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Next-Generation Total Synthesis of Vancomycin

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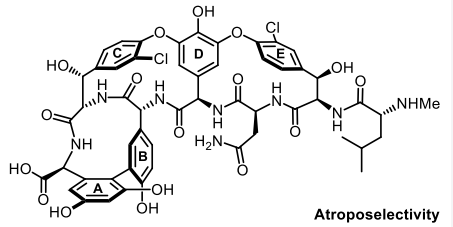
ABSTRACT: A next-generation total synthesis of vancomycin aglycon is detailed that was achieved in 17 steps (longest linear sequence, LLS) from the constituent amino acid subunits with kinetically controlled diastereoselective introduction of all three elements of atropisomerism. In addition to new syntheses of three of the seven amino acid subunits, highlights of the approach include a ligand-controlled atroposelective one-pot Miyaura borylation−Suzuki coupling sequence for introduction of the AB biaryl axis of chirality (>20:1 dr), an essentially instantaneous and scalable macrolactamization of the AB ring system nearly free of competitive epimerization (>30:1 dr), and two room-temperature atroposelective intramolecular S_NAr cyclizations for sequential CD (8:1 dr) and DE ring closures (14:1 dr) that benefit from both preorganization by the preformed AB ring system and subtle substituent effects. Combined with a protecting group free two-step enzymatic glycosylation of vancomycin aglycon, this provides a 19-step total synthesis of vancomycin. The approach paves the way for large-scale synthetic preparation of pocket-modified vancomycin analogues that directly address the underlying mechanism of resistance to vancomycin.



Total Syntheses	LLS	Overall Yield	Atroposelectivity AB	CD	DE
Evans (1998)	32	0.4%	<1:20	5:1	5:1
Nicolaou (1998)	24	0.05%	2:1	1:1	1:3
Boger (1999)	25	0.2%	1:1	1:1	8:1
This Work (2020)	17	5%	>20:1	8:1	14:1

INTRODUCTION

Vancomycin (**1**), first disclosed[1] and introduced into the clinic over 60 years ago, remains one of the clinically most effective and important antibiotics for treatment of life-threatening Gram-positive bacterial infections,[2] including methicillin-resistant *S. aureus* (MRSA). Its complex structure,[3] the three strained macrocyclic ring systems interwoven into the highly functionalized and rigid tricyclic heptapeptide core, the unusual centers of axial or planar chirality (atropisomers), and its glycosylation present formidable synthetic challenges (Figure 1). Three total syntheses of vancomycin aglycon[4−6] and two total syntheses of vancomycin[7,8] have been disclosed to date. Related efforts have detailed total syntheses of orienticin C,[9] teicoplanin,[10,11] and ristocetin A[12] aglycons, and a near endless number of methodology studies have been conducted that address synthetic challenges posed by their structures. The comprehensive reviews of Perkins,[13] Williams,[14,15] Nicolaou,[16] Courvalin,[17,18] Walsh,[19,20] Kahne,[2] ourselves,[21,22] and others[23−25] provide summaries of the rich history on the isolation, structure elucidation, biosynthesis, semisynthetic and synthetic studies, mechanism of action, and mechanisms of resistance of the glycopeptide antibiotics.

The only clinically significant resistance to vancomycin even after >60 years of use first emerged in enterococci (VanA and VanB VRE, 1987)[26] and more recently in *S. aureus* (VRSA, 2002).[18] It was co-opted from nonpathogenic source organisms that use an intricate mechanism to protect themselves while producing vancomycin.[27] It is induced upon detection[28−31] of a glycopeptide challenge that initiates an orchestrated response, resulting in late-stage remodeling of the N-terminus of peptidoglycan precursors from D-Ala-D-Ala to D-Ala-D-Lac.[32] This single-atom exchange in the cell wall precursors reduces vancomycin binding (1000-fold) and its derived antimicrobial activity (1000-fold).[33]

An extension of our efforts on the total synthesis of the glycopeptide antibiotics has targeted vancomycin analogues, containing deep-seated compensatory single-atom exchanges in the binding pocket,[34] that exhibit dual D-Ala-D-Ala/D-Ala-D-Lac binding and antimicrobial activity against both vancomycin-sensitive and -resistant organisms (**2**[35] and **3**[36]). These efforts, along with peripheral modifications that introduce additional independent mechanisms of action,[37,38] have provided extraordinarily potent analogues that display especially durable antimicrobial activity (Figure 1). We have shown that incorporation of two simple peripheral modifications (CBP and C1) independently (e.g., **4**−**6**) or simultaneously (**8**) into the more accessible of our pocket-modified analogues at the time, [ψ[CH_2NH]Tpg^4]vancomycin (**2**), provided potent antimicrobial agents with up to three synergistic mechanisms of action, each of which is individually effective against both vancomycin-resistant and vancomycin-sensitive bacteria and two of which are independent of D-Ala-D-Ala-D-Lac binding.[37] The exceptional potency (MIC 0.01−0.005 μg/mL, VRE) and

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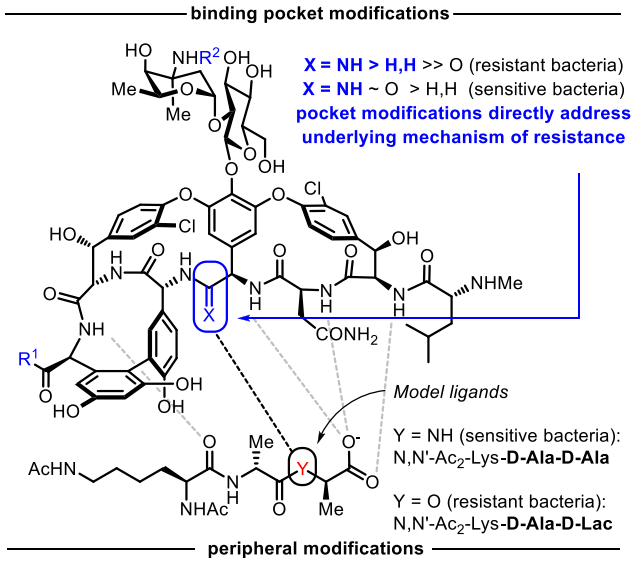

antimicrobial activity

	R¹	R²	X	VanA VRE (MIC, μg/mL)[a] *E. faecalis*[b]	*E. faecium*[c]
1,	OH	H	O	250	250
2,	OH	H	H,H	31	31
3,	OH	H	NH	0.5	0.5
4,	OH	CBP	O	2.5	2.5
5,	OH	CBP	H,H	0.13	0.06
6,	OH	CBP	NH	0.005	0.005
7,	C1	CBP	O	0.25	0.5
8,	C1	CBP	H,H	0.01	0.005
9,	C1	CBP	NH	tbd	tbd

Figure 1. (Top) Structure of vancomycin and key analogues with H-bonding interactions between vancomycin and model ligands indicated by dashed lines. (Middle) Added peripheral modifications that synergistically improve activity by inducing membrane permeabilization (C1) or directly inhibiting transglycosylase (CBP), both independent of D-Ala-D-Ala-D-Lac binding. (Bottom) VanA VRE antimicrobial activity.

remarkable durability of the prototypical members, CBP-[ψ[C(=NH)NH]Tpg⁴]vancomycin (**6**) and C1,CBP-[ψ-[CH₂NH]Tpg⁴]vancomycin (**8**), has inspired our continued examination of new analogues, including **9**, and their expanded preclinical examination.

