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Atroposelective Total Synthesis of Darobactin A

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Abstract

A concise, modular synthesis of the novel antibiotic darobactin A is disclosed. The synthesis successfully forges the hallmark strained macrocyclic ring systems in a sequential fashion. Key transformations include two atroposelective Larock-based macrocyclizations, one of which proceeds with exquisite regioselectivity despite bearing an unprotected alkyne. The synthesis is designed with medicinal chemistry considerations in mind, appending key portions of the molecule at a late stage. Requisite unnatural amino acid building blocks are easily prepared in an enantiopure form using C–H activation and decarboxylative cross-coupling tactics.

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Supporting Information

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Experimental procedures, analytical data (^{1}H and ^{13}C NMR, MS) for all new compounds, supplemental data and discussion (PDF)

Complete contact information is available at: https://pubs.acs.org/10.1021/jacs.2c05892

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Darobactin A (**1**, Figure 1), isolated in 2019 from *Photorhabdus* bacteria by the Lewis group,[1] features a highly strained fused macrocyclic peptide-based ring system. It exhibits selective antibiotic activity against Gram-negative pathogens in both in vitro and animal infection models and functions through an intriguing new mechanism of action. The rigid structure of darobactin A is likely responsible for its activity, on the basis of crystallographic studies.[2] The fact that all natural analogues retain the unique bicyclic core,[3,4] which acts as a β-strand mimetic,[2] supports this notion. Architecturally ornate peptide-based macrocycles have a rich history as promising antibacterial agents, from the clinically validated vancomycins[5–8] to the more recently pursued arylomycins.[9–11] Darobactin A contains multiple bonds that have not been previously described in antibiotic natural products[12,13]— namely, an aliphatic-aromatic ether moiety between the two tryptophan residues and a C–C bond between the β-carbon of lysine and C6 of the central tryptophan indole. Presumably, these bonds are formed in nature via post-translational macrocyclizations of a linear peptide.[1,14] These unusual features form the basis of the fused bis-macrocyclic core (14- and 15-membered rings) containing atropisomeric indoles.[15,16] A fully synthetic route to darobactin A would not only increase the available supply of material for further study, but also provide a framework for medicinal chemistry explorations.[2,17] In this Communication, a modular total synthesis of **1** that effectively addresses its structural and stereochemical challenges is disclosed.[18]

Although the final retrosynthetic blueprint for **1** is depicted in Figure 1, it is the culmination of multiple failed routes that preceded it. Strategies wedded to macrolactamization, Suzuki, and Heck tactics were all extensively investigated.[19] Ultimately, an approach featuring Larock-based cyclizations[15,20–22] successfully accessed **1** using inexpensive amino acids and arenes as building blocks (**2**–**8**, Figure 1).

To begin, two of the key unnatural amino acids (AAs) required for the synthesis were fashioned using decarboxylative cross-coupling methodology (Scheme 1A). Previously, such derivatives were prepared from serine in two steps (requiring the use of CuCN)[23–26] or from 2-prop-2-ynoxyoxane in four steps[22,27] requiring Schöllkopf's auxiliary.[28] By employing decarboxylative alkynylation,[29] a scalable synthesis of **9** was achieved in one step from commercially available **6**. Intermediates **12** and **14** could be divergently accessed from **9**, either through oxidation (via **10**/**11**) followed by peptide coupling or through peptide coupling (via **13**) followed by deprotection, respectively. Although diastereomer **11** ultimately proved to be the desired isomer, both **10** and **11** were needed in this study (vide infra).

Construction of the core of **1** (Scheme 1B) began with formation of the lysine-tryptophan C–C bond. Employing C–H functionalization logic to access this unique connectivity,[30] an 8-aminoquinoline (AQ)-directed Pd-catalyzed C–H arylation[31–33] was performed using conditions developed by Schreiber and co-workers.[34] Pyroglutamate derivative **15** (synthesized from **4**, see SI) and arene **2** were coupled in a *syn* fashion on a multigram scale to give **16** (51% yield). Reductive ring opening[35] of **16** followed by benzoyl protection provided intermediate **17** (69% yield). Following Cbz deprotection,[36] **12** was appended (75% yield over 2 steps) to set the stage for testing the Larock cyclization.

