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Scalable Synthesis of the Desoxy-biphenomycin B Core

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ABSTRACT: We describe the evolution of a kilogram-scale synthesis of the protected cyclic tripeptide desoxy-biphenomycin B, based on an early discovery route. The retrosynthetic concept included a macrolactamization strategy to build the core ring system of biphenomycin B in combination with a double catalytic asymmetric hydrogenation protocol for the construction of the *ansa*-tripeptide precursor. Eventually, the kilogram process comprised a 16-step sequence with an overall yield for the longest linear sequence of 19.5%.

INTRODUCTION

The biphenomycin B antibiotic represents a complex lead structure from natural sources.[1] In the past, several academic groups have elaborated viable routes to synthesize the biphenyl-bridged cyclopeptide based on two key steps: transition metal catalyzed biphenyl coupling and macrolactamization.[2]

For our in-house research and development program, the accessibility of the macrolactam core of biphenomycin B in multigram up to kilogram quantities was a prerequisite. In particular, we were interested in analogues derived from desoxy-biphenomycin B, which lack the hydroxyl group substitution in the hydroxy ornithine side chain. Our experience in route scouting and scale-up for manufacturing of target molecule **2** are detailed in this manuscript.

The Bayer routes towards protected desoxy-biphenomycin **2** (Scheme 1) were based on the pioneer work of Schmidt el al. who reported the first total synthesis of biphenomycin B in 22 linear steps with an overall yield of 9%.[3] Our discovery synthesis introduced an increased level of convergence by using the higher functionalized dehydroamino acid building blocks **5** and **6** as substrates for a Suzuki coupling. Both intermediates could be accessed commencing from phosphonate **3** and substituted benzaldehyde **4a**, respectively. In the key step, the catalytic asymmetric double hydrogenation of Suzuki product **7** furnished intermediate **8** in excellent yield (98%) and high enantioselectivity (>99.5% ee), which demonstrated the wide scope and robustness of this methodology. The final steps towards target molecule **2** consisted of the introduction of the protected (*S*)-ornithine and the subsequent macrolactamization of the ornithine N^{α} employing Schmidt's pentafluorophenyl ester protocol. In total, our discovery route towards **2** comprised 18 steps (15 steps starting from **3** and **4a**) with good chemical yields (21%, for the longest linear sequence of 11 steps) from commercially available raw materials.[4]

The route depicted in Scheme 1 has been applied to deliver multigram quantities of the protected desoxy-biphenomycin B (**2**). Nevertheless, with respect to scale-up towards an economically feasible production process, we encountered some limitations of this pathway, which were (1) the overall synthesis is still a lengthy process, and the majority of transformations are unproductive protecting group manipulations; (2) the boronate **6** is introduced on a highly functionalized intermediate, rendering the use of costly pinacolato diboron mandatory; and (3) several intermediates had to be purified by chromatography.

As the project advanced, the demand for key intermediate **2** increased readily reaching the kilogram scale. Accordingly, we revised the discovery route and embarked on the development of a scaleable route for **2**. Our endeavours towards this multi-kilogram-scale synthesis are outlined in the following chapters.

RESULTS AND DISCUSSION

Retrosynthesis of the Process Route. On the basis of our experience with the discovery route, we planned to utilize the double asymmetric hydrogenation as a key step, however, on the higher functionalized substrate **9** later in the sequence (cf. Scheme 2). This would offer an avenue to a more convergent synthesis as building blocks **10**−**12** should each be synthesized by a comparable level of complexity. Furthermore, the number of linear steps from intermediate **9** to the target molecule would be reduced substantially.

The macrocyclization should remain between the N^{α}-ornithine and the corresponding carboxylic acid as established by Schmidt, who reported a good yield (85%) for this step. In addition, we learned from the research phase that this transformation can be run at fairly high concentrations (4 mmol/L) to reach complete conversion within minutes. Therefore, we anticipated obtaining good volume productivity for this crucial step at scale.

Furthermore, we planned to abolish the costly 2-(trimethylsilyl)-ethyl (TMSE)-protecting group, which would have the benefit of decreasing the overall number of steps. The differentiation between the two carboxy groups in **9** would then be achieved by simply leaving one carboxylic acid unprotected. Additionally, the need for elaborate chromatographic purification of several intermediates in the discovery route had been attributed to the presence of the TMSE-ester. Thus, we speculated that workup procedures might be simplified if the TMSE-group could be replaced.

The synthesis of dehydroamino acid **10** should be straightforward by a Wadsworth−Emmons protocol using aldehyde **4b** and

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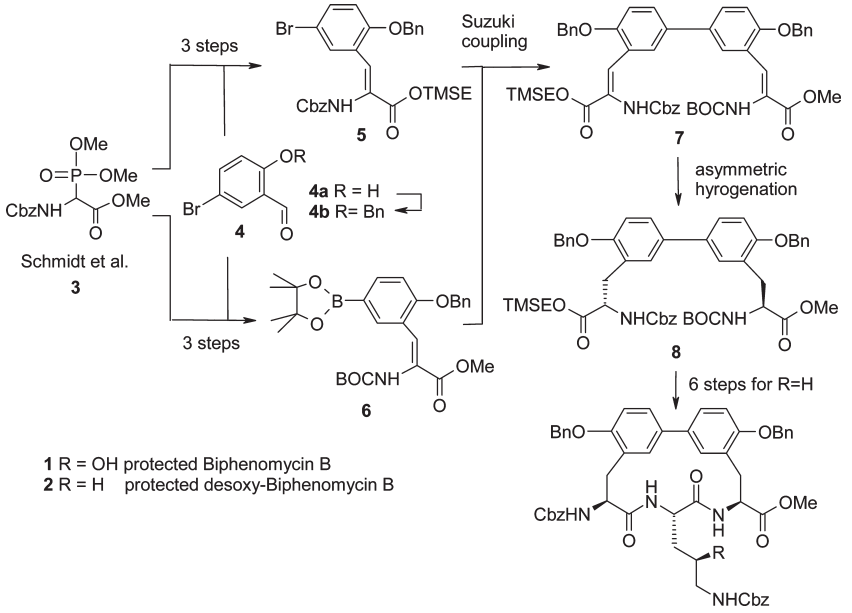
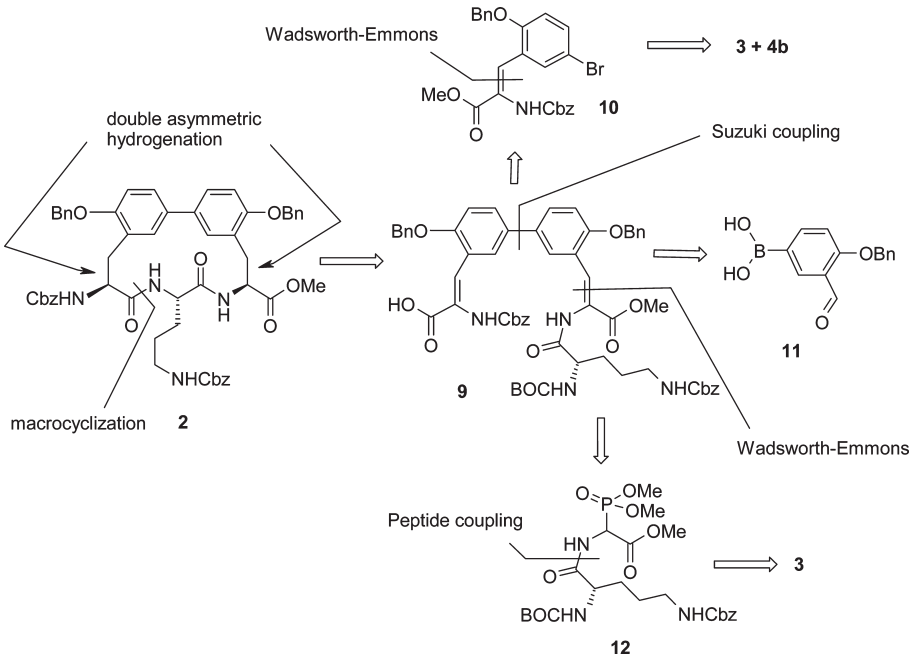
Scheme 1. Bayer's discovery route to protected desoxy-biphenomycin B (2)



Scheme 2. Retrosynthesis for the process route to protected desoxy-biphenomycin B (2)



phosphonate **3** (Scheme 1), while the boronate **11** has been reported in the literature and offers the opportunity to circumvent costly bispinacolato diboron at scale.[5]

Access to the phosphoryl dipeptide **12** was anticipated by a standard peptide coupling employing the deprotected phosphonate **3** (Scheme 1), which is additionally required as reagent for the synthesis of **10**.