In order to facilitate these studies, we have now developed a scalable total synthesis that could provide sufficient amounts of each analogue within the confines of an academic lab for initial preclinical assessment of in vivo efficacy and safety. In contrast to what many might think would be the most challenging feature, we earlier reported a scalable enzymatic conversion of the aglycon to the fully decorated natural product[8] (2 steps) or its pocket-modified analogues[39] that is conducted without protecting groups. However, the total synthesis of vancomycin aglycon (**10**) and its residue 4 modified analogues required redesign to overcome practical issues, including a long step count (25), low overall yield (0.2% without atropisomer recycling), lengthy syntheses of several of the unnatural amino acid subunits, and lack of kinetic atroposelectivity in the construction of the AB and CD ring systems. While the latter issue was originally addressed in our efforts by a defined order of macrocyclizations that permitted thermal equilibration[40] and recycling of the unnatural atropisomers, it required two atropisomer separations that limit material throughput. Therefore, we embarked on and herein detail a streamlined 17-step total synthesis of vancomycin aglycon (**10**) that achieves high kinetic diastereoselectivity for formation of each macrocyclic ring and improves the overall yield of **10** more than 20-fold.

RESULTS AND DISCUSSION

Improved Synthesis of the A, C, and D Ring Amino Acid Subunits. Our synthesis of vancomycin aglycon begins with seven unnatural amino acid subunits (**11**−**17**), four of which are either commercially available or readily prepared. The remaining three were each prepared in a modest overall yield (ca. 20%) in our previous efforts (Figure 2). The A ring[6]

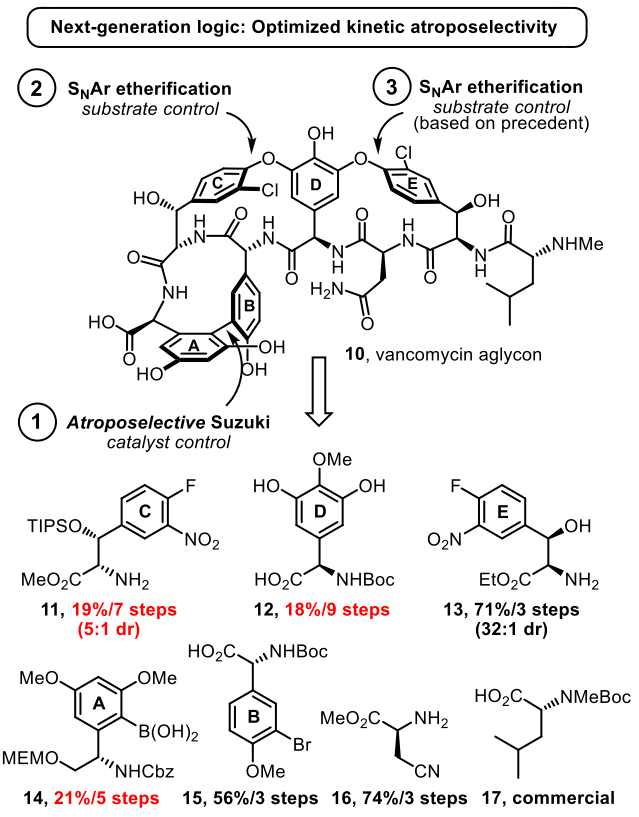

Figure 2. Key retrosynthetic disconnections and initial starting subunits.

(**14**) and D ring[41] (**12**) subunits were accessed in 5 and 9 total steps, respectively, with the requisite absolute stereochemistry introduced by Sharpless asymmetric aminohydroxylations,[42,43] while the C ring subunit[12] (**11**) was prepared by a modestly diastereoselective (5:1 syn:anti) Schöllkopf aldol reaction[12,44] with full control of the α-amino acid stereochemistry. Substantially improved syntheses of these three subunits were developed during the course of this work. With these improvements, five of the subunits are now derived from inexpensive chiral pool starting materials and only two require

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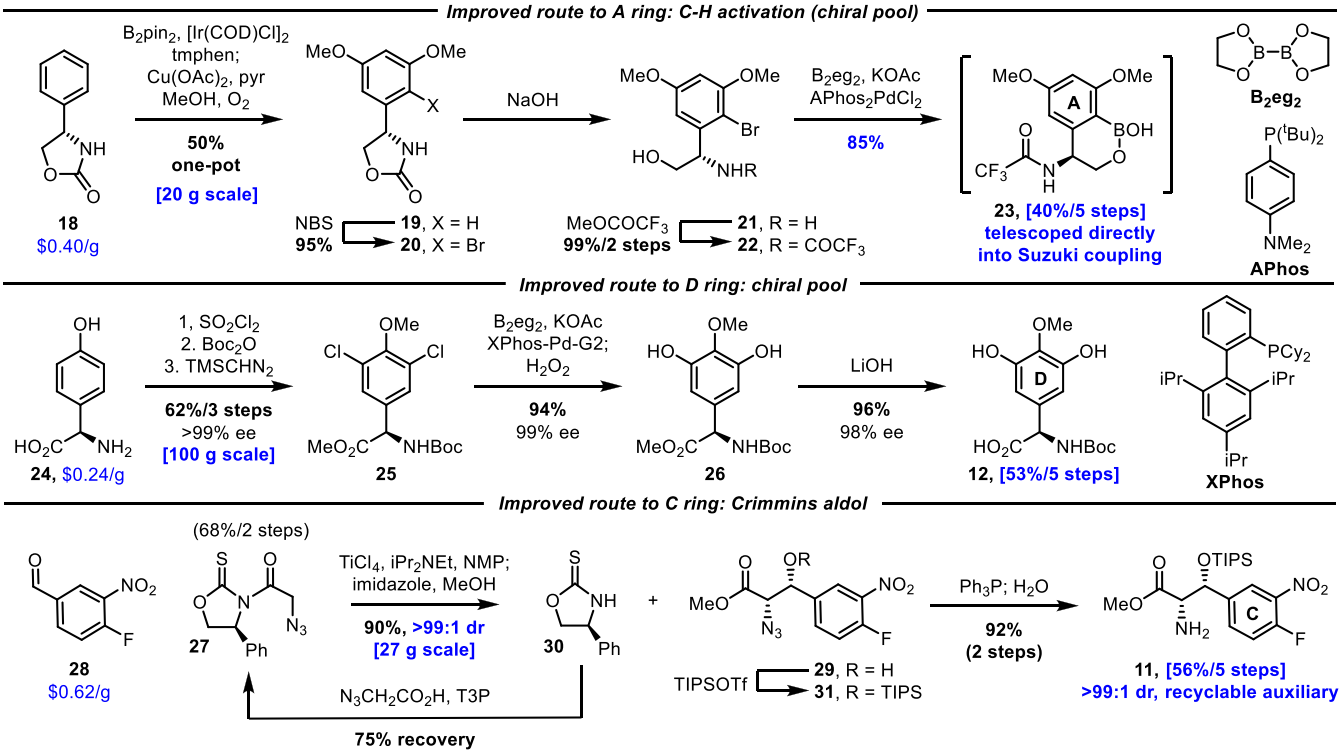
Scheme 1. Improved Synthesis of Starting Subunits



asymmetric synthesis. All but one of the subunits are now available in >50% overall yield.