Over a year of research went into identifying conditions for this reaction and the related cyclization derived from alkyne **10**. Early development was performed on **18** and suffered from poor scalability, deleterious byproduct formation,[37] and the use of superstoichiometric Pd. As depicted in the inset table (Scheme 1B, step 6), multiple ligands were evaluated with tBu$_3$P·HBF$_4$ emerging as the most effective. Using 4 equiv of Pd, a 20% yield of macrocycle **20′** was obtained on a small scale. Unfortunately, this reaction suffered from scalability issues above 100 mg. In accord with studies from the Reisman[26] and Boger[22] laboratories, preformed Pd(PtBu$_3$)$_2$ was evaluated. This catalyst provided a glimmer of hope by allowing for a slight reduction in Pd loading (3.5 equiv) and delivering 41% yield of **20′** albeit with a significant amount of protodehalogenation product formation. Since oxidative addition on **18** must be facile (as judged by the extensive debromination), a combination of unfavorable steric interactions and strain likely inhibit this cyclization. Traditionally, the Larock indole synthesis requires a hindered alkyl- or silyl-terminated alkyne to provide the desired regiochemical outcome.[38] It was reasoned that in this specific context, a triethylsilyl (TES) blocking group on **18** may be superfluous as the regiochemistry might be controlled by the conformation of the cyclization precursor. Gratifyingly, unprotected alkyne **19** engaged smoothly in Larock macrocyclization to deliver **20** in up to 60% yield using 1.9 equiv of Pd. Interestingly, the Pd loading could be reduced to 30 mol % without a detrimental impact to the yield, rendering the reaction catalytic. Ultimately the macrocyclization could be efficiently conducted on gram-scale, isolating **20** as a single atropisomer in 61% yield. A small amount of byproducts with the same mass yet different polarity can be observed on LC; however, appreciable amounts could not be isolated for characterization. A rationalization for the observed atroposelectivity is proposed (see the SI for further details and discussion).

Given the success of the Larock reaction in forging the central ring, this strategy was also pursued as a means to perform the second macrocyclization. This disconnection would require installation of an ortho bromoaniline functionality onto the hydroxyl group of **20** along with inversion of the stereochemistry at C-17. A variety of etherification tactics were evaluated using alkynes derived from both **10** and **11** in order to test both stereoretentive (e.g., S$_N$Ar) and stereoinvertive strategies. Ultimately, displacement of the alcohol with 3-bromo-2-nitrophenol (**7**) under Mitsunobu conditions proved most effective. Through extensive screening (see inset table, step 7), it was revealed that the unusual combination of PMe$_3$ and TMAD[39–43] in toluene afforded **21** in 60% yield (500 mg scale). Historically such reagents have been employed for carbon-based nucleophiles; in this case, the use of these conditions led to increased reactivity with considerable suppression of byproduct formation.[44] With the key bromo arene installed with the proper stereochemistry, Boc-deprotection of **21** and subsequent coupling with **14** afforded **22** (59% yield). Gratifyingly, the second Larock cyclization of the corresponding crude aniline (from nitro group reduction[45] of **22**) was successful using similar conditions to the first macrocyclization to give **23** as a single atropisomer in 67% isolated yield over two steps.

Inherent to the design of this synthesis was a desire to probe the SAR of the lysine residue, as crystallographic studies indicate its interactions with the phosphate moieties of lipids.[2] Late-stage homologation to arrive at **1** is predicated upon the ability to access a

library of analogues diversified at this position. Thus, the desired side chain installation was accomplished through a simple four step sequence: (a) methanolysis of **23** to **24** (76% yield), (b) mesylation and concomitant TES deprotection in the presence of methane-sulfonic acid, (c) cyanation under phase transfer conditions, and (d) reduction of both the nitrile and AQ ring with in situ generated nickel boride followed by in situ Boc protection.[46] The fortuitous reduction of the AQ allowed for its removal[47] using conditions developed by Geyer and co-workers[48] (as inspired by methodology from the Dawson group).[49] Consequently, treatment of **25** with triphosgene followed by hydrolysis with trimethyltin hydroxide[50] delivered **26** in 33% yield over 5 steps. With the carboxylic acid revealed, conditions were evaluated to install the side chain **27** (synthesized from **5** and **8**, see the SI). Numerous peptide coupling reagents were screened; however, only a combination of EDCI and HOAt led to appreciable quantities of the desired amide bond formation.[51] The coupled product **28** was then subjected to ammonolysis to convert the aspartate residue to the requisite asparagine. At this stage, all that remained was global deprotection, which was realized by treatment with a combination of TMSOTf, TFA, *p*-cresol, and ethane dithiol[52] (see theSI for optimization data) to complete the synthesis of **1** in 26% yield for the final sequence (18 steps LLS from **15**).