Pilot-Plant Synthesis of Phosphonate 3. The phosphonate **3** is a very useful reagent for the synthesis of dehydroamino acids, and we embarked on a multikilogram synthesis based on the procedures reported by Schmidt et al. (cf. Scheme 3).[6] The condensation reaction between glyoxylic acid monohydrate **13** and benzyl carbamate **14** is reported to proceed in diethyl ether in good yields.[7] For safety reasons, we replaced diethyl ether by toluene and observed no detrimental effects on the reaction rate or the product quality. On a 26-L scale, we obtained 5 kg of the desired mono condensation product **15** in 96% yield and 97% purity (HPLC area %). To our dismay, we could not transfer this process to pilot-plant scale without further adaptations. When we ran the lab-protocol on a 150-L scale, we observed the formation of 7% of the bis-adduct **17** (cf. Figure 1), which had not been observed in significant amounts in lab or kilogram/lab scale before.

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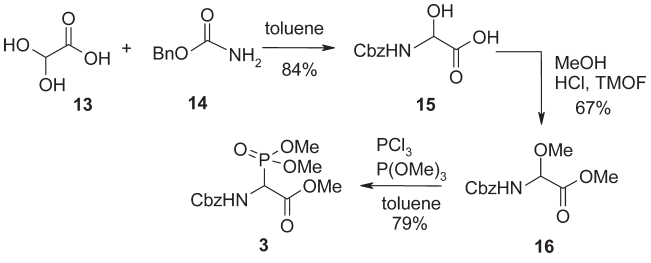
We scrutinized a range of parameters such as reaction time, stoichiometry, time for dosing, mixing, quality of raw materials etc., however, we could not identify the origin of the formation of **17** at scale. Therefore, we focused on a purification strategy to remove **17** by a subsequent operation. Upon workup, the bis-adduct **17** cocrystallized with the desired product **15** under a variety of conditions since **17** proved to be poorly soluble in organic solvents. Accordingly, mixtures of **15** and **17** were resuspended in methanol, unsoluble **17** was removed by filtration, and the filtrate containing **15** was directly used in the next step.

The formation of benzyloxy carbonyl (Cbz)-protected methoxy glycine ester **16**[8] was accomplished by reaction of **15** with methanol in the presence of trimethyl orthoformate (TMOF) and catalytic amounts of HCl to yield **16** in 98% purity with an average yield of 67%.

The formation of **3**, as outlined in Scheme 3, is a two step process comprising of a chlorination and a phosphorylation reaction.[6a,b,7c,9] The chlorination was accomplished employing PCl_3 in toluene, and subsequently the phosphonate was introduced upon reaction with trimethyl phosphite. In lab scale we observed significant variations in reaction time for the chlorination step. In most cases the chlorination was complete within hours, while conversion of some batches of **16** were incomplete even after 24 h at reflux. We could trace down this effect to the quality of the starting material **16**. Batches of **16**, which had been synthesized by an old procedure using sulphuric acid as catalyst were converted to **3** at a significantly faster rate. Thus, we added a catalytic amount of sulphuric acid (0.3%) to the chlorination cocktail, and, to our delight, we observed high reproducibility for the chlorination step independent of the batch history of **16**. In all cases chlorination was complete within 16 h and subsequent phosphorylation proceeded smoothly within 2.5 h.

Scheme 3. Synthesis of phosphonate 3



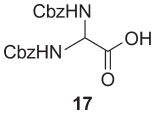


Figure 1. Side product arising from double condensation of **13** and **14**.

In total, four batches of the phosphorylglycin ester **3** were synthesized in our pilot plant to give 153 kg of the desired reagent with an average yield of 79% and an average purity of 98% (assay).

Synthesis of Dehydroamino Acid (10). The synthesis of dehydroamino acid **10** was achieved by a two step protocol starting from 5-bromo salicylaldehyde (**4a**). Benzyl protection was achieved by the use of benzyl chloride in the presence of sodium carbonate and catalytic amounts of sodium iodide.[3a,10] Accordingly, the protected intermediate **4b** was obtained in an average yield of 98% on a 26-L scale (cf. Scheme 4).

The Wadsworth–Emmons reaction of phosphonate **3** with aldehydes has been pioneered by Schmidt et al.[6a,b,11] In our hands, the highest diastereoselectivity was obtained in THF at −20 °C with tetramethylguanidine as a base. We ran seven batches on a 20-L scale to give a total of 14 kg of **10** with an excellent average yield of 97% and a *Z*/*E* ratio of 97:3. The diastereoselectivity could be improved to 99% by recrystallization from a 2.5:1 mixture of methyl *tert*-butyl ether and dioxane. However, we abandoned this purification step and used the crude *E*/*Z* mixture since the minor *E*-isomer could completely be removed during the workup in the following Suzuki coupling.

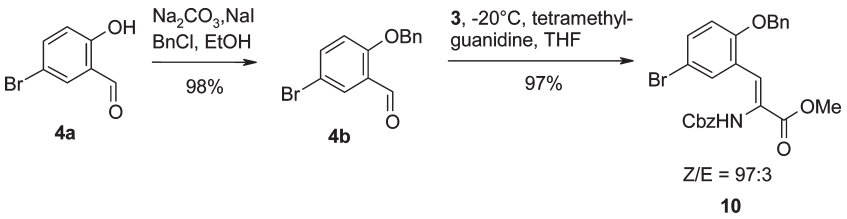
Pilot-Plant Synthesis of Boronate 11. The benzyl protected bromo salicylaldehyde **4b** was converted to cyclic acetal **18** upon reaction of **4b** with 1,3-propanediol in cyclohexane in the presence of catalytic amounts of sulphuric acid and azeotropic removal of water.[3a,b,5b] On a 25-L scale the process proved highly reproducible (eight batches) to deliver acetal **18** in an average yield of 89%.

The introduction of the boronic acid was accomplished by Schmidt et al. via a Grignard protocol.[5b,12] We considered a low temperature (−75 °C) bromo–lithium exchange pathway more attractive for scale-up since the reaction was dose-controlled and could be monitored more precisely.[13] The price to pay was a higher dilution (0.45 mol/L) in order to keep the reaction mixture agitated at −75 °C.

The reaction, as outlined in Scheme 5, was run on a 250-L scale to yield 7.0–7.6 kg of the boronic acid (99% purity) which corresponds to an average yield of 69%. The moderate yield is partly explained by an incomplete crystallization process from a toluene methylcyclohexane mixture leaving a significant amount of **11** in the mother liquor. The second source for a product loss is the formation of deborylated impurity **19**, which accumulates in the mother liquor (cf. Figure 2). This impurity is found at a level of approximately 6 area % after aqueous workup, and we assume that it is formed by hydrolysis of **11** after the aqueous quench. The sensitivity of **11** towards a protodeborylation mechanism was also confirmed by the short half-life of **11** under the aqueous conditions of the subsequent Suzuki coupling.

Synthesis of Phosphonate 12. The hydrogenolytic removal of the Cbz-group in **3** and the subsequent peptide coupling of the resulting amine **20** has been reported before.[6b,14] In our hands, deprotected intermediate **20** proved very unstable and readily

Scheme 4. Synthesis of dehydroamino acid 10



polymerized upon storage.[15] In contrast, a dilute solution of **20** in THF could be stored at 4 °C for several days without significant decomposition. Thus, we abandoned further experiments towards product isolation and carried on with the crude product solution after filtration.

The THF solution of **20** was directly coupled with commercially available Boc-ornithine(Cbz)−OH employing 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) and *N*-methylmorpholine (NMM). The latter coupling cocktail was chosen on the basis of costs over other alternatives (e.g., EDC) which worked equally well. After aqueous workup and removal of the solvents, phosphonopeptide **12** was obtained as an oil in 90% yield with a purity of 76 area % (see Scheme 6). It was used in the following Wadsworth−Emmons protocol without further purification.

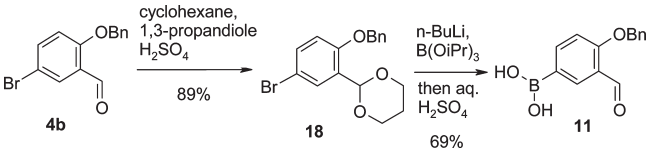
Synthesis of Bis-dehydroamino Acid 9. The synthesis of the biphenyl derivative **21** was achieved via a Suzuki coupling between bromoaryl **10** and boronic acid **11**.[5a,12,16] The solvent of choice was a 97:3 mixture of toluene and water from which the desired product conveniently crystallized directly from the reaction mixture.

We observed that under the aqueous conditions at elevated temperatures (approximately 90 °C) the half-life of the boronic acid was dependent on the nature of the base. Accordingly, we found potassium carbonate to be the best compromise between reaction rate and the deborylation pathway leading to the formation of **19** (cf. Figure 2). Thus, complete conversion was achieved with as little as 1.2 equiv of the boronic acid **11** if an aqueous solution of potassium carbonate was dosed to the reaction mixture containing **10**, **11**, 1% palladium acetate and 4% tri-*o*-tolylphosphine at reflux temperature within 45 min. The workup included a hot filtration through a bed of charcoal, crystallization upon cooling, and isolation by filtration. Eventually, the product was isolated as pure *Z*-isomer with an average chemical purity of 97% (assay) and an average yield of 94% (Scheme 7).

