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Synthesis of a Potent Pan-Serotype Dengue Virus Inhibitor Having a Tetrahydrothienopyridine Core

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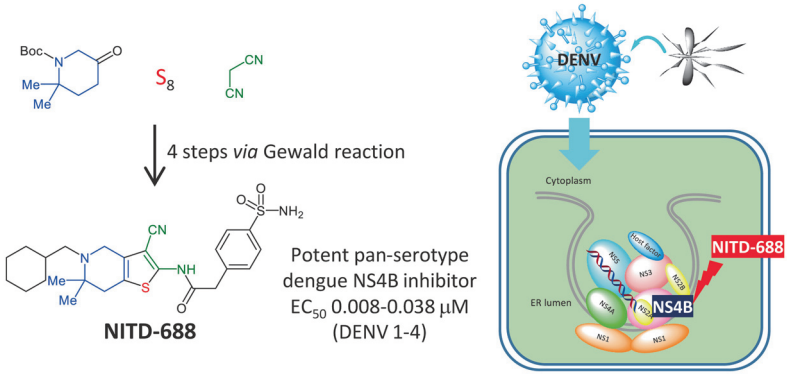

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Abstract A synthesis of the first-in-class pan-serotype dengue virus inhibitor NITD-688 is presented. The Gewald reaction of *N*-(*tert*-butoxycarbonyl)-6,6-dimethylpiperidin-3-one with malononitrile and sulfur in the presence of L-proline as a catalyst gave *tert*-butyl 2-amino-3-cyano-6,6-dimethyl-6,7-dihydrothieno[3,2-c]pyridine-5(4*H*)-carboxylate. This was coupled with [4-(aminosulfonyl)phenyl]acetic acid by using propanephosphonic acid anhydride. A subsequent reductive alkylation with cyclohexanecarboxaldehyde gave NITD-688. Preliminary results of our attempts to control the regioselectivity of the Gewald synthesis of the 2-amino-3-cyanothiophene core are also presented.

Key words medicinal chemistry, Gewald reaction, tetrahydrothienopyridines, regioselectivity, dengue virus inhibitors

Dengue is a mosquito-borne viral disease that is widespread in tropical and subtropical countries. Climate change in nonendemic regions is increasing the incidence of dengue transmission, and more than half the world's population is currently exposed to the risk of dengue infection.[2] The dengue virus is a single-stranded RNA flavivirus that can be divided into four distinct but closely related serotypes, all of which can cause the disease. Infection with the any of four serotypes is believed to induce protective immunity against that particular serotype; cross-immunity to the other serotypes is only partial and temporary. Secondary infection with a different serotype is known to increase the risk of developing severe dengue disease, which is a major cause of hospitalization and death.[3] One dengue vaccine, Dengvaxia, has been licensed and indicated for the prevention of dengue disease caused by all four viral serotypes. However, use of this vaccine in people with no prior infection causes a higher risk of more-severe dengue and hospitalization, and consequently it has been approved for use only in persons aged 9 to 45 years who have had at least one previous documented dengue virus infection.[4] To date, no antiviral agents are available for the treatment of dengue disease and, consequently, there is an urgent need to develop an effective and safe pan-serotype dengue virus inhibitor. The Novartis Institute for Tropical Diseases (NITD), in collaboration with academia and other companies, has been attempting to develop such an antiviral for more than 15 years.[5] As one of several screening campaigns aimed at identifying chemical starting points for medicinal-chemistry optimization,[6,7] we conducted a cell-based high-throughput screening (HTS) on the Novartis corporate compound collection by using dengue-infected cell lines. Subsequent careful characterization of screening hits led to the identification of the tetrahydrobenzothiophene **1** (Figure 1) as a validated hit with submicromolar potency against all four dengue serotypes. Following extensive structure–activity relationship (SAR) studies, an optimization of physicochemical properties resulted in the identification of the *N*-substituted tetrahydrothienopyridine derivative NITD-688 (**2**), which showed a strong potency against all four serotypes (EC_{50} = 0.008–0.038 μM) and showed excellent oral efficacy in the DENV-2 infected AG129 mouse model, with a 1.31 log viremia reduction at a daily oral dose of 30 mg/kg for three days. Selection of resistance mutations and binding studies with recombinant protein using NMR analysis indicate that the molecular target of NITD-688 (**2**) is dengue NS4B protein, which is a nonenzymatic transmembrane protein and a component of the viral replication complex.

NITD-688 (**2**) is currently under preclinical development as a first-in-class pan-serotype dengue virus inhibitor.[7,8] Here, we present our medicinal-chemistry synthesis route to NITD-688 (**2**), together with preliminary results of our attempts to control the regioselectivity of the synthesis of the 2-amino-3-cyanothiophene core of **2**.