An asymmetric aminohydroxylation (AA) reaction reported by Sharpless[42] was used to prepare the A ring amino acid in our prior efforts,[6] proceeded in 68% yield (90% ee) on 2 mmol (328 mg) scale, and provided regioisomeric products (3:1 rr). However, the yield of this reaction diminished on larger scales (>10 g, 33−41%) in our hands, and the product was invariably contaminated with an additional inseparable ring-chlorinated byproduct. An alternative, chiral pool route with complete control of the absolute stereochemistry was developed based on C−H activation and meta functionalization of the commercially available inexpensive ($0.40/g) phenylglycine-derived Evans auxiliary **18** (Scheme 1). A one-pot sequential Ir-mediated bis-C−H borylation[45]/Chan−Lam methoxylation[46] furnished **19** (50% overall) in a reaction that was easily scaled to >20 g. Bromination of **19** followed by recrystallization provided **20** (95%). To date, this three-step sequence has provided more than 300 g of intermediate **20**. Subsequent oxazolidinone hydrolysis and *N*-trifluoroacetamide protection of **21** provided **22** quantitatively. The lithium−halogen exchange/$B(OMe)_3$-trapping procedure previously used[6] to prepare the boronic acid **14** (59% yield) was superseded by a modified Miyaura borylation, providing high yields (85%) of cyclic boronate **23**. In addition and importantly, the crude reaction mixture containing boroxane **23** could be telescoped directly into a subsequent atroposelective Suzuki coupling (see below). Hence, the new route to the A ring subunit provides the cyclic boronate **23** in 40% yield (5 steps), a 2-fold improvement over the asymmetric aminohydroxylation route (21%) with complete (chiral pool) control of the absolute stereochemistry. Further, its implementation provides direct access to **23**, which is functionalized (trifluoroacetamide vs NHCbz) for more effective use in the final streamlined total synthesis detailed herein. The successful implementation of this route hinged on the use of 3,4,7,8-tetramethyl-1,10-phenanthroline (tmphen) as the ligand in the C−H borylation,[47,48] the inclusion of pyridine in the Chan−Lam methoxylation (Supporting Information Figure S1),[49] and the development of an efficient Miyaura borylation of the hindered aryl bromide **22** with bis(ethyleneglycolato)diboron (B_2eg_2), whereas typical Miyaura borylation conditions [B_2pin_2/$PdCl_2$(dppf)][50] delivered only trace product.

The D ring phenylglycine **12** was previously the most cumbersome subunit to prepare on scale, requiring 9 steps and relying on an asymmetric aminohydroxylation[42,43] (AA) to install the needed chiral center (69%, 96% ee, 7:1 regioselectivity).[41] We developed a new chiral pool 5-step route to the D ring subunit starting from inexpensive ($0.24/g) D-4-hydroxyphenylglycine (**24**), which was first converted to dichlorinated methyl ester **25** in 62% yield (59% for 3 steps on 100 g scale, Scheme 1) by modification of a reported procedure.[51] Although **25** proved sensitive to racemization (Et_3N, 23 °C), net hydroxylation of both chlorides in **25** could be accomplished in a one-pot Miyaura borylation/oxidation sequence (94%) with negligible loss of optical purity (99% ee for **26**). Saponification (LiOH, 96%, 98% ee) provided the required 3,5-dihydroxy-4-methoxyphenylglycine **12** now available in a much shorter (5 vs 9 steps) and easily scalable sequence with a 3-fold improvement in yield (56% yield for 5 steps) over our previous route (18% for 9 steps) and with complete control (chiral pool) of the absolute stereochemistry. Notably, the use of dichloride **25** as a substrate for the key borylation−oxidation reaction was essential, whereas use of the corresponding dibromide or diiodide substrates resulted in considerable ring halogenation upon exposure to H_2O_2.

Preparation of the C ring subunit **11** previously was achieved by zirconium-mediated addition of a Schöllkopf reagent[44]

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(commercially available for >$100/g, or in 4 steps[52] from D-valine), proceeding in 5:1 dr (syn:anti) and 50% yield (syn isomer). In order to avoid the needed separation of the alcohol diastereomers and lengthy preparation or costly reagent purchase, we turned to a Ti-mediated aldol reaction of the known oxazolidinethione **27**,[53] which proceeds with near perfect diastereoselection and enlists a more readily prepared (2 steps, 68%) and recyclable chiral auxiliary (**27**) (Scheme 1). Addition of **27** to 4-fluoro-3-nitrobenzaldehyde (**28**) under conditions designed and disclosed by Crimmins[54] to provide the syn aldol product, followed by in situ methanolysis, cleanly provided methyl ester **29** (90%, >99:1 dr, 27 g scale). In addition, the recovered auxiliary **30** was recycled in a single step to regenerate **27**, *without* prior separation of **29** from **30**. Subsequent TIPS protection of alcohol **29** and Staudinger reduction of **31** provided **11** in high yield (92% for 2 steps, >99% ee, 56% overall for 5 steps) virtually free of the anti-diastereomer (>99% de). To date, >320 g of **11** has been prepared by this route.

Formal Total Synthesis of Vancomycin Aglycon: Kinetically Controlled Diastereoselective Introduction of All Three Elements of Atropisomerism. Concurrent with the above efforts, we undertook studies on the atroposelective construction of the AB biaryl axis of chirality as well as diastereoselective formation of the CD and DE macrocyclic diaryl ethers. Efficient elements of the synthesis of the vancomycin core structure developed in our prior efforts were maintained, enlisting a macrolactamization[6] for closure of the 12-membered biaryl AB ring system and two aromatic nucleophilic substitution reactions for macrocyclization of the 16-membered diaryl ethers in the CD[42]/DE[6] ring systems but conducted in an altered order. The availability now of robust methods for atroposelective Suzuki biaryl coupling reactions suggested that we set the AB biaryl stereochemistry first. Then following macrolactamization and using the preformed AB macrocycle as an element of preorganization, we hoped to achieve high kinetic atroposelectivity in the closure of the CD ring system under substrate control, although precedent suggested this might be improbable.[9,55] If successful and based on our previous work,[6] we could anticipate that the subsequent DE ring closure would proceed with excellent substrate-controlled atroposelectivity (see Figure 2).