The saponification of **21** using lithium hydroxide in dioxane/water proceeded smoothly with full conversion after 17 h at 20 °C. However, we faced serious problems during product isolation, since the free carboxylic acid of **22** precipitated in very fine crystals yielding a non-filterable suspension. Thus, we decided to isolate the Li-salt, which precipitated from the reaction mixture by the addition of water. More importantly, due to its salt character **22** grew suitable crystals for filtration. Finally, saponification of **21** was conducted on a 3.3-kg scale with convenient filtration times yielding **22** in an average yield of 99% (Scheme 7).

The bis-dehydroamino acid building block **9** is a key intermediate in the synthesis of target molecule **2**. The tetramethyl guanidine-mediated Wadsworth−Emmons protocol towards **9** proceeded within hours in methanol or acetonitrile, while in THF the conversion was not complete within one day at 20 °C. Interestingly, the side-product profile was also solvent dependent. In methanol and acetonitrile only traces of the diester byproduct **24** were formed, while *E*-isomer **23** occurred in levels of up to 6% (cf. Figure 3). In THF the *Z*/*E* selectivity was increased to >97:3; however, up to 8% of the diester **24** was detected in the reaction mixture. This implies a mechanism involving methyl phosphonate species as the only reasonable source for methyl ester formation. Accordingly, we speculated that an aqueous *in situ* quench of alkylating phosphonate intermediates might reduce diester formation. Indeed, addition of 1.25% water to the THF reaction mixture reduced the formation of **24** from 8% to 2−3%. Moreover, the addition of water led to a drastic

Scheme 5. Synthesis of boronic acid 11



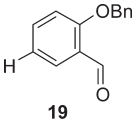
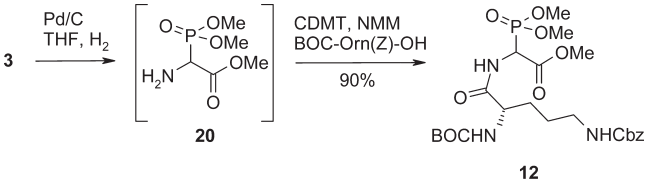
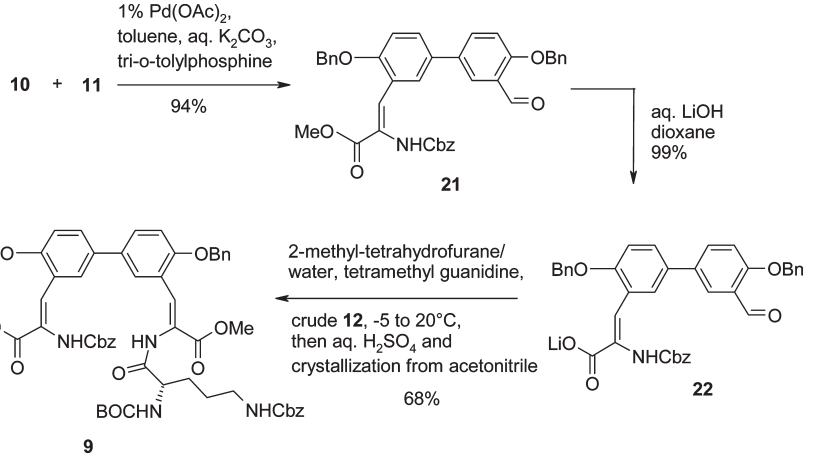


Figure 2. Protonated side product during the formation of **11**.

Scheme 6. Synthesis of phosponopeptide 12



Scheme 7. Synthesis of bis-dehydroamino acid derivative 9



improvement of the solubility of the reaction components **22** and **9**. This was beneficial for the volume productivity as well as for the reaction rate, leading to a complete conversion within several hours. Notably, the Wadsworth−Emmons protocol was compatible with the presence of the carboxylic acid in **22**. This observation confirms our initial assumption that the TMSE-protecting group used in the discovery route can easily be replaced by the free carboxy group. This simplification of the protecting group strategy adds considerable value to the overall synthesis.

Later in the project we learned that the presence of inorganic impurities in **9** had a detrimental effect on the efficiency and reproducibility of the enantioselective hydrogenation protocol. Thus, we replaced THF by 2-methyl-THF in order to conduct an aqueous workup with good phase separations. Eventually, the bis-dehydroamino acid **9** was crystallized from acetonitrile to deliver **9** with good chemical (>98%, assay) and optical purity (*Z*/*E* >99:1) in 68% yield based on **22** (Scheme 8).

Synthesis of target molecule 2. The hydrogenation of **9** using the achiral Wilkinson's catalyst delivered a random mixture of diastereomers indicating that the stereogenic center in the ornithine side chain did not control a side selective hydrogenation. Therefore, we screened a variety of commercially available rhodium catalysts with bidentate chiral phosphine ligands. Among the catalysts tested the phospholane type ligands[17] (cf. Figure 4) were identified as the ligands of choice with respect to stereoselectivity and conversion.[18]

In the course of the studies, we observed that reproducibility of the hydrogenation was dependent on the batch history of starting material **9**. In particular, old preparations of **9**, which had been worked-up with hydrochloric acid were among the most problematic batches since the latter did not show any conversion with as much as 10 mol % of catalyst at 80 bar of hydrogen pressure. Since we carefully excluded the presence of oxygen and we used batches of **9** with purities >98%, only, we speculated that the presence of inorganic salts might contribute to this phenomenon. Indeed, the critical batches of **9** were completely converted to **25** with quantitative yield and high selectivity (>99% de) with 1 mol % of (*S*,*S*)-Et-DuPHOS-Rh using a 1 mol % silver triflate as additive. This observation strongly suggested that the lack of reproducibility could be traced back to halide impurities. Accordingly, as described above, we introduced an aqueous workup during the isolation of **9** and replaced hydrochloric acid by sulphuric acid. With this measure in place, we obtained starting material **9** in quality consistently free of halides, which smoothly underwent hydrogenation with a high level of reproducibility.[19]

The conversion to **25** was a clean reaction. Thus, we decided to use the filtered reaction mixture for the next step without further purification.

The introduction of the pentafluorophenyl (PFP) ester was accomplished via an activation of the carboxylic acid by a mixed anhydride.[20] The PFP ester **26** was isolated by crystallization in 98% yield for two steps.

The final macrolactamization was a two-step process including removal of the ornithine *tert*-butoxy)carbonyl (BOC)-group followed by liberation of the resulting HCl salt with concomitant ring closure. Cleavage of the BOC group was accomplished by treatment of **26** with HCl in dioxane (20 h at 23 °C) and the resulting solution of the hydrochloride salt was added within 70 min to a solution of an excess triethylamine in THF. The protected desoxy-biphenomycin **2** was poorly soluble in organic solvents—a fact that could conveniently be exploited for product isolation. Thus, **2** was collected by filtration, and after extensive washing a quality of 97 area % was achieved, which was sufficient for further use.

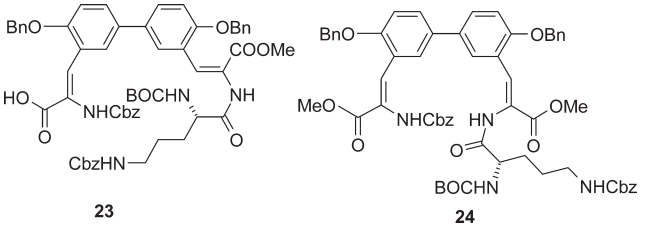


Figure 3. Side products during the formation of **9**.

