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Synthesis of a 2-indolylphosphonamide derivative with inhibitory activity against yersiniabactin biosynthesis

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Abstract—We report the synthesis of an adenosyl-derived indolylphosphonamide analogue of salicyladenosylmonophosphate involved in the plague and tuberculosis siderophore biosyntheses. The compound proved to be a potent inhibitor of the *Yersinia pestis* salicylate adenylation domain YbtE catalyzing the initial step of yersiniabactin biosynthesis.

Siderophore biosynthesis is the most common mechanism developed by bacteria, including numerous human pathogens, to extract iron from extracellular sources.[1] Among siderophores, salicyl-capped peptides such as mycobactins **1**[2] and yersiniabactin **2**[3] produced by the etiologic agents of tuberculosis (*Mycobacterium tuberculosis*) and plague (*Yersinia pestis*), respectively, have drawn substantial attention as important virulence factors.[4] Indeed, siderophore biosynthesis by nonribosomal peptide synthetases has been recognized as decisive for virulence development and survival of these bacteria in the host, pointing out the high therapeutic potential of siderophore synthesis inhibition. In this sense, hydrolytically stable phosphonate **4** as well as sulfamate analogs **5**–**7** of salicyladenosylmonophosphate (salicyl-AMP) **3**, the initial substrate for nonribosomal synthesis of **1** and **2** have been recently synthesized.[5] While the two β-keto derivatives **4** and **5** did not inhibit significantly the growth of *Mycobacterium tuberculosis* cultivated under iron-limiting conditions,[5c,d] acylsulfamate **6**[5a–c] and sulfamide **7**[5c] showed MIC_{50} below 0.1 μM. These differences in activity were very recently correlated with the ability of the linker between the adenosyl and hydroxyphenyl moieties to adopt a planar geometry through a hydrogen-bonding arrangement as shown for the natural substrate **3**.[5d] We have thus initiated the synthesis of bicyclic hetero-arylic compounds as stable rigid analogs of salicyl-AMP and report here our efforts to synthesize the phosphorylated indolyl derivatives **8** and **9**.

We envisioned that indolylphosphonamidate **8** could be obtained as shown in Scheme 1, using an Atherton–Todd reaction[6] between 5-deoxy-5-amino-adenosine **10** and a protected indolyl-*H*-phosphonate derivative as a key step. The Atherton–Todd reaction, involving a nucleophilic attack on a phosphorochloridate resulting from the CCl_4-mediated oxidation of a *H*-phosphonate precursor, is recognized as a method of choice for the preparation of phosphonamidates.[6] We anticipated that only the more nucleophilic 5-amino group in **10** would react, thus allowing us to work with unprotected 5-amino-5-deoxyadenosine. R_1 and R_2 were chosen among a series of protecting groups that are labile under mild basic conditions, as it is well known that the phosphonamide bond is sensitive to acidic conditions.[7]

The preparation of indolyl partner **12** from *N*-boc-indole (Scheme 2) initially met with difficulties. In a first series of experiments, the 2-lithio-indolyl derivative resulting from *n*BuLi deprotonation of *N*-boc-indole was trapped by addition of freshly prepared chloro(diisopropylamino)methylphosphite **11**[8] at −50 °C. The reaction was allowed to proceed for 15 min at this temperature, then quenched with a saturated NH_4Cl aqueous solution