In addition to exquisite control of core stereochemical features, this synthesis highlights the unique ability of the Larock reaction to generate two unprecedented strained ring systems. In one of those instances specific geometrical and conformational constraints guide regiochemistry; this allows for the use of an unprotected alkyne, rendering it catalytic in Pd, and providing a single atropisomeric outcome. Rapid access to key unnatural AA building blocks from inexpensive natural AAs is enabled by scalable C–H activation and decarboxylative coupling methods. Although nonstrategic from an efficiency standpoint, homologation to install the lysine side chain is deliberately implemented toward the end of the synthesis solely for medicinal chemistry explorations of this portion of the molecule. For the same reasons, a late stage incorporation of the C-terminus dipeptide was pursued. The atroposelective and modular synthesis of **1** sets the stage for the generation of analogues that are currently unavailable through engineered biosynthetic approaches and enables future exploration of the potent antibiotic activity of this important new family of peptidic natural products.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

(1). Imai Y; Meyer KJ; Iinishi A; Favre-Godal Q; Green R; Manuse S; Caboni M; Mori M; Niles S; Ghiglieri M; Honrao C; Ma XY; Guo JJ; Makriyannis A; Linares-Otoya L; Boehringer N; Wuisan ZG; Kaur H; Wu R; Mateus A; Typas A; Savitski MM; Espinoza JL; O'Rourke A; Nelson KE; Hiller S; Noinaj N; Schäberle TF; D'Onofrio A; Lewis K A new antibiotic selectively kills Gram-negative pathogens. Nature 2019, 576, 459–464. [PubMed: 31747680]

(2). Kaur H; Jakob RP; Marzinek JK; Green R; Imai Y; Bolla JR; Agustoni E; Robinson CV; Bond PJ; Lewis K; Maier T; Hiller S The antibiotic darobactin mimics a beta-strand to inhibit outer membrane insertase. Nature 2021, 593, 125–129. [PubMed: 33854236]

(3). Wuisan ZG; Kresna IDM; Bohringer N; Lewis K; Schäberle TF Optimization of heterologous Darobactin A expression and identification of the minimal biosynthetic gene cluster. Metab. Eng 2021, 66, 123–136. [PubMed: 33872780]

(4). Böhringer N; Green R; Liu Y; Mettal U; Marner M; Modaresi SM; Jakob RP; Wuisan ZG; Maier T; Iinishi A; Hiller S; Lewis K; Schäberle TF Mutasynthetic Production and Antimicrobial Characterization of Darobactin Analogs. Microbiol. Spectr 2021, 9, e01535–21. [PubMed: 34937193]

(5). Bruniera FR; Ferreira FM; Saviolli LRM; Bacci MR; Feder D; Pedreira MDG; Peterlini MAS; Azzalis LA; Junqueira VBC; Fonseca FLA The use of vancomycin with its therapeutic and adverse effects: a review. Eur. Rev. Med. Pharmacol 2015, 19, 694–700.