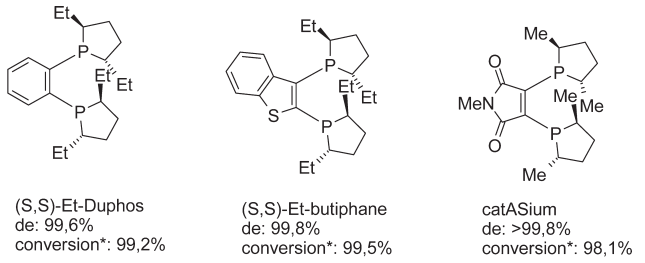
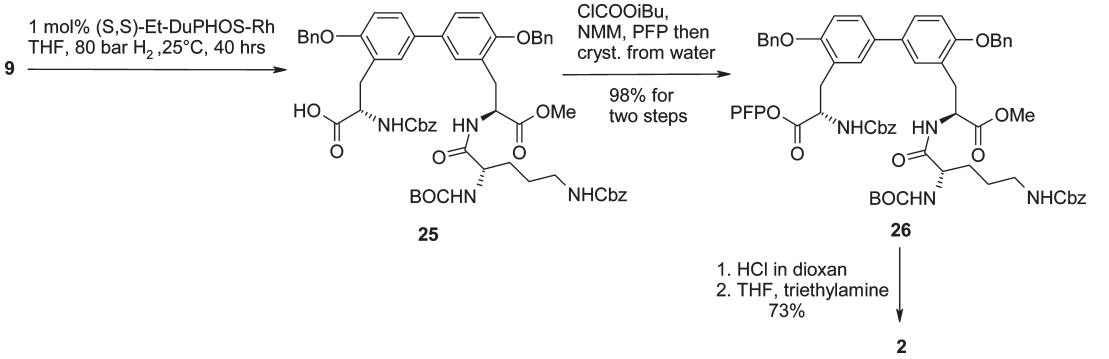


Figure 4. Most efficient phospholane-type ligands for the asymmetric double hydrogenation.

Scheme 8. Synthesis of target molecule 2[a]



[a] NMM = *N*-methylmorpholine; PFP = pentafluorophenol.

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To our delight, the sequence could be run with a fairly high concentration of up to 57 mmol/L of **26**. Along these lines, we assume that both functional groups are preorientated for the ring closure by the rigid biphenyl backbone and the three-dimensional conformation controlled by the (*S*,*S*,*S*) stereochemistry. This hypothesis is supported by the observation that the (*R*,*S*,*R*) diastereomer of **26** derived from hydrogenation with (*R*,*R*)-Et-DuPHOS-Rh cyclized with a much slower rate and led to a sluggish reaction at a comparable concentration.

On a 20-L scale, approximately 1 mol of **26** was converted to target molecule **2** in 73% yield. This translates into some 700 g of the desired product per batch.

CONCLUSION

In conclusion, we present an efficient kilogram synthesis of protected desoxy-biphenomycin B (**2**). The salient features of the process route are (1) reduction of the overall number of steps from the discovery route to the process route from 18 to 16 with 14 isolated intermediates, only; (2) all tedious chromatographic separations were successfully replaced by crystallization or extraction protocols; (3) the overall yield for the longest linear sequence (**13** to **2**) was 19.5%; and (4) the convergence of the synthesis was increased which led to an improved economy in both resources and raw materials.

The new development route is the basis for an efficient manufacturing process, and we could demonstrate the superiority of this route by obtaining a total of 1.4 kg of protected desoxy-biphenomycin B as a result of the first production campaign.

EXPERIMENTAL SECTION

General Remarks. Melting points (uncorrected) were determined in capillaries using an apparatus manufactured by Büchi. Routine NMR spectra were recorded using a Bruker AC500 instrument. All spectra are calibrated against tetramethylsilane as an internal standard ($\delta = 0$). Coupling constants (J) are given in Hz. HRMS were acquired on a Micromass LCT, and IR-spectra were recorded on a Bruker IFS66 V.

For HPLC analysis several methods were elaborated using a Hewlett-Packard 1100. All HPLC analyses were conducted using a Phenomenex Prodigy ODS3 (symmetry: 150 mm × 3 mm, 3 μm) column. *HPLC method A*: column: Phenomenex Prodigy ODS3, column symmetry: 150 mm × 3 mm, 3 μm; *T*: 40 °C; eluent: A = acetonitrile, B = aqueous phosphate buffer pH 2.4, gradient: 0−15 min 50% A, 15−25 min from 50% A to 90% A, 25−45 min 90% A; flow rate: 0.5 mL/min; detection at 220 nm. *HPLC method B*: *T*: 40 °C eluent: A = aqueous phosphate buffer pH 2.4, B = acetonitrile, gradient: 0−45 min 90% A to 20%, flow rate: 0.5 mL/min; detection at 220 nm. *HPLC method C*: *T*: 25 °C eluent: A = aqueous phosphate buffer pH 2.4, B = acetonitrile, gradient: 0−25 min 90% A to 30% A; 25−45 min 20% A, flow rate: 0.5 mL/min; detection at 220 nm. *HPLC method D*: *T*: 25 °C eluent: A = aqueous phosphate buffer pH 2.4, B = acetonitrile, gradient: 0−20 min: 70% A, to 20% A; 20−40 min 20% A; flow rate: 0.5 mL/min; detection at 210 nm. *HPLC-method E*: analogous to method C, detection at 210 nm.

Benzyloxycarbonyl Aminohydroxyacetic Acid (15). A solution of 25.0 kg (165.3 mol) of benzylcarbamate, 16.8 kg (182.5 mol) of dihydroxyacetic acid monohydrate in 170 kg of toluene was stirred at 40 °C for 90 min. Subsequently, 40 kg of toluene was distilled off (40 °C, 100 mbar). Additionally, 50 kg of toluene was removed by distillation with concomitant addition of 50 kg of fresh toluene. It was stirred at 40 °C for 2 h and then cooled to 20 °C. The mixture was maintained at this temperature for one hour and then it was filtered. It was washed with 35 kg of toluene and dried in vacuo to yield 30.0 kg (81%) of the desired product. Analytical characterization revealed that the material was obtained in a purity of 91 area % containing 7% of side product **17**. The crude product was taken to the next step without purification.

In total, seven batches of **15** were produced in the pilot plant to yield 220.3 kg of hydroxyglycine **15** with an average yield of 84%. On a 3.3-kg benzylcarbamate scale the yield increased to 96%, and the purity rose to 97 area %. For analytical characterization, a laboratory-scale batch with 99.9 area % was analyzed. Melting point: 155 °C dec; HPLC (method C): R_t = 10.0 min, 99.9 area %; ^{1}H NMR (500 MHz, D_6-DMSO): δ = 5.06 (s, 2H), 5.23 (d, J = 8.8 Hz, 1H), 6.28 (s, broad, 1H), 7.31−7.39 (m, 5H), 8.14 (d, J = 8.8 Hz, 1H), 12.81 (s, broad, 1H); ^{13}C NMR (125 MHz, D_6-DMSO): δ = 65.4, 73.1, 127.7, 128.3 (3C), 136.7, 155.4, 171.0; HRMS (ESI$^+$): Calc. mass ($C_{10}H_{11}NO_5$) = 151.0633; found = 151.0635; IR (KBr): ν (cm^{-1}) = 532, 574, 607, 647, 695, 716, 738, 788, 893, 916, 989, 1004, 1020, 1093, 1249, 1272, 1334, 1378, 1407, 1453, 1498, 1531, 1700, 1732, 2535, 2719, 2943, 3039, 3335.

Benzyloxycarbonyl Amino Methoxyacetic Acid Methyl Ester (16). A suspension of 31.3 kg (139.0 mol) of **15** in 91 kg of methanol was heated to 55 °C and then stirred at 10 °C for 10 h. The side product **17** was filtered off, and it was washed with 37 kg of methanol. To the filtrate were added 29.6 kg (279.0 mol) of trimethyl orthoformate and 5 kg of a 1.25 M solution of HCl in methanol, and it was stirred at 56 °C for 30 min. Subsequently, at a temperature of 58−67°, the solvent was partially (115 kg) distilled off. It was cooled to 35 °C, 37.0 kg of diisopropyl ether was added, and after the addition of seed crystals it was cooled to 2 °C within 4 h. The product was isolated by filtration, and it was washed twice with 22.0 kg of diisopropyl ether. Finally, it was dried in vacuo at 45 °C to yield 22.9 kg of the Cbz-methoxy-glycinester **16** in 65% yield.

One campaign comprising seven batches amounted to a total of 163.3 kg of **16**. This corresponds to an average yield of 67% per batch. Melting point: 77 °C; HPLC (method C): R_t = 16.0 min, 98 area %; ^{1}H NMR (500 MHz, D_6-DMSO): δ = 3.26 (s, 3H), 3.67 (s, 3H), 5.08 (s, 2H), 5.16 (d, J = 8.8 Hz, 1H), 7.33−7.40 (m, 5H), 8.49 (d, J = 8.5 Hz, 1H); ^{13}C NMR (125 MHz, D_6-DMSO): δ = 52.1, 54.6, 65.8, 80.3, 127.8, 127.9,128.3 (3C), 136.5, 155.8, 167.8; HRMS (ESI$^+$): Calc. mass ($C_{12}H_{15}NO_5$) = 221.0688; found = 221.0692; IR (KBr): ν (cm^{-1}) = 521, 563, 581, 615, 663, 698, 736, 762, 790, 847, 899, 937, 981, 1031, 1103, 1196, 1226, 1262, 1362, 1440, 1455, 1533, 1688, 1753, 2844, 2946, 2973, 3035, 3313.