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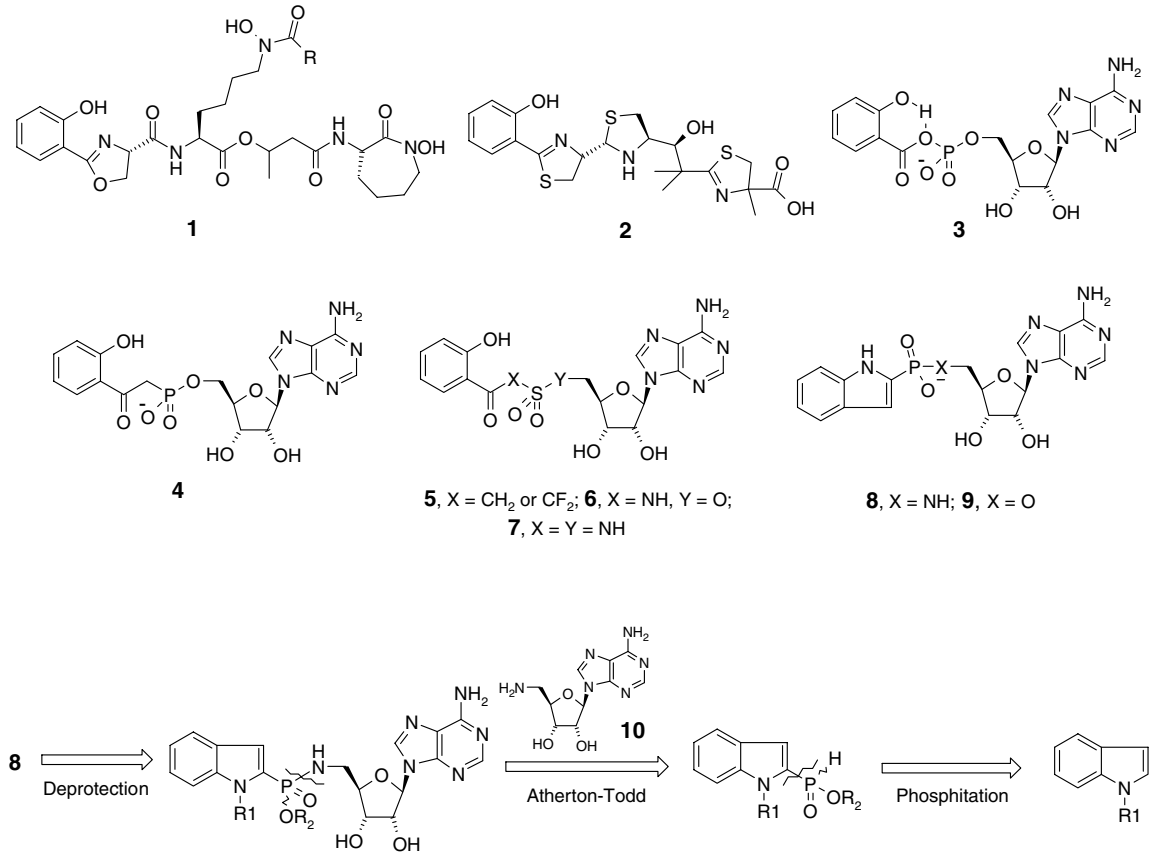
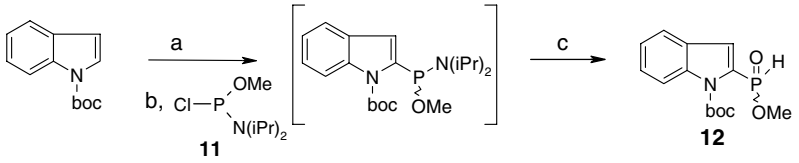


Scheme 1. Retrosynthetic scheme for the preparation of **8**.



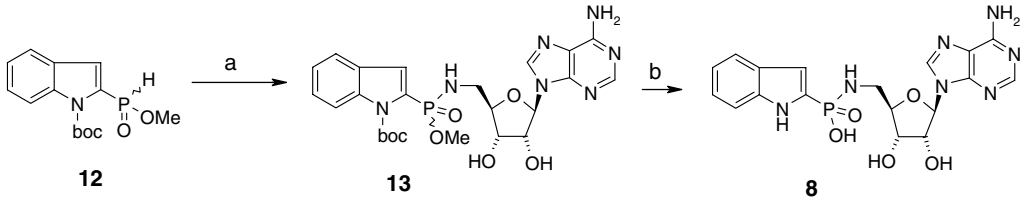
Scheme 2. Reagents and conditions: (a) *n*BuLi, −65 °C, 75 min; (b) −50 °C, **11**, 2 min; (c) HCl aq (2 N), 10 min, 20 °C, 45%.

before the temperature was raised to room temperature. Under these conditions we obtained **12** contaminated with ca. 25% of diisopropylamino(methyl)phosphoramidite (due to the hydrolysis of **11**) in a maximum estimated yield of 30%. In subsequent experiments, prolonged reaction times up to 30 min or 3 h at −50 °C did not lead to any improvement.[9] An acceptable reproducible yield of 95% pure **12** as confirmed by NMR[10] could be finally obtained by shortening the reaction time to 2 min and transferring the reacting medium via cannula into a 2 N HCl aqueous solution.