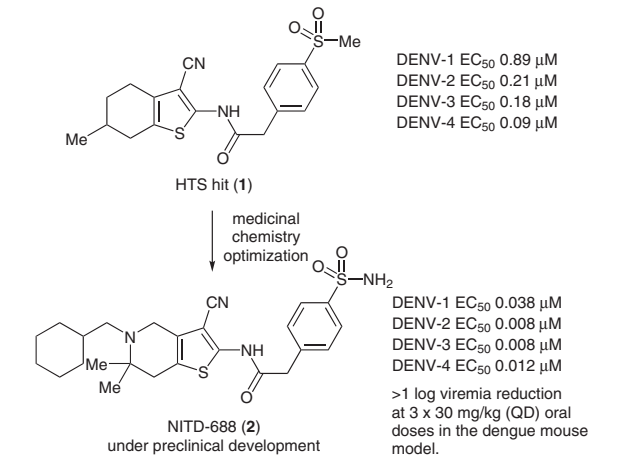
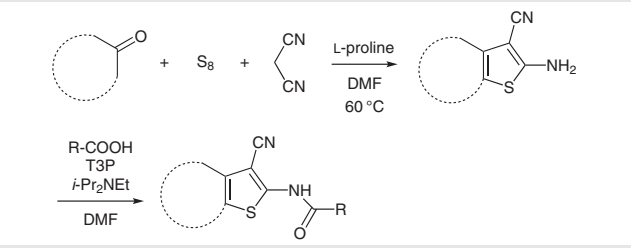


Figure 1 Chemical structures of the dengue cell-based HTS hit (**1**) and the advanced lead compound, NITD-688 (**2**), and their antidengue activities

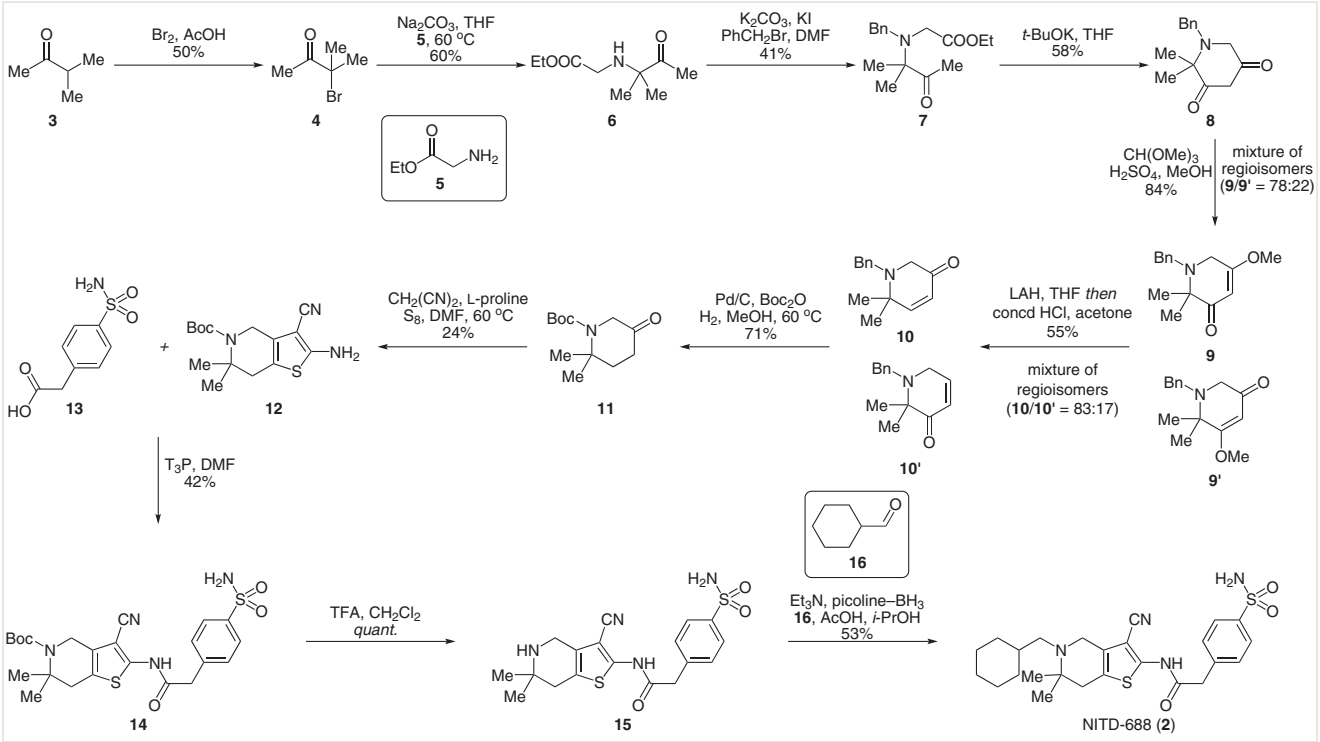
In our medicinal-chemistry optimization of the screening hit compound **1**, the 2-amino-3-cyanothiophene cores were synthesized by a Gewald reaction of malononitrile, the appropriate ketone, and sulfur with L-proline as a catalyst (Scheme 1).[9] The resulting 2-amino-3-cyanothiophenes were coupled with various carboxylic acids by using propanephosphonic acid anhydride (T3P)[10] to provide rapid access to a collection of 3-cyanothiophene analogues for SAR studies of antidengue activity.