Although not incorporated into their total synthesis of vancomycin, Nicolaou and co-workers later reported[56] an atroposelective Suzuki biaryl coupling conducted on model substrates similar to our own that was mediated by (*R*)-BINAP and $Pd(OAc)_2$ (3:1 ligand:Pd). We suspected that the active catalyst was actually the palladium-ligated bisphosphine mono-oxide of (*R*)-BINAP [(*R*)-BINAP(O)−Pd^0] based on the frequently underappreciated role of such ligand complexes[57,58] that are most often unknowingly generated in situ. Consistent with this proposal, the coupling of **32** with **14** that employed a 2:1 combination of (*R*)-BINAP(O):Pd_2dba_3[59] (1:1 ligand:Pd) afforded biaryl **33** with a consistently high yield and atroposelectivity (>20:1 dr, up to 89% yield on 8 g scale) (Figure 3). In contrast, the combination of (*R*)-BINAP and Pd_2dba_3 was ineffective, confirming that mono-oxidation of the BINAP ligand was essential to catalytic activity. While the (*R*)-BINAP(O):Pd_2dba_3 system provided more than sufficient material for initial studies, the use of a noncommercial ligand as well as the variable quality of commercial Pd_2dba_3 (Pd-black contamination)[60] introduced practical challenges that we hoped to circumvent by direct in situ generation of the

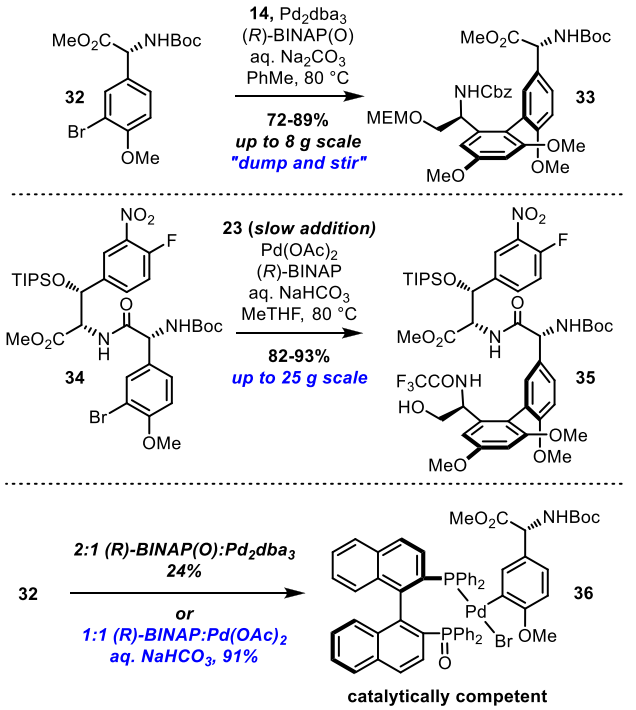

Figure 3. Key observations in the development of a catalyst-controlled diastereoselective synthesis of AB axis of chirality.

catalyst [(*R*)-BINAP(O)-Pd^0] from 1:1 (*R*)-BINAP/$Pd(OAc)_2$. Our initial screen of available chiral ligands for the coupling of **32** with **14** revealed that the (*R*)-BINAP/$Pd(OAc)_2$ combination was among the most effective of those examined (Supporting Information Figure S2). However, these initially favorable results from the Pd^{II}/BINAP system proved difficult to maintain as we scaled the reaction, which we found was due to precipitation of the active (*R*)-BINAP(O)−Pd^0 catalyst as a cherry-red solid prior to substrate addition. Fortunately, highly reproducible results were obtained by first heating a mixture of $Pd(OAc)_2$, (*R*)-BINAP, and aryl bromide in the presence of aqueous $NaHCO_3$, thereby trapping the active catalyst as the stable and soluble oxidative addition complex, followed by slow addition of the boronic acid. The superiority of this latter procedure was confirmed in subsequent studies of the coupling of **34** with **23** that consistently provided high yields of **35** (82−93%) on scales up to 25 g. Finally, the oxidative addition complex **36** was isolated in excellent yield (91%) from the reaction of (*R*)-BINAP, $Pd(OAc)_2$, and **32** in the absence of boronic acid, and **36** was demonstrated to be catalytically competent in the coupling of **32** with **14** (TON = 60, Supporting Information Figure S4). In comparison, the oxidative addition to **32** employing Pd_2dba_3 and (*R*)-BINAP(O) furnished **36** in a more modest yield (24%), although it is worth highlighting the simplicity and effectiveness of the "dump-and-stir" Pd_2dba_3/BINAP(O) method, which may be preferable for small-scale experiments.

Biaryl **33** was converted to the macrolactamization precursor **42** as shown in Figure 4. The A ring Cbz protecting group was exchanged for Alloc (H_2, Pd/C; AllocCl, 83% for 2 steps) to allow eventual amine deprotection under conditions that avoid reduction of the C ring nitro group. Subsequent saponification (LiOH, 99%), coupling of the carboxylic acid with **11** (DMTMM, 91%), Alloc deprotection ($PhSiH_3$, $Pd(PPh_3)_4$, 91%), and methyl ester saponification (LiOH, 99%) provided

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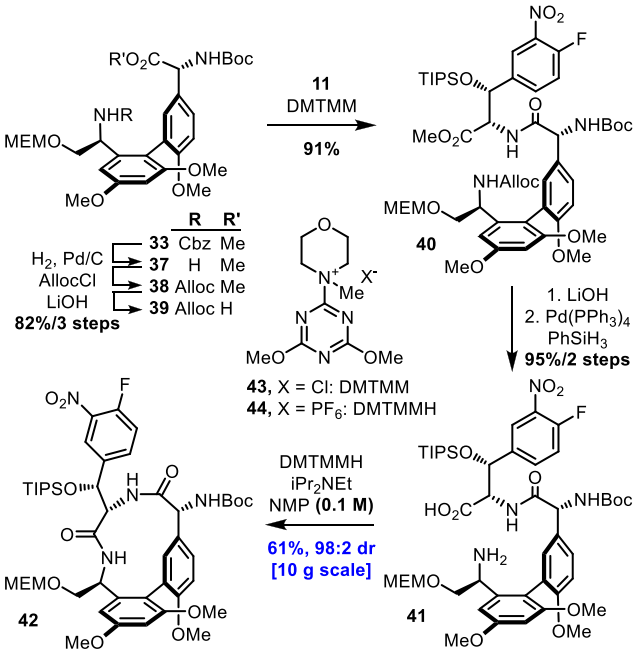

reagent screening at 1 mM [C]

reagent (eq.)	base (eq.)	solvent	yield	dr
DMTMM (10)	-	**DMF**	**29%**	**>95:5**
PyOxim (3)	iPr_2NEt **(6)**	**DMF**	**25%**	**>95:5**
DEPBT (10)	NMM (10)	THF	48%	75:25
PyAOP (8)	NMM (10)	THF	45%	66:34
PyBOP (8)	NMM (10)	THF	9%	32:68
T3P (5)	NMM (10)	THF	0%	-

optimization under simulated high-dilution conditions

reagent	base	solvent	[C]	yield
DMTMMH	**iPr_2NEt**	**NMP**	**0.1 M**	**59%**
DMTMMH	iPr_2NEt	NMP	0.01 M	58%
DMTMMH	NMM	NMP	0.01 M	31%
DMTMMH	iPr_2NEt	DMF	0.01 M	46%
PyOxim	iPr_2NEt	DMF	0.01 M	43%
COMU	iPr_2NEt	DMF	0.01 M	24%

Figure 4. (Top) Synthesis of the AB ring system. (Bottom) Optimization of the macrolactamization reaction.

41. Macrolactamization of **41** under previously optimized conditions[36] [3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one (DEPBT),[61] *N*-methylmorpholine (NMM), 1 mM in THF, 3:1 dr (inseparable), 48% combined yield] proved disappointing. Prior iterations on this route with smaller C ring alcohol silyl ether protecting groups (TBS, TBDPS) did not suffer from low yields or competitive epimerization (60%, no epimerization and 60%, 13:1 dr, respectively), demonstrating that the increased steric bulk of the TIPS protecting group impeded productive cyclization. However, TIPS protection proved essential to the success of a subsequent diastereoselective CD ring closure, requiring an improved method for the macrolactamization of **41** that avoids epimerization of the C ring α-stereocenter.