Benzyloxycarbonyl Amino(dimethoxyphosphoryl)acetic Acid Methyl Ester (3). To a solution of 0.05 kg (0.51 mol) of sulfuric acid and 37.5 kg (148.1 mol) of Cbz-methoxyglycinester **16** in 210 kg of toluene was added 24.0 kg (174.8 mol) of phosphorus trichloride at 75 °C within 1 h. It was stirred for 16 h at 75 °C. Subsequently, the excess phosphorus trichloride and part of the solvent was distilled off at 70 °C and 250 mbar. To the reaction mixture 20.7 kg of (166.8 mol) trimethyl phosphite was added within 1 h at 75 °C, and it was stirred at 90 °C for 1.5 h. Then it was cooled to 20 °C, and the pH was adjusted to pH 6.6, employing an ammonia solution (3% in water). The phases were separated, and the aqueous layer was extracted with 25 kg of toluene. The combined organic layers were washed with 30 kg of water, and the solvent was partly distilled off in vacuo (200 mbar)

at 60 °C. To the residue were added 53 kg of diisopropyl ether and seed crystals. It was stirred at 5 °C for 1 h. Subsequently, it was filtered and washed twice with 28 kg of diisopropyl ether. Finally, it was dried for 12 h at 50 °C and 30 mbar to yield 42.6 kg (87%) of the desired product. In one production campaign, four batches were manufactured to yield a total of 152.6 kg of **3** with an average yield of 79%.

Melting point: 80 °C; HPLC (method C): R_t = 14.1 min, 98 area %; ^{1}H NMR (500 MHz, D$_6$-DMSO): δ = 3.71 (m, 9H), 4.85 (dd, J = 9.1 Hz, 23.6 Hz, 1H), 5.09 (s, 2H), 7.32−7.38 (m, 5H), 8.36 (d, J = 8.8 Hz, 1H); ^{13}C NMR (125 MHz, D$_6$-DMSO): δ = 51.4, 52.6, 52.7, 53.6, 66.0, 127.7 (2C), 127.8, 128.3 (2C), 136.6, 156.1, 167.2; HRMS (ESI$^+$): Calc. mass ($C_{13}H_{18}NO_7P$) = 332.0894; found = 332.0890; IR (KBr): ν (cm^{-1}) = 526, 577, 603, 672, 704, 734, 757, 785, 831, 843, 862, 919, 962, 985, 1003, 1030, 1061, 1172, 1212, 1239, 1274, 1333, 1427, 1455, 1467, 1532, 1716, 1749, 2859, 2964, 3033, 3230, 3418.

2-Benzyloxy-5-bromobenzaldehyde (4b). Under a nitrogen atmosphere a 26 L-HC4 vessel was charged with a solution of 3.02 kg (15.0 mol) of 5-bromosalicylaldehyde and 22.5 g (0.15 mol) of sodium iodide in a mixture of 6.0 L of ethanol and 1.5 L of water. Subsequently, 954 g (9.0 mol) of sodium carbonate was added at 22 °C, and it was heated to reflux for 3 h. To the mixture was added 2.09 kg (16.5 mol) of benzylchloride, it was stirred at reflux for 5 h and at 25 °C overnight. For workup, 4.5 L of water was added; it was filtered and washed four times with 1.0 L of aq sulphuric acid (2% in water) and five times with 1.0 L of water. The product was dried in vacuo at 40 °C to yield 4.31 kg (14.8 mol, 99% yield) of the benzyl ether **4b**. In one campaign, 42.6 kg of the title compound was synthesized in 10 batches with an average yield of 98%.

Melting point: 71 °C; HPLC (method A): R_t = 22.3 min, 99%; ^{1}H NMR (500 MHz, D$_6$-DMSO): δ = 5.29 (s, 2H), 7.31 (d, J = 8.9 Hz, 1H), 7.37 (m, 1H), 7.42 (m, 2H), 7.52 (m, 2H), 7.76 (d, 1H), 7.79 (m, 1H), 10.34 (s, 1H); ^{13}C NMR (125 MHz, D$_6$-DMSO): δ = 70.2, 112.7, 116.7, 125.9, 127.5 (2C), 128.0, 128.5 (2C), 129.9, 136.0, 138.3, 159.5, 187.9; HRMS (ESI$^+$): Calc. mass ($C_{14}H_{11}BrO_2$) = 289.9942; found = 289.9939; IR (KBr): ν (cm^{-1}) = 530, 665, 694, 735, 812, 883, 1024, 1032, 1124, 1185, 1237, 1274, 1307, 1396, 1409, 1450, 1476, 1485, 1496, 1590, 1678, 2566.

3-(2-Benzyloxy-5-bromophenyl)-2-benzyloxycarbonyl Aminoacrylic Acid Methyl Ester (10). To a solution of 1.83 kg (6.30 mol) of 2-benzyloxy-5-bromobenzaldehyde and 2.30 kg (6.93 mol) of the phosphonate **3** in 12.2 L of THF 0.84 kg (7.25 mol) of *N*,*N*,*N*′,*N*′-tetramethylguanidine was added at −20 °C within 22 min. It was maintained at this temperature for 4.5 h and subsequently warmed up to 20 °C. To the reaction mixture was added 24.4 kg of water, and the pH was adjusted to 1−2 using conc. sulphuric acid. It was stirred at 3 °C for 1.5 h, filtered, and washed three times with 3.75 L of water. The product was dried at 40 °C in vacuo to obtain 3.06 kg (5.10 mol, 98% yield) of the desired dehydroamino acid **10**, which was isolated in 96% purity (HPLC, method A) with a *Z*/*E* ratio of 97:3. For an analytical characterization, a sample was recrystallized from methyl *tert*-butyl ether/dioxane (2.5:1) to yield >99.5% area % of the *Z*-isomer. In one production campaign, seven batches of **10** were synthesized as described above to give 14.3 kg of the desired material with an average yield of 97%.

Melting point 156 °C; HPLC (method A): R_t = 25.1 min, 99.7%; ^{1}H NMR (500 MHz, D$_6$-DMSO): δ = 3.70 (s, 3H), 5.11 (s, 2H), 5.20 (s, 2H), 7.12 (d, J = 8.83 Hz, 1H), 7.33−7.46 (m, 11H), 7.53 (J = 8.83 Hz, 1H), 7.85 (s, 1H), 9.28 (s, breit, 1H); ^{13}C NMR (125 MHz, D$_6$-DMSO): signals for aromatic carbon partially overlap δ = 52.3, 65.8, 69.9, 112.1, 115.1, 124.2, 126.7, 127.1, 127.3 (2C), 127.4, 127.6, 127.8, 127.9, 128.3, 128.5 (2C), 131.3, 133.1, 136.3, 154.4, 155.5, 165.3; HRMS (ESI$^+$): Calc. mass ($C_{25}H_{22}BrNO_5$) = 496.0755; found = 496.0754; IR (KBr): ν (cm^{-1}) = 526, 607, 629, 651, 698, 736, 764, 786, 811, 886, 961, 1019, 1045, 1131, 1220, 1235, 1254, 1299, 1367, 1385, 1408, 1437, 1453, 1485, 1511, 1586, 1693, 1733, 3029, 3331.

2-(2-Benzyloxy-5-bromophenyl)-[1,3]dioxolane (18). In a nitrogen atmosphere 4.30 kg (14.8 mol) of benzyloxy-5-bromobenzaldehyde was suspended in 16.3 L of cyclohexane, and 29.4 g (0.30 mol) of sulphuric acid and 13.8 kg (17.8 mol) of 1,3-propandiol were added. It was heated to reflux, and 290 mL of water was separated by azeotrope distillation, while the temperature rose from 77 to 83 °C. It was cooled to 60 °C, and within 15 min a solution of 39.2 g sodium carbonate in 165 mL of water was added. It was cooled to 10 °C, stirred for 30 min, and filtered. The filter cake was washed twice with 3.0 L of water, and the product was dried in vacuo at 40 °C to yield 4.65 kg (13.3 mol, 90% yield) of the dioxolane **18**. In eight batches, a total of 35.3 kg was produced, which corresponds to an average yield of 89%.

Melting point: 93 °C; HPLC (method A): R_t = 24.05 min, 98%. ^{1}H NMR (500 MHz, D$_6$-DMSO): δ = 1.40 (d, J = 13.4 Hz, 1H), 2.02 (m, 1H), 3.90 (t, J = 10.6 Hz, 2H), 4.12 (m, 2H), 5.15 (s, 2H), 5.79 (s, 1H), 7.05 (d, J = 8.8 Hz, 1H), 7.32 (m, 1H), 7.38−7.48 (m, 5H), 7.55 (d, J = 2.4 Hz, 1H); ^{13}C NMR (125 MHz, D$_6$-DMSO): δ = 25.3, 66.8 (2C), 69.6, 95.4, 111.9, 115.0, 127.0 (2C), 127.7, 128.4 (2C), 129.4, 129.5, 132.2, 136.7, 154.4; HRMS (ESI$^+$): Calc. mass ($C_{17}H_{17}BrO_3$) = 349.0434; found = 349.0436.