(6). Okano A; Isley NA; Boger DL Total Syntheses of Vancomycin-Related Glycopeptide Antibiotics and Key Analogues. Chem. Rev 2017, 117, 11952–11993. [PubMed: 28437097]

(7). Okano A; Isley NA; Boger DL Peripheral modifications of [Ψ[CH2NH]Tpg4]vancomycin with added synergistic mechanisms of action provide durable and potent antibiotics. Proc. Natl. Acad. Sci. U.S.A 2017, 114, E5052–E5061. [PubMed: 28559345]

(8). Moore MJ; Qu S; Tan C; Cai Y; Mogi Y; Jamin Keith D; Boger DL Next-Generation Total Synthesis of Vancomycin. J. Am. Chem. Soc 2020, 142, 16039–16050. [PubMed: 32885969]

(9). Liu J; Smith PA; Steed DB; Romesberg F Efforts toward broadening the spectrum of arylomycin antibiotic activity. Bioorg. Med. Chem. Lett 2013, 23, 5654–5659. [PubMed: 24012184]

(10). Smith PA; Koehler MFT; Girgis HS; Yan D; Chen Y; Chen Y; Crawford JJ; Durk MR; Higuchi RI; Kang J; Murray J; Paraselli P; Park S; Phung W; Quinn JG; Roberts TC; Rouge L; Schwarz JB; Skippington E; Wai J; Xu M; Yu Z; Zhang H; Tan MW; Heise CE Optimized arylomycins are a new class of Gram-negative antibiotics. Nature 2018, 561, 189–194. [PubMed: 30209367]

(11). Peters DS; Romesberg FE; Baran PS Scalable Access to Arylomycins via C-H Functionalization Logic. J. Am. Chem. Soc 2018, 140, 2072–2075. [PubMed: 29381350]

(12). Swain JA; Walker SR; Calvert MB; Brimble MA The tryptophan connection: cyclic peptide natural products linked via the tryptophan side chain. Nat. Prod. Rep 2022, 39, 410–443. [PubMed: 34581375]

(13). Crich D; Banerjee A Expedient synthesis of threo-beta-hydroxy-alpha-amino acid derivatives: phenylalanine, tyrosine, histidine, and tryptophan. J. Org. Chem 2006, 71, 7106–7109. [PubMed: 16930077]

(14). Guo S; Wang S; Ma SZ; Deng ZX; Ding W; Zhang Q Radical SAM-dependent ether crosslink in daropeptide biosynthesis. Nat. Commun 2022, 13, 2361. [PubMed: 35487921]

(15). Garfunkle J; Kimball FS; Trzupek JD; Takizawa S; Shimamura H; Tomishima M; Boger DL Total Synthesis of Chloropeptin II (Complestatin) and Chloropeptin I. J. Am. Chem. Soc 2009, 131, 16036–16038. [PubMed: 19839632]

(16). Reisberg SH; Gao Y; Walker AS; Helfrich EJN; Clardy J; Baran PS Total synthesis reveals atypical atropisomerism in a small-molecule natural product, tryptorubin A. Science 2020, 367, 458–463. [PubMed: 31896661]

(17). Gross S; Panter F; Pogorevc D; Seyfert CE; Deckarm S; Bader CD; Herrmann J; Müller R Improved broad-spectrum antibiotics against Gram-negative pathogens via darobactin biosynthetic pathway engineering. Chem. Sci 2021, 12, 11882–11893. [PubMed: 34659729]

(18). While this manuscript was being written, a total synthesis was disclosed by the Merck–Sarlah team:Nesic M; Ryffel DB; Maturano J; Shevlin M; Pollack SR; Gauthier DR Jr.; Trigo-Mouriño

P; Zhang L-K; Schultz D; McCabe Dunn JM; Campeau L-C; Patel NR; Petrone DA; Sarlah D Total Synthesis of Darobactin A ChemRxiv, May 24, 2022. DOI: 10.26434/chemrxiv-2022-c4mz6 (accessed on May 24, 2022).

(19). See the SI for an overview of these failed routes; further details to be disclosed in a full account of this work.

(20). Shimamura H; Breazzano SP; Garfunkle J; Kimball FS; Trzupek JD; Boger DL Total Synthesis of Complestatin: Development of a Pd(0)-Mediated Indole Annulation for Macrocyclization. J. Am. Chem. Soc 2010, 132, 7776–7783. [PubMed: 20469945]

(21). Breazzano SP; Poudel YB; Boger DL A Pd(0)-Mediated Indole (Macro)cyclization Reaction. J. Am. Chem. Soc 2013, 135, 1600–1606. [PubMed: 23298368]

(22). Isley NA; Endo Y; Wu ZC; Covington BC; Bushin LB; Seyedsayamdost MR; Boger DL Total Synthesis and Stereochemical Assignment of Streptide. J. Am. Chem. Soc 2019, 141, 17361–17369. [PubMed: 31577142]