Extensive reagent screening identified DMTMM[62] (**43**) and its PF_6^- salt[63] (DMTMMH) (**44**), PyOxim,[64] and COMU[65] as the only reagents capable of suppressing epimerization (>95:5 dr) in the macrolactamization of **41**, consistent with their capabilities for promoting the couplings of hindered carboxylic acids (Figure 4). Further optimization of solvent, base, and reagent under simulated high-dilution conditions (i.e., slow addition of substrate to reagents) revealed that DMTMMH provided the highest yields, particularly under conditions that promote fast cyclization (Base: iPr_2NEt > NMM. Solvent: NMP (*N*-methylpyrrolidone) > DMF > MeCN.). These studies culminated in a rapid macrolactamization reaction that is complete on addition, conducted at a practical concentration (0.1 M), and capable of furnishing **42** in 61% yield on 10 g scale with negligible (≤2%) epimerization. Subsequent Boc deprotection (HCO_2H) of **42** and coupling of the free amine with the D ring subunit carboxylic acid **12** (DEPBT, 72% for 2 steps) set the stage for studies on the key CD ring closure.

The proposal to use the AB macrocycle as an element of preorganization for the CD ring closure was first validated by an improvement in the CD cyclization dr from 1:1[42] in the absence of the AB macrocycle to 3:1 in its presence with preferential formation of natural atropisomer **46b** when the C ring alcohol was protected as a TBS ether. The macrocyclization of substrate **45b** was also unusually facile, proceeding readily at 23 °C in DMF (Scheme 2). Unfortunately, thorough screening of solvent, base, temperature, and additives did not improve the reaction diastereoselectivity beyond 3:1. Moreover, the TBS ether proved sensitive to desilylation as noted in our previous work. Conducting the cyclization on the unprotected alcohol **45a** reduced the diastereoselectivity to 2:1, suggesting that the sterically bulky TBS ether improved the S_NAr atroposelectivity. Indeed, a progressive increase in the size of the silyl ether protecting group improved the diastereoselectivity (TBS, 3:1 dr; TBDPS, 5:1 dr; TIPS, 7:1 dr). As an added benefit, TIPS-protected **45d** was considerably less prone to desilylation than either **45b** or **45c**, although strict anhydrous conditions were still required for optimal results. The challenge of installation and removal of even larger silyl ether protecting groups (e.g., BIBS[66] and supersilyl), as well as their potential to further decelerate the macrolactamization, led us to select the TIPS ether as the preferred alcohol protecting group.

A similar study of the CD macrocyclization of substrates **47b–d** prior to macrolactamization of the AB ring system displayed the same trends, where increasing the size of the silyl ether protecting group improved the selectivity, although diastereoselections were lower than those obtained for substrates **45a–d** that contain the intact AB ring system (Scheme 2). In addition, the cyclizations of **47b–d** proceeded 2–3 times more slowly than **45b–d** bearing the intact AB ring system under identical conditions (Cs_2CO_3, DMF, 23 °C, 10 h vs 3–5 h, respectively). Collectively, these studies illustrate that the rate and diastereoselectivity of the CD ring closures of **45a–d** benefit from a combined preorganization provided by the AB ring system, which preferentially adopts an inherent cis amide conformation[6] poised for cyclization and a subtle tunable substituent effect that sterically further directs the nitro group distal to the large silyl ether. An analogous study of the S_NAr cyclization of the isolated DE ring system (substrates **49a–d**) demonstrated that an increase in the size of the silyl ether protecting group on the E ring hydroxyl also progressively favored the natural DE atropisomers **50a–d**. However, the diastereoselectivities achieved were modest, and only the TIPS-protected substrate **49d** favored formation of the natural atropisomer (1.1:1 dr), whereas all other substrates examined slightly favored formation of the unnatural DE atropisomer (1:1.7–1:1.3 dr). Nonetheless, the consistent correlation between the size of the silyl ether protecting group and the reaction diastereoselectivity was ultimately key to

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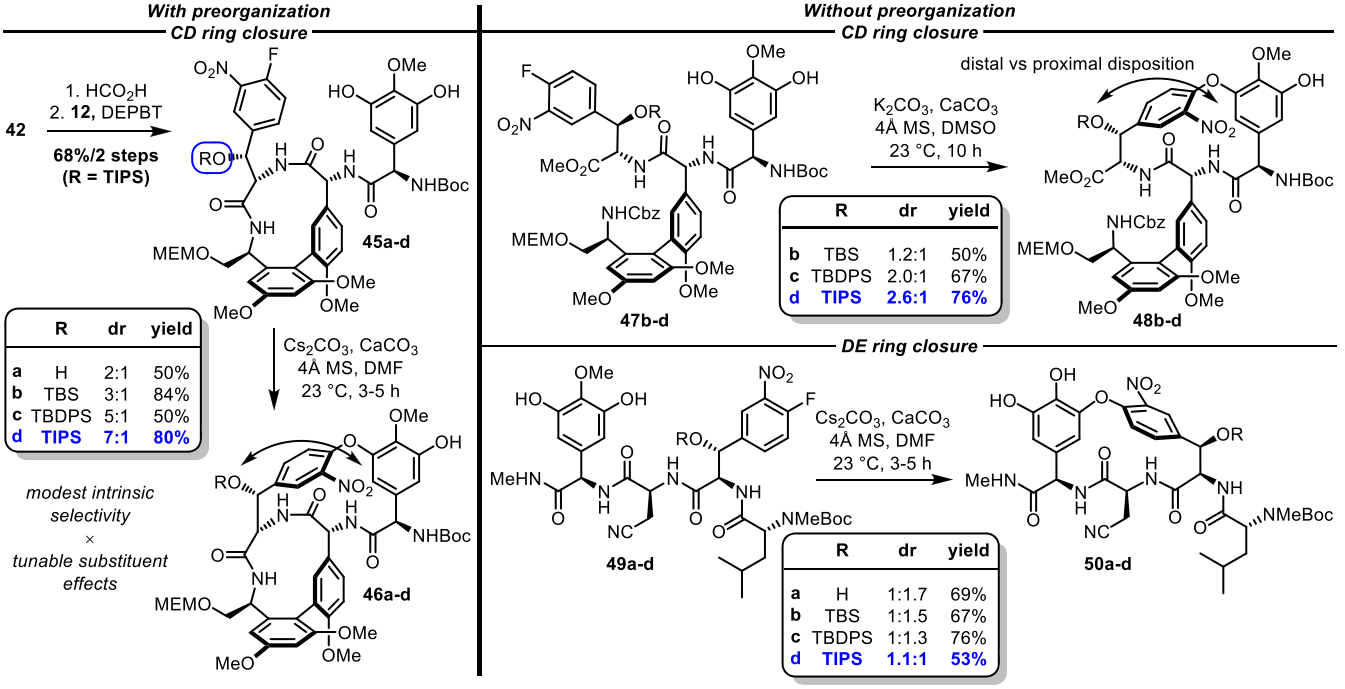
Scheme 2. CD and DE Ring Macrocyclization Studies