Scheme 1 General synthetic route to 3-cyanothiophene-2-amide analogue dengue inhibitors

Once NITD-688 (**2**) was identified as a candidate for preclinical development, our original synthetic route[11] was modified to permit access to sufficient amounts of this material to support formulation and in vivo safety studies (Scheme 2). Large-scale synthesis of the corresponding ketone intermediate for NITD-688 (**2**) commenced with bromination of methyl isopropyl ketone (**3**) with bromine to provide 3-bromo-3-methylbutan-2-one (**4**) in 50% yield. *N*-Alkylation of glycine ethyl ester (**5**) with the bromide **4**,[12] gave amine **6** in 60% yield. Subsequent benzylation with benzyl bromide gave the *N*-benzylglycine derivative **7** in 41% yield. To form the piperidine core, we conducted an intramolecular Claisen cyclization of **7** with potassium *tert*-butoxide to afford the piperidine-3,5-dione **8** in 58% yield.[13] Treatment of this intermediate with trimethyl orthoformate under acidic conditions gave a 78:22 mixture of the regioisomeric methyl enol ethers **9** and **9′** in 84% combined yield. Subsequent 1,2-reduction of the ketone moiety in **9** and **9′** with lithium aluminum hydride, followed by acidic hydrolysis of the methyl enol ether group, gave enones **10** and **10′** as an 83:17 regioisomeric mixture in 55% yield.[12] This regioisomeric mixture was further purified by recrystallization from 5% ethyl acetate in hexane to give the single isomer **10**. Hydrogenolysis of the *N*-benzyl protecting group in **10**, with reduction of the enone moiety, followed by concomitant protection of the secondary amine gave the pure ketone **11** in 71% yield. With ketone **11**, the stage was set for the formation of the 2-amino-3-cyanothiophene core through a Gewald pathway. This was accomplished by treating ketone **11** with malononitrile and elemental sulfur with L-proline as a catalyst at 60 °C.[9] The desired regioisomer **12** was separated at this stage by column chromatography to give an isolated yield of 24%. Moving forward, simple T3P-mediated coupling of **12** with acid **13** furnished amide **14** in 42% yield. In the final stretch of the synthesis, the secondary amine in **15** was obtained by removal of the *tert*-butoxycarbonyl (Boc) protecting group of **14** in quantitative yield through the action of trifluoroacetic acid. Finally, a reductive amination reaction with picoline–borane complex was used to couple aldehyde **16** and amine **15** to furnish NITD-688 (**2**) in 53% yield.[13] Overall, NITD-688 (**2**) was assembled from simple industrial feedstocks in 11 linear steps with a serviceable yield.

Our early to mid-stage medicinal-chemistry investigations were adequately supported by the successful implementation of the aforementioned synthetic route to NITD-688 (**2**). However, as the program matured, the need for greater quantities of NITD-688 (**2**) of higher quality and with improved synthetic efficiency became increasingly apparent. Hence, the synthesis was scrutinized for areas that could be further optimized. Specifically, the formation of the desired methyl enol ether **9** from the 1,3-diketone **8** showed a low regioselectivity. Furthermore, material throughput of the 2-amino-3-cyanothiophene core **12** from the ketone **11** through the Gewald pathway was suboptimal, and screening efforts were unable to improve on the low <30% isolated yield, due to poor regioselectivity. The lack of robust regiocontrol in the coupling was also of concern from a cost-of-goods standpoint, because separation of the isomers required extensive column chromatography. Hence, a proof-of-concept campaign was launched in an attempt to address the regioselectivity of the critical thiophene-formation step. A review of the literature revealed