(23). Trost BM; Rudd MT Chemoselectivity of the ruthenium-catalyzed hydrative diyne cyclization: Total synthesis of (+)-cylindricine C, D, and E. Org. Lett 2003, 5, 4599–4602. [PubMed: 14627393]

(24). Newhouse T; Lewis CA; Baran PS Enantiospecific Total Syntheses of Kapakahines B and F. J. Am. Chem. Soc 2009, 131, 6360–6361. [PubMed: 19374357]

(25). Newhouse T; Lewis CA; Eastman KJ; Baran PS Scalable total syntheses of N-linked tryptamine dimers by direct indole-aniline coupling: psychotrimine and kapakahines B and F. J. Am. Chem. Soc 2010, 132, 7119–7137. [PubMed: 20426477]

(26). Chuang KV; Kieffer ME; Reisman SE A Mild and General Larock Indolization Protocol for the Preparation of Unnatural Tryptophans. Org. Lett 2016, 18, 4750–4753. [PubMed: 27598827]

(27). Noichl BP; Durkin PM; Budisa N Toward Intrinsically Colored Peptides: Synthesis and Investigation of the Spectral Properties of Methylated Azatryptophans in Tryptophan-Cage Mutants. Biopolymers 2015, 104, 585–600. [PubMed: 26250482]

(28). Schöllkopf U; Groth U; Deng C Enantioselective Synthesis of (R)-Amino Acids Using L-Valine as Chiral Agent. Angew. Chem., Int. Ed 1981, 20, 798–799.

(29). Smith JM; Qin T; Merchant RR; Edwards JT; Malins LR; Liu ZQ; Che GD; Shen ZC; Shaw SA; Eastgate MD; Baran PS Decarboxylative Alkynylation. Angew. Chem., Int. Ed 2017, 56, 11906–11910.

(30). Gutekunst WR; Baran PS C-H functionalization logic in total synthesis. Chem. Soc. Rev 2011, 40, 1976–1991. [PubMed: 21298176]

(31). Zaitsev VG; Shabashov D; Daugulis O Highly regioselective arylation of sp^3 C-H bonds catalyzed by palladium acetate. J. Am. Chem. Soc 2005, 127, 13154–13155. [PubMed: 16173737]

(32). Reddy BVS; Reddy LR; Corey EJ Novel acetoxylation and C-C coupling reactions at unactivated positions in alpha-amino acid derivatives. Org. Lett 2006, 8, 3391–3394. [PubMed: 16836413]

(33). Feng YQ; Chen G Total Synthesis of Celogentin C by Stereoselective C-H Activation. Angew. Chem., Int. Ed 2010, 49, 958–961.

(34). Verho O; Maetani M; Melillo B; Zoller J; Schreiber SL Stereospecific Palladium-Catalyzed C-H Arylation of Pyroglutamic Acid Derivatives at the C3 Position Enabled by 8-Aminoquinoline as a Directing Group. Org. Lett 2017, 19, 4424–4427. [PubMed: 28809506]

(35). Soai K; Oyamada H; Takase M The Preparation of N-Protected Amino-Alcohols and N-Protected Peptide Alcohol by Reduction of the Corresponding Esters with Sodium-Borohydride - an Improved Procedure Involving a Slow Addition of a Small Amount of Methanol. Bull. Chem. Soc. Jpn 1984, 57, 2327–2328.

(36). Olah GA; Narang SC; Gupta BGB; Malhotra R Synthetic Methods and Reactions. 62. Transformations with Chlorotrimethylsilane Sodium Iodide, a Convenient in Situ Iodotrimethylsilane Reagent. J. Org. Chem 1979, 44, 1247–1251.

(37). Including the protodebromination product and acyl-transfer derived species (from indole nitrogen to hydroxy group) as determined by NMR studies.

(38). Larock RC; Yum EK Synthesis of Indoles Via Palladium-Catalyzed Heteroannulation of Internal Alkynes. J. Am. Chem. Soc 1991, 113, 6689–6690.