IR (KBr): ν (cm^{-1}) = 554, 673, 695, 733, 816, 859, 880, 899, 930, 958, 990, 1011, 1022, 1034, 1103, 1134, 1147, 1186, 1246, 1275, 1384, 1412, 1430, 1452, 1464, 1498, 1597, 2848, 2954, 2976.

[4-(Benzyloxy)-3-formylphenyl]boronic Acid (11). In a 250-L alloy vessel was added 21.0 kg (49.0 mol) *n*-butyl lithium (1.6 M in hexane) to a solution of 15.0 kg (43.0 mol) of 2-(2-benzyloxy-5-bromophenyl)-[1,3]-dioxolane in 83 L of THF at −75 °C within 1.5 h. It was stirred for 30 min followed by the addition of 9.30 kg (49.0 mol) of triisopropylborate. Within 3 h it was warmed to −10 °C, and 16.8 kg of dilute sulphuric acid (20% in water) was added. It was maintained at 40 °C for 4 h. Subsequently, the organic layer was washed four times with 13.5 kg water; 67.3 L of toluene was added, approximately 150 L of solvent was distilled off in vacuo, and 97.4 L of methylcyclohexane was added. It was cooled to 0 °C, filtered, and washed twice with 18.8 L of methylcyclohexane. Finally, the product was dried at 30 mbar and 60 °C for 16 h to yield 7.6 kg (69%) of the boronic acid **11**. A second batch delivered an additional 7.0 kg of the boronic acid **11**.

Melting point: 199 °C; HPLC (method B): R_t = 17.6 min, 99%; ^{1}H NMR (500 MHz, D$_6$-DMSO): δ = 5.32 (s, 2H), 7.30 (d, J = 8.5 Hz, 1H), 7.36 (m, 1H), 7.42 (t, J = 7.6 Hz, 2H), 7.53 (d, J = 7.4 Hz, 2H), 8.08 (dd, J = 1.2 Hz, 8.3 Hz, 1H), 8.24 (d, J = 1.2 Hz, 1H), 10.50 (s, 1H); ^{13}C NMR (125 MHz, D$_6$-DMSO): δ = 69.7, 112.9, 123.7, 127.5 (2C), 127.9, 128.5 (2C), 134.4, 136.4, 142.2, 162.0, 189.3; CHN ($C_{14}H_{13}BO_4$): calc. C 65.7% H 5.2%; found C 65.9% H 5.2%; MS (ES$^+$): [M + H]$^+$ = 257, [M + Na]$^+$ = 279; IR (KBr): ν (cm^{-1}) = 523, 617, 651, 699, 740, 802, 825, 915, 934, 1015, 1042, 1075, 1116, 1158, 1223, 1270, 1282, 1296, 1315, 1350, 1381, 1427, 1454, 1463, 1499, 1572, 1604, 1673, 2870, 3315 (broad).

(5-Benzyloxycarbonylamino-2-*tert*-butoxycarbonyl Amino-pentanoylamino)(dimethoxyphosphoryl)acetic Acid Methyl Ester (12). A solution of 547 g (1.54 mol) of **3** and 37.0 g of 5% palladium on charcoal (50 weight % in water) in 2.1 L of THF was exposed to an 80 bar hydrogen atmosphere for 4 h at 20 °C. The reaction mixture was passed through a plug of celite, and it was washed with 400 mL of THF. To the combined filtrates were added 513 g (1.4 mmol) of Boc-Orn-(*Z*)-OH and 246 g (1.4 mmol) of 2-chloro-4,6-dimethoxy-1,3,5-triazine, and it was cooled to 0−5 °C. Subsequently, 154 mL (1.4 mmol) of *N*-methylmorpholine was added dropwise within 20 min, and it was stirred at 20 °C for 18 h. The solvent was removed in vacuo, and the residue was redissolved in ethyl acetate. It was filtered off, and the filter cake was washed twice with 250 mL of ethyl acetate. The combined filtrates were washed with 1.05 L of a sat. aqueous $NaHCO_3$ solution, twice with 1.05 L of aqueous HCl (1 molar), again with 1.25 L of a sat. aqueous $NaHCO_3$ solution and with 1.0 L of water. Finally, the solvent was distilled off *in vacuo* to give 758 g (1.39 mol, 90% yield) of the desired product as an oil. According to HPLC analysis (method A), the crude product had a purity of 77 area %. It was used in the next step without further purification. For analytical characterization, a sample of the above material was purified by preparative HPLC. Conditions for the preparative HPLC separation: Phenomenex Prodigy ODS3, column symmetry: 250 mm × 50 mm, 10 μm; eluent: A = water, B = acetonitrile, gradient = 0−22 min: 60% A, to 20% A; 22−23 min 20% A to 60% A; 23−28 min 60% A; flow rate = 140 mL/min; detection at 240 nm.

HPLC (method D): R_t = 10.16 min, 99 area %; ^{1}H NMR (500 MHz, D_6-DMSO): δ = 1.37 (s, 9H), 1.33−1.58 (m, 4 H), 2.98 (m, 2H), 3.66−3−73 (m, 9H), 4.09 (m, 1H), 5.00 (m, 2H), 5.08 (m, 1H), 6.94 (dd, *J* = 9.52 Hz, 21.44 Hz, 1H), 7.24 (m, 1H), 7.35 (m, 5H), 8.66 (dd, *J* = 8.20 Hz, 18.6 Hz, 1H); ^{13}C NMR (125 MHz, D_6-DMSO): δ = 26.0, 28.2 (3C), 29.1, 40.0, 49.0, 52.8, 53.5, 53.7, 53.8, 65.2, 78.1, 127.8 (2C), 128.4 (3C), 137.3, 155.3, 146.1, 166.9, 172.6; HRMS (ESI$^+$): Calc. mass ($C_{23}H_{36}N_3O_{50}P$) = 546.2212; found = 546.2209; IR (KBr): ν (cm^{-1}) = 631, 699, 740, 779, 841, 868, 916, 1036, 1168, 1256, 1368, 1393, 1439, 1455, 1529, 1715, 1751, 2959, 3034, 3318.

2-Benzyloxycarbonyl Amino-3-(4,4′-bis-benzyloxy-3′-formylbiphenyl-3-yl)acrylic Acid Methyl Ester (21). In a nitrogen atmosphere, a 36-L stainless steel vessel was charged with a solution of 1.58 kg (3.0 mol) of the arylbromide **10** (containing 3.4 area % *E*-isomer), 0.92 kg (3.60 mol) of the boronic acid **11**, 37.0 g (0.12 mol) tris-*o*-tolylphosphine and 6.7 g (0.03 mol) of palladium(II)acetate in 11.4 L of toluene and 305 mL of water at 20 °C. It was heated to reflux temperature (approximately 88−91 °C) and 1.05 L of a 4 M aqueous potassium carbonate solution was dosed within 45 min. It was further kept at this temperature for 3.5 h followed by a hot filtration through a bed of charcoal. The filtrate was cooled to 20 °C overnight and subsequently cooled to 0−5 °C. It was filtered off, washed twice with 2.3 L of toluene and three times with 1.5 L of water. In order to remove residues of water-soluble salts, the crude product was suspended in 10.1 L of water, stirred at 22 °C for 1 h, filtered, and washed with 2.0 L of water. Finally, it was dried in vacuo at 40 °C to give 1.90 kg (99% yield) of the desired Suzuki coupling product **21**. In 10 batches, a total of 19.5 g of **21** was produced with an average yield of 94%.

Melting point: 142 °C; HPLC method B: HPLC: R_t = 38.3 min, 98.9%; ^{1}H NMR (500 MHz, D_6-DMSO): δ = 3.72 (s, 3H), 5.12 (s, 2H), 5.25 (s, 2H), 5.35 (s, 2H), 7.22−7.48 (m, 15H), 7.54 (d, *J* = 7.41 Hz, 2H), 7.65 (m, 2H), 7.85 (dd, *J* = 2.05, 8.67 Hz, 1H), 7.93 (m, 1H), 8.01 (m, 1H), 9.29 (m, breit, 1H), 10,48 (s, 1H); ^{13}C NMR (125 MHz, D_6-DMSO): δ = 52.2, 65.8, 69.8, 70.0, 113.3, 114.7, 122.4, 124.7, 125.1, 126.0, 126.6, 127.1, 127.3 (2C), 127.4 (2C), 127.5 (2C), 127.7, 127.8, 127.9, 128.2 (2C), 128.4 (2C), 128.5 (2C), 128.8, 131.0, 132.2, 133.8, 136.4, 136.6, 136.7, 154.5, 155.9, 159.7, 165.5, 189.0; HRMS (ESI$^+$): Calc. mass ($C_{39}H_{33}NO_7$) = 628.2330; found = 28.2346; IR (KBr): ν (cm^{-1}) = 559, 623, 656, 698, 743, 776, 803, 865, 912, 1011, 1071, 1127, 1141, 1182, 1231, 1247, 1263, 1294, 1376, 1435, 1454, 1483, 1504, 1605, 1639, 1692, 1721, 3271.