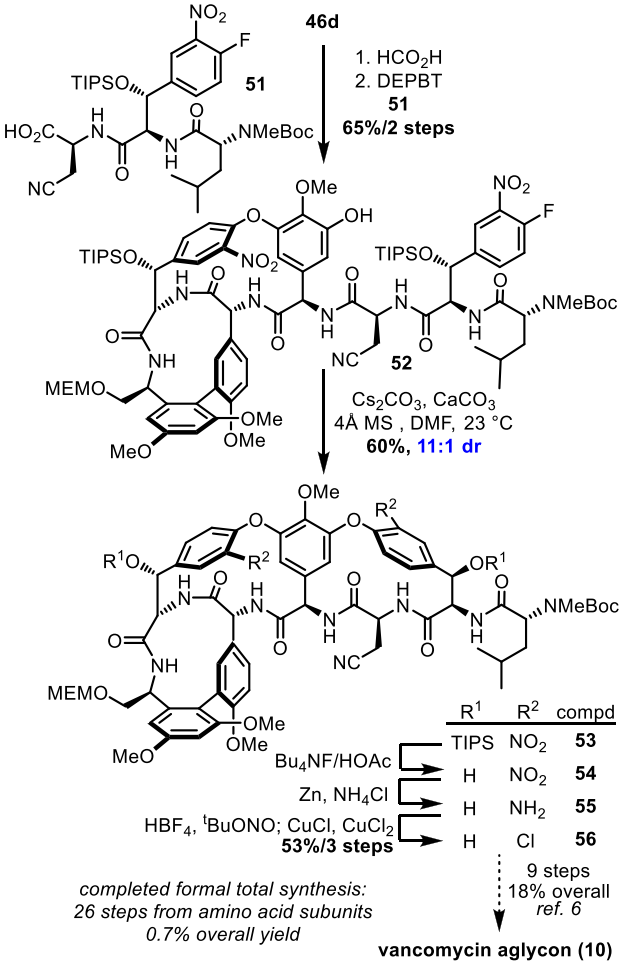


realizing the highly diastereoselective construction of the CD and DE biaryl ethers.

Completion of the formal synthesis required introduction of the DE ring system (Scheme 3). The high diastereoselection (8:1) obtained for the room-temperature DE ring closure in our previous work[6] with substrates bearing the intact ABCD ring system was observed with the E ring free alcohol substrate (7:1) herein and was further improved to 11:1 by the subtle impact of TIPS protection of the E ring hydroxyl group, mirroring the results obtained above. Simultaneous C/E ring TIPS deprotection (Bu_4NF/HOAc, 98%), nitro group reduction (Zn/NH_4Cl), and double-Sandmeyer chlorination (^tBuONO, HBF_4; CuCl/$CuCl_2$, 54% for 2 steps) provided known intermediate **56**, thereby completing a formal total synthesis of vancomycin aglycon **10** in 26 projected steps[6] from the individual amino acid subunits and 0.7% overall yield (major diastereomer only) *without* thermal atropisomer equilibrations. This result compares favorably with our previous synthesis,[6] which proceeded in 25 steps but with a more modest 0.2% overall yield when material recovered by thermal atropisomer equilibrations is excluded.

Concurrent with the above efforts, we undertook further optimization of the Sandmeyer chlorination to minimize protodediazoniation, which in practice often leads to copolar deschloro reaction byproducts. In previous efforts, we mitigated this issue by employing a large excess of CuCl/$CuCl_2$ in a mixed CH_3CN/H_2O solvent system at low temperature.[39,42] In these prior studies, we also observed that Sandmeyer chlorination of the C ring substituent was especially challenging, whereas the less hindered D ring substitution was more straightforward. In this work, Sandmeyer substitution of model substrate **57** for the challenging C ring substitution was problematic at 0 °C (3:1 **58**:**59**, 65%) (Figure 5). Further lowering the reaction temperature (−35 °C) suppressed protodediazoniation to acceptable levels (18:1), although the more difficult model

Scheme 3. Completion of Formal Total Synthesis



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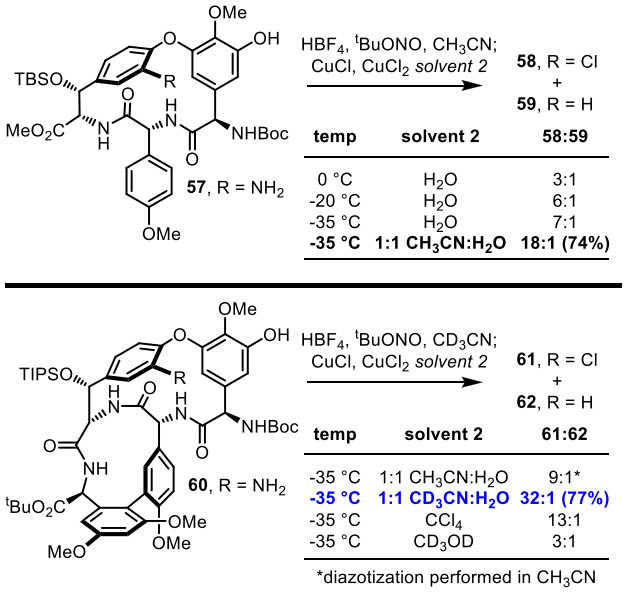

Figure 5. Model studies of the Sandmeyer chlorination reaction. General conditions: HBF_4 (1.3 equiv), ^tBuONO (1.3 equiv), solvent 1, 0 °C for 30 min, cooled to the specified temperature, treated with CuCl (50 equiv) and $CuCl_2$ (60 equiv) in solvent 2, and warmed to room temperature over 1 h. Product ratios were determined by LC/MS analysis of the crude reaction mixture. Isolated yields are given in parentheses.

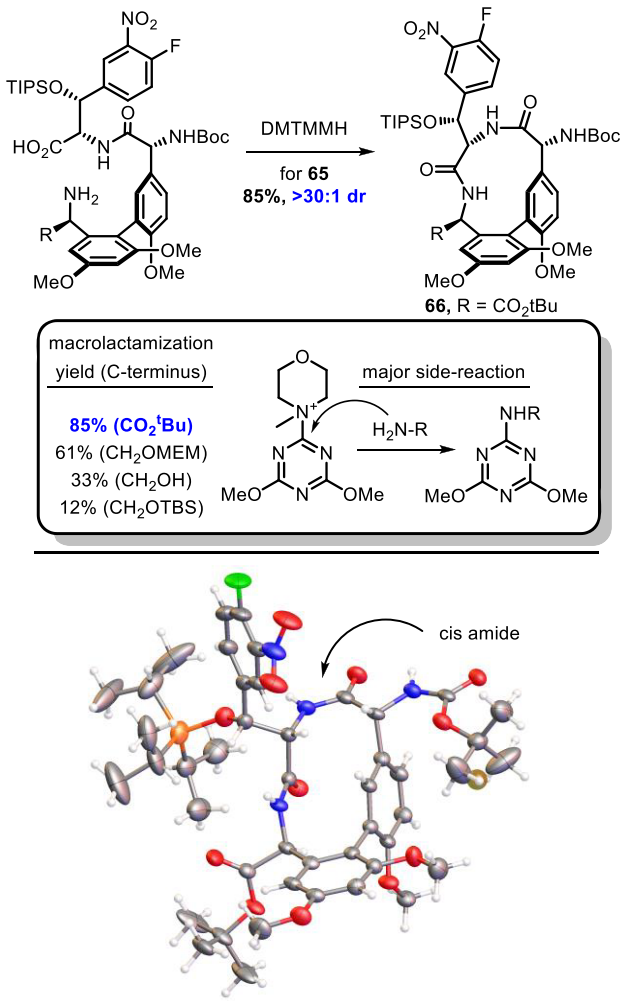

Figure 6. (Top) Substituent impact on macrolactamization of the AB ring system. (Bottom) X-ray crystal structure of **66**.