(39). But TYS; Toy PH The Mitsunobu reaction: Origin, mechanism, improvements, and applications. Chem. Asian J 2007, 2, 1340–1355. [PubMed: 17890661]

(40). Swamy KC; Kumar NN; Balaraman E; Kumar KV Mitsunobu and related reactions: advances and applications. Chem. Rev 2009, 109, 2551–2651. [PubMed: 19382806]

(41). Reynolds AJ; Kassiou M Recent Advances in the Mitsunobu Reaction: Modifications and Applications to Biologically Active Molecules. Curr. Org. Chem 2009, 13, 1610–1632.

(42). Fletcher S The Mitsunobu reaction in the 21st century. Org. Chem. Front 2015, 2, 739–752.

(43). Panday SK Advances in the Mitsunobu Reaction: An Excellent Organic Protocol with Versatile Applications. Mini-Rev. Org. Chem 2019, 16, 127–140.

(44). β-Elimination and intramolecular cyclization of the N-Boc group to an oxazolidinone.

(45). Ramadas K; Srinivasan N Iron-Ammonium Chloride - a Convenient and Inexpensive Reductant. Synth. Commun 1992, 22, 3189–3195.

(46). Caddick S; Judd DB; Lewis AKD; Reich MT; Williams MRV A generic approach for the catalytic reduction of nitriles. Tetrahedron 2003, 59, 5417–5423.

(47). Fitzgerald LS; O'Duill ML A Guide to Directing Group Removal: 8-Aminoquinoline. Chem. Eur. J 2021, 27, 8411–8436. [PubMed: 33559933]

(48). Nicke L; Horx P; Harms K; Geyer A Directed C(sp3)-H arylation of tryptophan: transformation of the directing group into an activated amide. Chem. Sci 2019, 10, 8634–8641. [PubMed: 31803437]

(49). Blanco-Canosa JB; Nardone B; Albericio F; Dawson PE Chemical Protein Synthesis Using a Second-Generation N-Acylurea Linker for the Preparation of Peptide-Thioester Precursors. J. Am. Chem. Soc 2015, 137, 7197–7209. [PubMed: 25978693]

(50). Nicolaou KC; Estrada AA; Zak M; Lee SH; Safina BS A mild and selective method for the hydrolysis of esters with trimethyltin hydroxide. Angew. Chem., Int. Ed 2005, 44, 1378–1382.

(51). Jiang WL; Wanner J; Lee RJ; Bounaud PY; Boger DL Total synthesis of the ramoplanin A2 and ramoplanose aglycon. J. Am. Chem. Soc 2002, 124, 5288–5290. [PubMed: 11996568]

(52). Yajima H; Fujii N; Funakoshi S; Watanabe T; Murayama E; Otaka A New Strategy for the Chemical Synthesis of Proteins. Tetrahedron 1988, 44, 805–819.