Lithium-(2*Z*)-2-{[(benzyloxy)carbonyl]amino}-3-[4,4′-bis-(benzyloxy)-3′-formylbiphenyl-3-yl]acrylate (22). A solution of 273 g (6.5 mol) LiOH in 4.6 L of water was added to a solution of 3.30 kg (5.12 mol) of the methyl ester **21** in 11.6 L of dioxane within 1 h at 20 °C. It was stirred at this temperature for 17 h followed by the addition of 13.2 L of water. It was filtered off, washed twice with 2.2 L of water and three times with 2.2 L of dioxane. The product was dried at 40 °C in vacuo to yield 3.16 kg (5.10 mol, 99% yield) with a purity of 98% (HPLC, method A). According to this protocol, five batches of **22** were manufactured to give a total of 15.3 kg, which corresponds to an average yield of 99%. Upon recrystallization the Li-salt dissociated. Thus, for an analytical characterization we generated the free carboxylic acid as follows: 125 g of the Li-salt was suspended in 2 L of water and treated with aqueous HCl (20%) to adjust the pH to 2. It was stirred for 3 h, filtered off, and washed three times with 0.5 L of water. The crude carboxylic acid was recrystallized from isopropanol/water (3:1).

Melting point: 163−165 °C; HPLC (method C): R_t = 31.4 min, 99.2 area %; ^{1}H NMR (500 MHz, D_6-DMSO): δ = 5.07 (s, 2H), 5.24 (s, 2H), 5.35 (s, 2H), 7.21−7−48 (m, 15H), 7.54 (d, *J* = 7.24 Hz, 2H), 7.63 (m, 2H), 7.83 (dd, *J* = 2.21 Hz, 8.51 Hz, 1H), 7.91 (m, 1H), 7.99 (s, 1H), 9.04 (s, broad, 1H), 10.46 (s, 1H), 12.79 (s, broad, 1H); ^{13}C NMR (125 MHz, D_6-DMSO): signals for aromatic carbons partially overlap δ = 65.7, 69.8, 70.0, 113.3, 114.8, 124.7, 125.1, 127.1, 127.4 (2C), 127.5 (2C), 127.7, 127.9, 128.0, 128.2 (2C), 128.5 (2C), 128.6 (2C), 131.0, 132.4, 133.8, 136.4, 136.7, 136.8, 154.5, 155.8, 159.7, 166.4, 189.1; HRMS (ESI$^+$): Calc. mass ($C_{38}H_{31}NO_7$) = 614.2174; found = 614.2199; IR (KBr): ν (cm^{-1}) = 696, 731, 803, 1006, 1022, 1072, 1128, 1180, 1245, 1270, 1286, 1376, 1430, 1453, 1483, 1498, 1507, 1519, 1605, 1627, 1692, 2852, 3034, 3252.

(2*Z*)-2-{[(Benzyloxy)carbonyl]amino}-3-[4,4′-bis(benzyloxy)-3′-((1*Z*)-2-{[N5-[(benzyloxy)carbonyl]-N2-(*tert*-butoxycarbonyl)-Lornithyl]amino}-3-methoxy-3-oxoprop-1-en-1-yl)biphenyl-3-yl]acrylic Acid (9). A solution of 177 g (5.52 mmol, 76 HPLC-area %) of phosphonate **12** in 338 mL of 2-methyltetrahydrofuran and 18 mL of water was added to a solution of 173 g (279 mmol) of lithium carboxylate **22** in 850 mL of 2-methyltetrahydrofuran. The resulting suspension was cooled to −5 to 0 °C, and within 90 min 98 g (818 mmol) *N*,*N*,*N*′,*N*′-tetramethylguanidine was added. It was slowly warmed to 20 °C and stirred for 18 h at this temperature. For workup, 700 mL of deionized water was added, and the pH was adjusted to 2.0, employing concentrated sulphuric acid. The layers were separated, and the organic layer was washed twice with 700 mL of deionized water. It was filtered through a bed of zeolite, and ∼900 mL of the solvent was destilled off in vacuo. The resulting suspension was diluted with 3.0 L of acetonitrile and heated to 50 °C for 3 h. Over a period of 18 h it was cooled to 20 °C. Subsequently, it was cooled to 0 °C, and the resulting solid was filtered off. It was washed three times

with 400 mL of cold acetonitrile and 1.0 L of deionized water. Finally, the product was dried in vacuo at 45 °C to yield 196 g (68%) of the desired product.

Melting point: 184 °C; HPLC (method E): R_t = 35.05 min, 98.3%; ^{1}H NMR (500 MHz, D$_6$-DMSO): δ = 1.22−1.62 (m, 13H), 2.90 (m, 2H), 3.66 (s, 3H), 4.10 (m, 1H), 4.98 (s, 2H), 5.05 (m, 2H), 5.19 (m, 2H), 5.25 (s, 2H), 6.91 (dd, J = 7.9 Hz, 1H), 7.09−7.48 (m, 25H), 7.66 (s, 1H), 7.72 (d, J = 8.2 Hz, 1H), 7.91 (m, 1H), 7.98 (m, 1H), 9.73 (s, 1H), 9.01 (s, breit, 1H), 12.77 (s, breit, 1H); ^{13}C NMR (125 MHz, D$_6$-DMSO): δ = 25.9, 28.1 (3C), 28.8, CH$_2$NHCbz covered by solvent signal, 52.0, 53.7, 65.0, 65.7, 69.7, 69.8, 77.9, (signals for aromatic-C partly overlap) 113.0, 113.2, 122.5, 124.0, 126.5, 126.6, 126.9, 127.1, 127.6 (2C), 127.7 (2C), 127.8 (2C), 128.2 (2C), 128.3 (2C), 128.4 (2C), 128.5 (2C), 128.9, 131.6, 132.0, 136.7, 137.1, 154.5, 155.3, 156.0, 165.4, 172.2; HRMS (ESI$^+$): Calc. mass ($C_{59}H_{60}N_4O_{13}$) = 1033.4230; found = 1033.4215; IR (KBr): ν (cm^{-1}) = 696, 736, 806, 1024, 1051, 1132, 1168, 1246, 1367, 1453, 1492, 1522, 1608, 1658, 1693, 2948, 3334.

(2*S*)-2-{[(Benzyloxy)carbonyl]amino}-3-{4,4′-bis(benzyloxy)-3′-[(2*S*)-2-({(2*S*)-5-{[(benzyloxy)carbonyl]amino}-2-[(*tert*-butoxycarbonyl)amino]pentanoyl}amino)-3-methoxy-3-oxopropyl]biphenyl-3-yl}propanoic Acid (25). In an 10-L autoclave, a solution of 562 g (544 mmol) of the dehydroamino acid **9** and 3.54 g (5.4 mmol) (*S*,*S*)-Et-DuPHOS-Rh in 3.5 L of THF was stirred in a hydrogen atmosphere at 80 bar and 25 °C for 40 h. The resulting solution was filtered through a plug of zeolite, and it was washed with 0.5 L of THF. After analytical release (98 area %; >99% de) the combined filtrates (4.26 L) were directly taken to the next step. For an analytical characterization, a portion of the product solution was passed through a plug of silica and subsequently crystallized three times from THF/acetonitrile.

