. Finally, to eliminate the possibility of a radical mechanism, we conducted a radical trapping experiment using TEMPO (Scheme S1). As anticipated, no trapping products were formed supporting the heterolytic mechanism.

Conclusions

In this study, we have described an unprecedented regio- and stereoselective C3-thioarylation reaction at the iminoKdo scaffold. Leveraging an acid-driven deprotection/aromatization sequence, the synthesized derivatives were transformed into novel 3,6-disubstituted chiral picolinates, reminiscent of the southern part of naturally occurring dichotomines. The mechanism of the thioarylation was elucidated through control experiments and NMR analyses. We revealed that, following the tandem SaW reaction of azido-containing keto ester **1**, an equimolar mixture of imine/enamine (**2a** and **2b)** is formed. Enamine **2a** then undergoes a Stork-like nucleophilic attack at the disulfide species to provide the coupling products. The picolinate derivatives and the iminoKdo, obtained in pure forms, were evaluated for their inhibition potency against a panel of enzymes, and compound **11f** exhibited selective inhibition of a mushroom tyrosinase. Ongoing work in our laboratory aims to assess the inhibitory activity of these compounds against various Kdo-processing enzymes.

Supporting Information

Synthetic procedures, experimental data, additional figures, schemes and tables, and NMR spectra for all new compounds. This material is available free of charge via the Internet.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Conflict of Interests

The authors declare no competing financial interests.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: iminosugars · 3-deoxy-D-*manno*-oct-2-ulosonic acid · thioarylation · Staudinger/aza-Wittig · picolinates

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