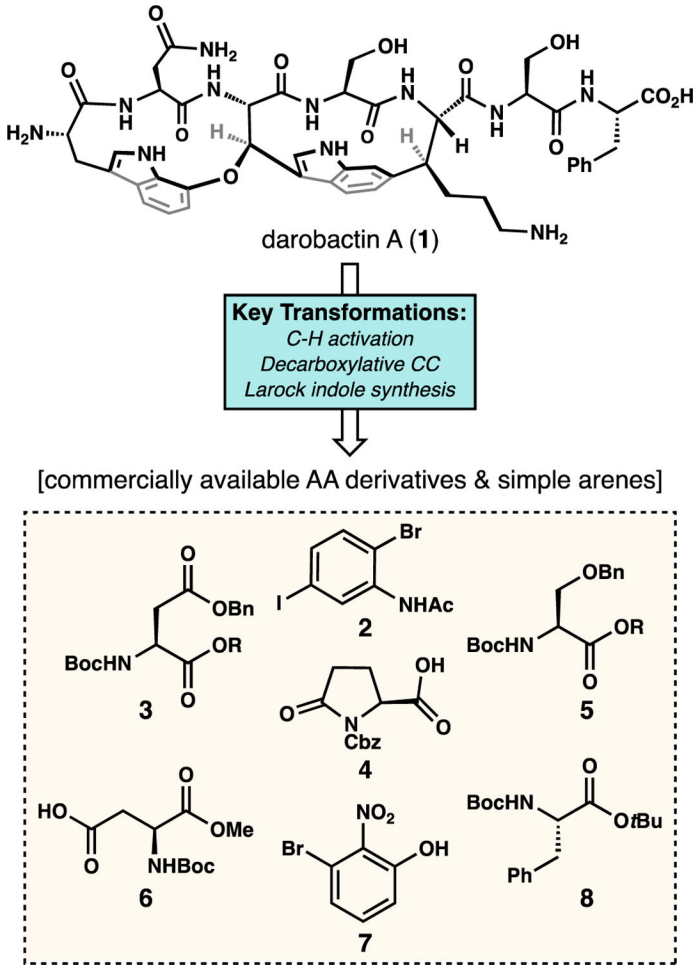
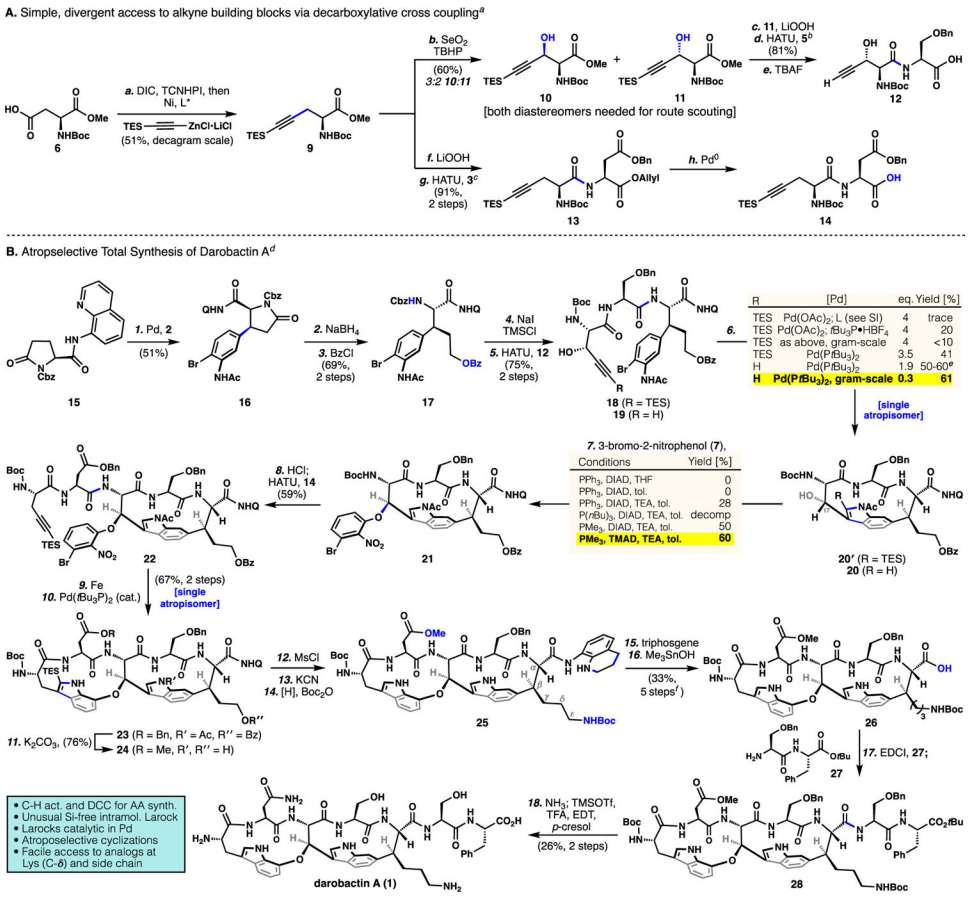


Figure 1.
Darobactin A (**1**): Retrosynthetic strategies and building blocks.



Scheme 1.