substrate **60** bearing the fully functionalized ABCD ring system yielded a less satisfactory but good 9:1 ratio of **61:62** under identical conditions. Disappointingly, inclusion of CCl_4 as an additional chlorine atom donor *increased* the amount of reduction byproduct by >2-fold, contrary to earlier claims by Zhu.[67] Fortunately, the product ratio improved >3-fold (to >32:1) in CD_3CN at −35 °C, indicating that reduction primarily occurs by solvent acetonitrile C−H abstraction. A full study of factors influencing the reaction is summarized in Supporting Information Figure S5. These optimal conditions (CD_3CN, −35 °C) translated smoothly to the simultaneous double-Sandmeyer substitution of substrate **72** bearing the fully functionalized ABCDE ring system in the further streamlined total synthesis below, providing the dichlorinated product **73** in 63% yield. Importantly, CD_3CN is inexpensive and available in bulk quantities, such that its use in the Sandmeyer chlorination of 1 g of a late-stage intermediate such as **72** would cost only $20.

Next-Generation Total Synthesis of Vancomycin. With high kinetic diastereoselectivity achieved for all three atropisomer elements as well as access to large quantities of the amino acid subunits, we proceeded to streamline the approach in anticipation of preparing significant quantities of pocket-modified analogues by total synthesis. Foremost among our concerns with the redesigned formal synthesis above were the step count (26 steps LLS), low overall yield (<1%), unnecessary A ring amine protecting group exchange, and late-stage conversion of the C-terminus MEM ether to the carboxylic acid. Although the latter feature was used to preclude C-terminus epimerization of a precursor methyl ester in prior studies, it introduced an inefficient deprotection−oxidation sequence (53%, 4 steps)[6] conducted toward the end of the synthesis that is even more challenging for substrates containing the oxidation-prone residue 4 thioamide[36] used to access pocket-modified analogues.

We incorporated the A ring subunit as the unprotected alcohol **23** also now bearing an orthogonal trifluoroacetamide protecting group (Scheme 4). The use of **22** in a direct, one-pot borylation/atroposelective Suzuki coupling with BC dipeptide **34**, prepared by coupling **11** with **15** (DMTMM, 88%), provided **35** in superb yield and outstanding diastereoselectivity (93%, unnatural atropisomer not detected), the development of which is detailed in Figure 3. To date, this reaction has been scaled to 25 g without loss of efficiency and avoids isolation and handling of the sensitive boronic acid **23**. Subsequent primary alcohol oxidation and esterification (TEMPO, $PhI(OAc)_2$;[68] *tert*-butyl trichloroacetimidate,[69] 82% for 2 steps, 26 g scale) secured the C-terminus in the required oxidation state in high yield and at an early stage prior to introduction of the D ring subunit (residue 4) that will bear a key thioamide in future syntheses of pocket-modified analogues. Alternative efforts to incorporate the C-terminus *tert*-butyl ester into the A ring subunit prior to the biaryl coupling resulted in racemization in studies conducted to date. Through judicious choice of the amine protecting group, simultaneous single-step deprotection of the C ring methyl ester and A ring trifluoroacetamide of **64** (LiOH, 82%) provided **65** without competitive *tert*-butyl ester hydrolysis and

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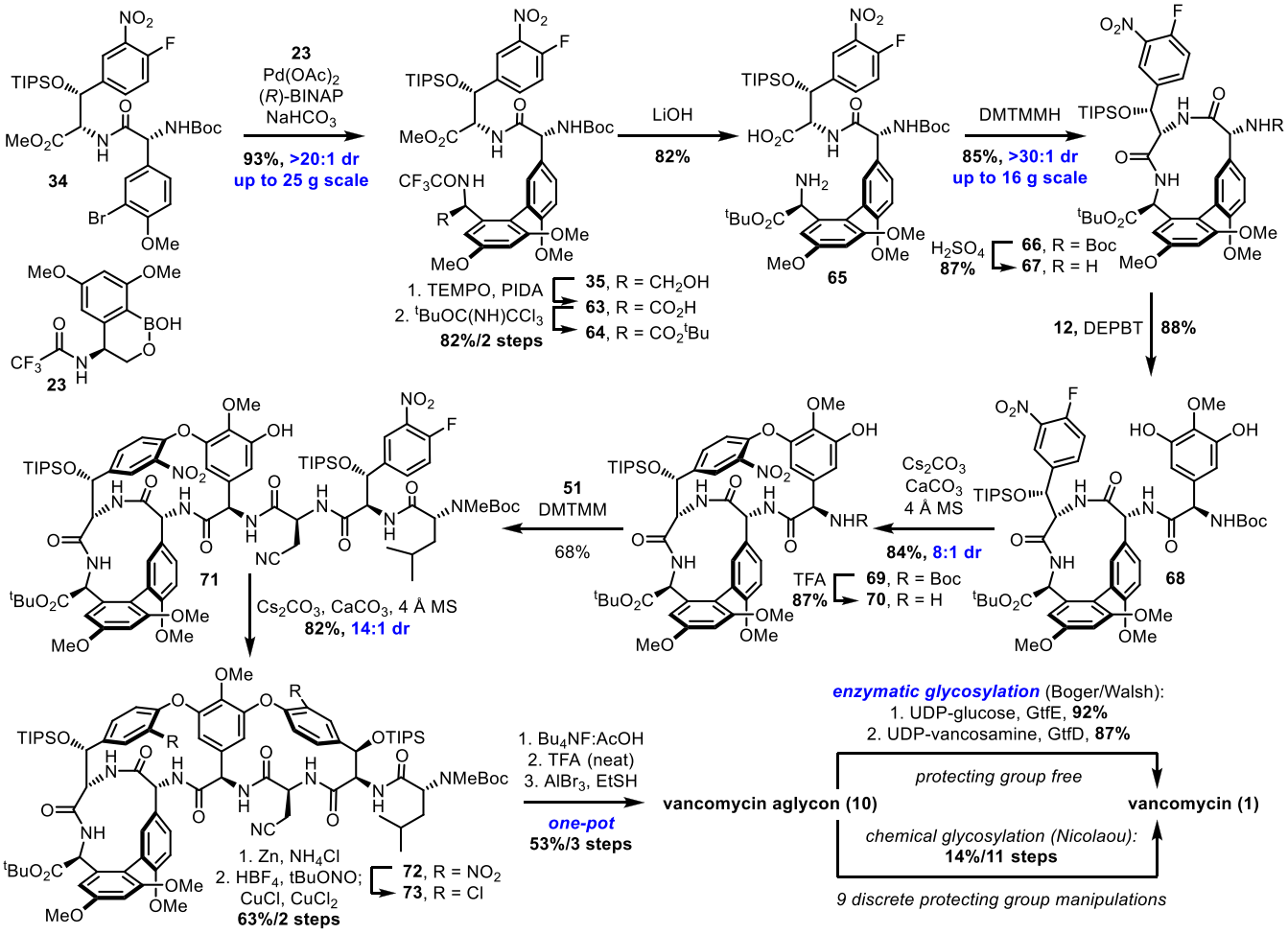
Scheme 4. Next-Generation Vancomycin Total Synthesis