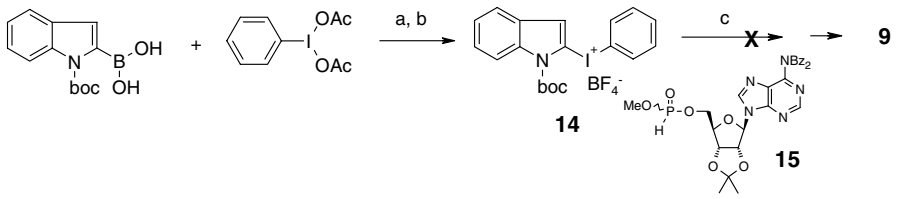
The Atherton–Todd coupling was then performed using freshly prepared *H*-phosphonate **12** and unprotected 5-amino-5-deoxyadenosine **10** as indicated in Scheme 3. The reaction proceeded as expected and yielded phosphonamidate **13**. Finally, simultaneous removal of the boc protecting group and hydrolysis of the phosphonamidate ester were possible under smooth conditions by action of LiOH. After tlc purification (SiO_2, EtOAc/MeOH/H_2O (3:1:0.1) as eluent), the free indolylphosphonamide derivative **8** was obtained in >95% purity as judged by NMR.[10]

For comparison purposes, we also attempted without success to prepare phosphonate **9**. Extension of Atherton–Todd conditions using commercial 2,3-*O*-isopropylidene-adenosine as a starting material was not successful and only complex mixtures were obtained.[11]

We also tried to use the copper-mediated coupling method of *H*-phosphonate and aryl-iodonium derivatives that we recently described.[12] Although the required new indolyliodonium salt **14** was successfully prepared from commercial *N*-boc-indolylboronic acid under the conditions described by Ochiai et al. (Scheme 4),[10,13] it appeared too unstable to survive the coupling conditions and, at best, only traces of coupling products with *H*-adenosylphosphonate **15**[12] were obtained after 4 h at 30 °C.



Scheme 3. Reagents and conditions: (a) CCl_4, Et_3N, molecular sieves, 5-deoxy-5-amino-adenosine **10**, rt, 2 h, (45%); (b) LiOH, 3 h, 40 °C, 60%.



Scheme 4. Reagents and conditions: (a) $BF_3{\cdot}Et_2O$, 45 min, 0 °C; (b) $NaBF_4$, then three washings with cold THF, 40% for both steps; (c) CuI, TMEDA, 40 min or 4 h, 30 °C, <5%.

As phosphonamidate **8** is expected to be at least as interesting as **9**, based on observations in the acylsulfamide/acylsulfamate series,[5c,d] synthesis of **9** was not further pursued. The inhibitory activity of phosphonamidate **8** was tested with the salicylate adenylation domain YbtE of *Y. pestis* and showed an IC_{50} of 6 μM.[14]

In conclusion, we have synthesized an indolylphosphonamidate **8** as a new lead compound that exhibits high inhibitory activity against a salicylation enzyme involved in virulence-associated siderophore biosynthesis. To the best of our knowledge, this is the first salicyl-AMP analogue featuring a phosphonamide junction showing activity. Compound **8** was straightforwardly synthesized by direct coupling of unprotected 5-deoxy-5-amino-adenosine to indolyl-*H*-phosphonate **12** under conditions that are classically used for Atherton–Todd reactions. Application of our approach to the preparation of other adenosyl/indolyl derivatives as potential inhibitors of salicyl-capped siderophore biosynthesis is in progress.