Total Synthesis of Darobactin A

[a]Reagents and conditions: (a) $NiCl_2{\cdot}6H_2O$ (40 mol %), L* = *t*Bubpy (40 mol %), TES-alkynyl-ZnCl·LiCl (4.5 equiv, see SI for preparation), DMF, rt, 51%. (b) SeO_2 (2.0 equiv), TBHP (4.0 equiv), MeCN, 50 °C, 60% (3:2 **10**:**11**). (c) LiOH (1 M aqueous, 1.5 equiv), H_2O_2 (30 wt % aqueous, 15 equiv), THF, 0 °C to rt. (d) **11**, hydrolyzed (1.1 equiv), **5** (1.0 equiv). [b]R = $(CH_2)_2SiMe_3$, Boc-deprotected, HATU (1.3 equiv), DIPEA (3.3 equiv), rt, 81%. (e) TBAF (3.0 equiv), THF, 0 °C. (f) LiOH (1 M aqueous, 1.5 equiv), H_2O_2 (30 wt % aqueous, 15 equiv), THF, 0 °C to rt. (g) **9**, hydrolyzed (1.6 equiv), **3** (1.0 equiv). [c]R = allyl, Boc-deprotected, HATU (1.6 equiv), DIPEA (6.0 equiv), rt, 91% (2 steps). (h) $Pd(PPh_3)_4$ (10 mol %), morpholine (5.0 equiv), THF, rt. [d]Reagents and conditions: (**1**) **2** (1.0 equiv), **15** (1.8 equiv), AgOAc (1.5 equiv), $(BnO)_2PO_2H$ (20 mol %), $Pd(OAc)_2$ (60 mol %), CPME, 120 °C, 51%. (**2**) $NaBH_4$ (4.0 equiv), 8:1 THF:*t*BuOH, rt. (**3**) BzCl (4.0 equiv), pyridine (6.0 equiv), DMAP (10 mol %), DCM, rt, 69% (2 steps). (**4**) NaI (25 equiv), TMSCl (15 equiv), rt. (**5**) **12** (2.0 equiv), HATU (2.0 equiv), DIPEA (10 equiv), rt, 75% (2 steps). (**6**) $Pd(P\mathit{t}Bu_3)_2$ (30 mol %), DIPEA (5.0 equiv), dioxane, 85 °C, 61%. (**7**) **7** (20 equiv), TEA (10 equiv), PMe_3 (1 M in toluene, 10 equiv), PMe_3 (1 M in THF, 3.0 equiv), TMAD (13 equiv), 38 °C, 60%. (**8**) HCl (4 M in dioxane, 60 equiv), DCM, 0 °C; **14** (1.9 equiv), HATU (1.9 equiv), DIPEA (9.0 equiv), rt, 59%. (**9**) Fe powder (320 equiv), NH_4Cl (85 equiv), AcOH, 50 °C. (**10**) $Pd(P\mathit{t}Bu_3)_2$ (50 mol %), DIPEA (5.0 equiv),

dioxane, 85 °C, 67% (2 steps). (**11**) K_2CO_3 (25 equiv), DCM:MeOH 1:4, rt, 76%. (**12**) TEA (22 equiv), MsCl (35 equiv), DCM, 0 °C; MsOH (2.3 equiv), DCM, rt. (**13**) KCN (142 equiv), KH_2PO_4 (42 equiv), TBHS (5.0 equiv), 1:1 D_2O:$CDCl_3$, 36 °C. (**14**) $NiCl_2{\cdot}6H_2O$ (3.5 equiv), Boc_2O (19 equiv), $NaBH_4$ (5.0 equiv), 3:1 MeOH:THF, −5 °C. (**15**) DIPEA (2.4 equiv), triphosgene (1.2 equiv), DCM, 0 °C to rt. (**16**) Me_3SnOH (3.5 equiv), DCE, 31 °C, 33% (5 steps). (**17**) **27** (6.0 equiv), HOAt (4.0 equiv), $NaHCO_3$ (6.0 equiv), EDCI (4.0 equiv), DMF:DCM 1:1, rt. (**18**) NH_3(*g*), MeOH, 33 °C; TFA:TMSOTf:*p*-cresol:EDT 17:3:2:1, 0 °C, 26% (2 steps). [e]LCMS yield. [f]Intermediates at steps 13 and 14 were column purified but contained inseparable contaminants derived from reaction conditions (e.g., tetrabutylammonium). Semipure fractions were carried forward, but an accurate yield could not be determined at these steps. Small samples were purified by PTLC for characterization.