Melting point: 137 °C; HPLC (method A): R_t = 26.9 min, 99.4%; determination (HPLC):[21] column KBD5287 (Bayer HealthCare chiral stationary phase based on poly(*N*-methylacryloyl-L-leucine-dicyclopropylmethylamide), column symmetry: 250 mm × 4.6 mm; T: 22 °C; eluent: isocratic ethyl acetate 30 min; flow rate: 1 mL/min; detection at 220 nm; R_t for (*S*,*S*,*S*)-diastereomer = 11.2 min, >99.5% de; ^{1}H NMR (500 MHz, D$_6$-DMSO): δ = 1.16−1.50 (m, 13H), 2.80−2.97 (m, 4H), 3.18−3.36 (m, 2H), 3.50 (s, 3H), 3.91 (m, 1H), 4.42 (m, 1H), 4.62 (m, 1H), 4.90−5.04 (m, 4H), 5.15−5.19 (m, 4H), 6.74 (d, J = 8.2 Hz, 1H), 7.07 (t, J = 8.2 Hz, 1H), 7.18−7.53 (m, 26H), 7.60 (d, J = 8.2 Hz, 1H), 8.23 (d, J = 6.9 Hz, 1H), 12.67 (s, broad, 1H); ^{13}C NMR (125 MHz, D$_6$-DMSO): δ = 25.8, 28.0 (3C), 29.5, 32.0, 32.1, CH$_2$NHCbz covered by solvent signal, 51.6, 52.0, 53.5, 53.6, 65.0, 65.1, 69.1, 69.2, (signals for aromatic-C partly overlap), 112.1, 112.2, 125.3, 125.5, 125.7, 126.1, 126.6, 126.8 (2C), 126.9 (2C), 127.4 (2C), 127.5, 127.2, 127.5, 127.6 (2C), 128.1 (2C), 128.2 (2C), 128.2 (2C), 128.3 (2C), 128.7, 129.2, 131.9, 132.2, 136.8, 137.1, 155.0, 155.3, 155.9, 156.0, 172.0, 173.6; HRMS (ESI$^+$): Calc. mass ($C_{59}H_{64}N_4O_{13}$) = 1037.4543; found = 1037.4546; IR (KBr): ν (cm^{-1}) = 631, 696, 733, 778, 799, 861, 1024, 1051, 1132, 1168, 1247, 1367, 1439, 1453, 1493, 1525, 1608, 1657, 1692, 2947, 3032, 3064, 3333.

Pentafluorophenyl (2*S*)-2-{[(Benzyloxy)carbonyl]amino}-3-{4,4′-bis(benzyloxy)-3′-[(2*S*)-2-({(2*S*)-5-{[(benzyloxy)carbonyl]amino}-2-[(*tert*-butoxycarbonyl)amino]pentanoyl}amino)-3-methoxy-3-oxopropyl]biphenyl-3-yl}propanoate (26). The crude THF solution obtained for carboxylic acid **25** (4.26 L) was divided into two batches of the same size (each of 2.13 L) and consecutively transformed into the pentafluorophenyl ester **26**. At 0−5 °C a solution of 79.5 g (786 mmol) *N*-methylmorpholine in 80 mL of THF was added to the crude solution of **25** (2.13 L) and 57.3 g (419 mmol) of isobutyl chloroformate within 45 min. It was stirred at 0 °C for 1 h. Subsequently, a solution of 77.2 g (419 mmol) of pentafluorophenol in 150 mL of THF was added within 45 min, and the reaction was allowed to warm to 20 °C overnight. For workup, 850 mL of water was added, and the pH was adjusted to 2.0 using 73.5 mL of an aqueous 20% HCl. The organic layer was separated and concentrated to a volume of 1.8 L. The resulting solution was slowly added to 2.0 L of water within 2 h, and it was stirred at 20 °C for 18 h to complete the crystallization process. It was filtered, washed four times with 0.5 L of water and six times with 0.5 L of methyl *tert*-butyl ether. Finally, it was dried in vacuo to yield 308 g (98% for two steps) of the desired pentafluorophenol **26** with a purity of 91 area % (HPLC method A). In total, 2.6 kg of the desired material was synthesized according to this procedure in laboratory scale. For an analytical characterization, a portion of the product was recrystallized three times from THF/acetonitrile (1:2).

Melting point: 177 °C; HPLC (method A): R_t = 30.40 min, 97 area %; ^{1}H NMR (500 MHz, D$_6$-DMSO): δ = 1.16−1.50 (m, 13H), 2.85 (m, 3H), 3.07 (m, 1H), 3.19 (m, 1H), 3.46 (m, 1H), 3.50 (s, 3H), 3.61 (m, 1H), 3.92 (m, 1H), 4.63 (m, 1H), 4.86−5.04 (m, 4H), 5.21 (m, 4H), 6.74 (d, J = 8.2 Hz, 1H), 7.06−7.62 (m, 28H), 8.22 (m, 1H); ^{13}C NMR (125 MHz, D$_6$-DMSO): δ = 25.8, 28.1 (3C), 29.5, 31.8, 32.1, (CH$_2$NHCbz covered by solvent signal), 51.6, 52.0, 53.5, 53.6, 65.1, 65.7, 69.2, 69.3, 77.9, (signals for aromatic-C partly overlap), 112.3, 112.4, 124.7, 125.3, 125.4, 125.7, 126.1, 126.9, 127.0 (2C), 127.4, 127.5 (2C), 127.6 (3C), 127.7 (2C), 127.8, 128.2 (3C), 128.3 (3C), 128.4 (2C), 128.8, 129.2, 132.0, 132.1, 136.5, 136.8, 137.0, 137.1, 137.2, 155.1, 155.4, 156.0, 168.8, 172.1, 173.6; HRMS (ESI$^+$): Calc. mass ($C_{65}H_{63}F_5N_4O_{13}$) = 1203.4385; found = 1203.4371; IR (KBr): ν (cm^{-1}) = 576, 628, 696, 731, 775, 802, 859, 902, 1000, 1025, 1048, 1117, 1134, 1171, 1249, 1311, 1367, 1377, 1440, 1453, 1498, 1521, 1608, 1659, 1693, 1800, 2947, 3033, 3065, 3337.

Methyl (8*S*,11*S*,14*S*)-5,17-Bis(benzyloxy)-14-{[(benzyloxy)carbonyl]amino}-11-(3-{[(benzyloxy)carbonyl]amino}propyl)-10,13-dioxo-9,12-diazatricyclo-[14.3.1.12,6]henicosa-1(20),2(21),3,5,16,18-hexaene-8-carboxylate (2). To a solution of 1.27 kg (1.06 mol) of pentafluorophenylester **26** in 7.8 L of dioxan was added 3.59 L of HCl in dioxane (4 M) at 24 °C within 30 min. It was stirred for 20 h at 23 °C. The resulting reaction mixture was added over a period of 70 min to a solution of 1.55 kg (15.3 mol) of triethylamine in 7.0 L of THF. It was maintained at 23 °C for 80 min, and subsequently 9.4 L of THF and 7.8 L of acetonitrile were added. It was filtered and washed three times with 5.1 L of acetonitrile, and five times with 6.1 L of water. The crude product was suspended in a mixture of 2.0 L of methanol and 1.5 L of water and stirred at 45 °C for 3 h. It was filtered and washed three times with 1.0 L of methanol and twice with 0.5 L of water. The washing protocol using methanol and water was repeated twice until the filtrate was free of water-soluble salts. The resulting product was finally dried in vacuo at 45 °C for 48 h to yield 705 g (77%) of the desired macrocycle **2**. In a second batch 662 g (68% yield) of **2** was obtained. In total, 1.37 kg of the building block **2** was synthesized in an average yield of 73%. The purity of the material was sufficient for further use: analytical HPLC (method A, sample injected as DMSO solution): R_t = 27.2 min, 97 area %.

For an analytical characterization a sample of **2** was further purified by precipitation from a DMSO solution using acetonitrile

followed by extensive washing with methanol and water: melting point: >260 °C; HPLC (method A, sample injected as DMSO solution): R_t = 27.2 min, 99 area %; ^{1}H NMR (500 MHz, D_6-DMSO): δ = 1.46−1.64 (m, 4H), 2.89 (m, 2H), 3.00 (m, 2H), 3.39 (m, 2H), 3.67 (s, 3H), 4.54 (m, 1H), 4.59 (m, 1H), 4.82−4.96 (m, 3H), 5.00−5.17 (m, 6H), 6.33 (d, J = 7.57 Hz, 1H), 7.05 (m, 4H), 7.25−7.48 (m, 23H), 8.66 (d, J = 9.1 Hz, 1H), 8.99 (d, J = 8.5 Hz, 1H); ^{13}C NMR (125 MHz, D_6-DMSO): δ = 25.8, 28.1, 30.4, 30.5, $\underline{C}H_2$NHCbz covered by solvent signal, 50.5, 51.4, 52.2, 54.0, 65.0, 65.3, 69.4 (2C), (signals for aromatic-C partly overlap), 112.1, 112.3, 124.2, 124.3, 124.9, 125.6, 126.4, 127.1, 127.2 (2C), 127.4. 127.5, 127.6 (2C), 127.7, 128.1 (2C), 128.2 (2C), 128.4 (2C), 128.9, 131.4, 131.6, 137.1, 137.2, due to the occurrence of amide rotamers, the carbonyl signals are partly doubled: 154.6, 154.9, 155.5, 156.0, 169.3, 171.8, 171.9; HRMS (ESI$^+$): Calc. mass ($C_{54}H_{54}N_4O_{10}$) = 919.3913; found = 919.3907; IR (KBr): ν (cm^{-1}) = 518, 576, 597, 626, 696, 778, 801, 817, 837, 858, 896, 912, 1020, 1054, 1128, 1180, 1245, 1381, 1435, 1454, 1498, 1533, 1639, 1665, 1692, 1744, 2664, 2949, 3032, 3064, 3273.

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