Acknowledgments

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8. Phosphoramidite **11** was prepared by adding diisopropylamine (2 equiv) at room temperature and under argon atmosphere to a THF solution of commercial chlorodimethylphosphite (1 equiv); after stirring for 2 h, a white solid was formed that was removed by filtration, and concentration in vacuo resulted in the crude phosphoramidite as a colorless liquid.
9. No improvement was obtained by using a reverse addition involving the transfer of the lithio-indolyl derivative at −50 °C to a THF solution of **11**.
10. *Selected analytical data*: Compound **8** ^{1}H NMR (400 MHz, CD_3OD, 300 K) δ 8.33 (1H, s), 8.25 (1H, s), 7.53 (1H, d, $J = 8.0$ Hz), 7.37 (1H, d, $J = 8.0$ Hz), 7.10 (1H, t, $J = 8.0$ Hz), 6.98 (1H, t, $J = 8.0$ Hz), 6.82 (1H, d, $J = 4.0$ Hz), 5.93 (1H, d, $J = 6.8$ Hz), 4.34 (1H, dd, $J = 2.8$ and 5.2 Hz), 4.13 (1H, m), 3.19 (2H, m). ^{13}C NMR (100.69 MHz, CD_3OD, 300 K) δ 157.98, 154.64, 151.32, 142.50, 139.78 (d, $J = 16.0$ Hz), 137.60 (d, $J = 293.7$ Hz), 130.11 (d, $J = 21.5$ Hz), 123.82, 122.41, 121.36, 120.79, 113.05, 109.20 (d, $J = 24.0$ Hz), 90.73, 88.19 (d, $J = 12.5$ Hz), 75.46, 73.29, 45.21. ^{31}P NMR (161.9 MHz, CD_3OD, 300 K) δ 8.29. HR MS (electrospray) calcd for $C_{18}H_{20}N_7O_5PH$ ($M+H^+$), 446.1342; found, 446.1336. Compound **12** ^{1}H NMR (400 MHz, $CDCl_3$, 300 K) δ 8.12 (1H, d, $J = 8.0$ Hz), 7.85 (1H, d, $J = 635.5$ Hz), 7.66 (1H, d, 8.0 Hz), 7.50 (1H, d, $J = 5.3$ Hz), 7.45 (1H, d, $J = 8.0$ Hz), 7.29 (1H, d, $J = 8.0$ Hz), 3.82 (3H, d, $J = 12.6$ Hz), 1.72 (9H, s). ^{13}C NMR (100.69 MHz, $CDCl_3$, 300 K) δ 149.90, 138.18 (d, $J = 5.9$ Hz), 129.65, 128.47 (d, $J = 13.4$ Hz), 127.42, 123.53, 122.89 (d, $J = 13.1$ Hz), 122.52, 115.69 (d, $J = 1.4$ Hz), 86.18, 52.40

(d, $J = 5.3$ Hz), 28.13. ^{31}P NMR (161.9 MHz, $CDCl_3$, 300 K) δ 17.49. Compound **14** ^{1}H NMR (400 MHz, $CD_2Cl_2/CDCl_3$ (9:1, v/v), 300 K) δ 8.19 (2H, d, $J =$ 7.8 Hz), 7.96 (1H, d, $J = 7.8$ Hz), 7.86 (1H, t, $J = 7.8$ Hz), 7.68 (1H, t, $J = 7.8$ Hz), 7.45 (1H, d, $J = 7.8$ Hz), 7.40 (1H, t, $J = 7.8$ Hz), 7.30 (1H, t, $J = 7.8$ Hz), 6.13 (1H, s), 1.77 (9H, s). ^{13}C NMR (100.69 MHz, $CD_2Cl_2/CDCl_3$ (9:1, v/v), 300 K). δ 152.43, 137.31, 136.63, 134.70, 134.14, 132.85, 132.70, 130.85, 126.79, 124.43, 121.47, 115.46, 115.23, 90.77, 28.07.

11. We also performed the reaction with (*N*,*N*)-dibenzoyl-2,3-*O*-isopropylidene-adenosine without better success. For each experiment, the reaction was followed for 12 h.
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14. Inhibition analysis was essentially performed as described in Ref. 5b. Briefly: Radioactive ATP-PP_i exchange assays were done with YbtE (200 nM) as target enzyme, and the concentration of indolylphosphonamidate inhibitor used for IC_{50} determination was varied between 1.0 and 75 μM. The assays were performed at 37 °C and stopped after 5 min. The cpm were detected with a liquid scintillation counter (PerkinElmer). Curves were plotted with Microcal™ ORIGIN™ 5.0 software (Microcal Software Inc., Northampton, MA, USA), and IC_{50} was calculated using the sigmoidal equation:

$$v_i/v_0 = (A_1 - A_2)/(1 + [x/IC_{50}]^p) + A_2$$

with v_i: reaction rate with inhibitor, v_0: reaction rate without inhibitor, A_1: initial curve value, A_2: final curve value, x: inhibitor concentration, p: curve slope.