set the stage for closure of the AB ring system. Gratifyingly, the macrolactamization conditions developed herein (see Figure 6) proved most effective for **65** bearing the C-terminal *tert*-butyl ester, despite the more electron-deficient nature of the reacting amine. Not only did the macrocyclization of **65** (DMTMMH, iPr_2NEt, NMP) proceed essentially instantaneously to provide **66** in higher yield (up to 85% yield, on scales of up to 16.4 g) than the C-terminal MEM ether (61% for **42**) but alternative substrates that bear a C-terminal free alcohol or TBS-protected alcohol also cyclized in inferior yields (33% and 12%, respectively), revealing that the substrate containing the *least* nucleophilic amine (**65**) cyclized most effectively (Figure 6). Although this trend may appear counterintuitive, adducts of the less effective starting amines with the coupling reagent DMTMMH were invariably observed, indicating that an amine triazinylation side reaction was competitive with macrocyclizations of substrates bearing more nucleophilic amines. Finally, the structure, relative stereochemistry, and cis amide linking residues 5 and 6 (vancomycin numbering) were confirmed in a single-crystal X-ray structure determination of **66** (Figure 6).[70] In addition, the suggested distal disposition of the large TIPS ether and aryl nitro substituent poised for diastereoselective CD ring closure was observed in the X-ray crystal structure of **66**. In total, the AB macrocycle was prepared in 53% yield (5 steps), representing a significant improvement over the formal synthesis above (37%/8 steps) with the added advantage of early-stage oxidation of the C-terminus that avoids 4 additional late-stage steps in the overall synthesis.

Completion of our next-generation total synthesis of vancomycin aglycon **10** is outlined in Scheme 4. Selective N-Boc deprotection of **66** under conditions that may permit reversible *tert*-butyl ester deprotection/reprotection[69] (H_2SO_4, ^tBuOAc, 86% yield, 98% brsm, 6.9 g scale) followed by coupling of amine **67** with the D ring subunit **12** provided **68** (88%) poised for CD ring closure. Further refinement of conditions for the room-temperature macrocyclization of this substrate provided the ABCD ring system **69** in superb yield and excellent diastereoselectivity (84%, 8:1 dr) without detectable epimerization of the C-terminal *tert*-butyl ester. Attentive reaction monitoring permitted N-Boc deprotection of **69** without significant *tert*-butyl ester deprotection (5% TFA-CH_2Cl_2, 87%). Coupling of the amine **70** with the E ring tripeptide **51** promoted by DMTMM provided **71** in excellent yield with minimal epimerization of the β-cyanoalanine residue (6:1 dr, 68% major diastereomer) that is ordinarily problematic for this segment coupling.[36,10] Room-temperature macrocyclization of the DE ring system proceeded with near exclusive formation of the desired DE atropisomer (14:1 dr) in high yield (82%), where the rate and substrate-controlled diastereoselectivity benefit from both the preorganization provided by the ABCD ring system observed in prior studies[6] as well as the now added influence of the E ring TIPS alcohol protecting group discovered in this work. Dual nitro group

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reduction followed by Sandmeyer chlorination under the conditions developed herein (^{t}BuONO/HBF_4; CuCl/$CuCl_2$, CD_3CN:H_2O, −35 °C) yielded dichloride **73** (63% for 2 steps), and this proved more effective than Sandmeyer substitution following removal of the two TIPS protecting groups. Without optimization, final conversion to vancomycin aglycon **10** was conducted in a now simplified three-step, one-pot sequence consisting of desilylation (Bu_4NF/HOAc, THF, 23 °C), nitrile hydration with concomitant Boc and *tert*-butyl ester deprotections (neat TFA, 23 °C), and global O-demethylation ($AlBr_3$, EtSH, 23 °C; 53−58% for 3 steps with removal of 9 protecting groups). This next-generation total synthesis of vancomycin aglycon was completed in 17 steps and in 5% overall yield from the amino acid subunits with excellent kinetic diastereoselectivity achieved for introduction of each atropisomer element.

Just as importantly, we previously implemented a practical solution to what many may think would be the most challenging feature of developing synthetic glycopeptide antibiotics, the late-stage steps used for scalable conversion of vancomycin aglycon to the fully decorated natural product or its analogues. This entailed enzymatic glycosylation of the aglycon(s) with the biosynthetic enzymes (glycosyltransferases GtfE and GtfD)[71−73] and commercial (UDP-glucose)[74] or readily accessible (UDP-vancosamine)[75] glycosyl donors for introduction of the disaccharide.[8] The two enzymes have been stably overexpressed in *E. coli* by Walsh, making them readily available as reagents for use on any scale, like the UDP-glycosyl donors.[71−73] Moreover, this utilizes the fully functionalized aglycon without introduction or removal of protecting groups and was found to work effectively on the vancomycin aglycon residue 4 thioamide that serves as our immediate precursor to binding pocket analogues.[8,38] Combined, this provides a 19-step total synthesis of vancomycin **1** that proceeds in 3.7% overall yield, substantially improving on prior approaches (Figure 7).

			Atroposelectivity			Glycosylation		
Total Syntheses	LLS	Yield	AB	CD	DE	method	steps	yield
Nicolaou (1998)	35	0.006%	2:1	1:1	1:3	chemical	11	14%
Boger (2014)	27	0.14%	1:1	1:1	8:1	enzymatic	2	80%
This work	**19**	**3.7%**	**>20:1**	**8:1**	**14:1**	**enzymatic**	**2**	**80%**

Figure 7. Comparison of the total syntheses of vancomycin (**1**), highlighting the late-stage enzymatic glycosylation. Yield and LLS start from individual amino acid subunits.

CONCLUSION

Despite the exceptional antimicrobial potency and durability of the pocket-modified vancomycin analogues discovered by our group, their preclinical evaluation has been slowed by the challenges presented by their total synthesis. Herein, we address this issue through the development of a scalable atroposelective total synthesis of vancomycin that considerably reduced the step count and increased the overall yield. With this new approach in hand, our efforts are focused on the preparation of new durable vancomycin analogues (e.g., **9**) and preclinical evaluation of the most promising candidates, including those that express three synergistic mechanisms of action (MOAs), two of which are independent of D-Ala-D-Ala/D-Lac binding and each of which is independently effective against vancomycin-resistant and vancomycin-sensitive bacteria. It is possible that such durable analogues that act by up to three MOAs might provide extraordinarily potent antibiotics, which display clinical lifetimes measured not in decades, or even the half-century of vancomycin, but perhaps in centuries. The total synthesis of vancomycin detailed herein provides the opportunity to prepare and assess such analogues.[76,77]

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.0c07433.

Full experimental details and copies of ^{1}H, ^{13}C, and 2D NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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