Scheme 2 A medicinal-chemistry synthetic route to NITD-688 (**2**)

that introduction of an electrophilic leaving group in the position α to the ketone was critical in controlling the regioselectivity of the formation of the heterocycle.[14] Thus, enone **10** was envisioned as a suitable intermediate to leverage regioselective halide installation through the enone moiety (Scheme 3). Conjugate reduction of this scaffold was unknown, to the best of our knowledge, and various hydride sources gave clean and exclusive 1,2-reductions.[15] However, Wilkinson's catalyst and triethylsilane smoothly afforded the silyl enol ether **17** in 72% isolated yield under buffered purification conditions.[16] Next, bromination and concomitant desilylation of **17** with pyridinium tribromide gave the α-oxo bromide **18**.[17] The unstable bromide **18** was then immediately subjected to Gewald conditions, followed by a silica-gel mediated cyclization of the putative Knoevenagel intermediate, to give the cyanothiophene core **19** as a single regioisomer in 20% isolated yield. Although there is room for improving the efficiency of the thiophene-formation step by further optimization and side-product profiling, we have nevertheless demonstrated a regioselective synthesis of the cyanothiophene core of NITD-688 (**2**) on a discovery scale by way of these currently unoptimized reaction and purification conditions.

In conclusion, we have disclosed the first-in-class panserotype dengue virus inhibitor NITD-688 (**2**). A medicinal chemistry route to **2**, which was used to support preclinical development, has been described.[18,19] Furthermore, potential adaptations to large-scale synthetic access were validated by exploratory chemistry. The feasibility of the modified route and its impact on the cost-of-goods is currently being evaluated at a process scale. We are actively investigating further ways to improve the synthesis, and the results will be reported in subsequent publications.

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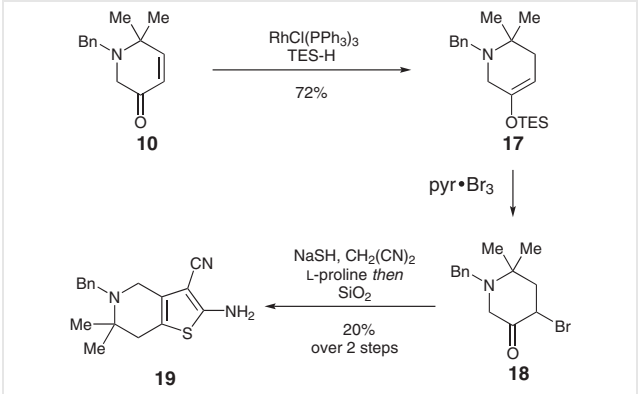

Scheme 3 Exploratory chemistry to develop a regiocontrolled synthesis of the 2-amino-3-cyanothiophene core of NITD-688 (**2**)

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Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/a-1323-4036.

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(18) ***tert*-Butyl 2-Amino-3-cyano-6,6-dimethyl-6,7-dihydrothieno-[3,2-*c*]pyridine-5(4*H*)-carboxylate (12)**
L-Proline (12.66 g, 110 mmol, 1.0 equiv) and sulfur (5.27 g, 165 mmol, 1.5 equiv) were added sequentially to a solution of **11** (25 g, 110 mmol) in DMF (100 mL) at rt, and the mixture was stirred at rt for 10–15 min. Malononitrile (8 g, 121 mmol, 1.1 equiv) was added, and mixture was stirred at 55–60 °C for 10–12 h. When the reaction was complete, the mixture was washed with hexane (3 × 50 mL). The bottom DMF layer was slowly added to cold H_2O (250 mL), and the precipitated solids were collected by filtration, washed with H_2O (50 mL), and dried. The wet product was purified by column chromatography (silica gel, CH_2Cl_2–MeOH) to give **12** as a pale brown color solid; yield: 8 g (26 mmol, 24%) (96.1% pure; 96.22:0.75 isomeric mixture based on HPLC).
^{1}H NMR (400 MHz, DMSO-d_6): δ = 17.13 (br s, 2 H), 4.18 (s, 2 H), 3.31 (s, 2 H), 2.54 (s, 2 H), 1.40 (s, 9 H), 1.38 (s, 6 H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 1163.71, 154.76, 128.71, 115.51, 114.31, 80.68, 79.24, 55.22, 42.66, 37.85, 28.06, 27.40. HRMS (ESI): *m/z* [M – H]$^-$ calcd for $C_{15}H_{20}N_3O_2S$: 306.1271; found: 306.1281.
(19) **2-Amino-5-benzyl-6,6-dimethyl-4,5,6,7-tetrahydro-thieno[3,2-*c*]pyridine-3-carbonitrile (19): Regiocontrolled Synthesis**
Pyridinium tribromide (382 mg, 1.194 mmol, 1.2 equiv) was added to a 0.1 M solution of silyl enol ether **17** (330 mg, 0.995 mmol, 1.0 equiv) in CH_2Cl_2 (10 mL) at 23 °C, and the mixture was stirred for 15 min. The reaction was then quenched with sat. aq $Na_2S_2O_3$ and extracted with CH_2Cl_2 (×3). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated in vacuo to provide bromide **18**, which was used immediately in the next step without further purification.
Anhyd DMF (3.32 mL, 0.1 M) was added to the crude bromide **18** (1.0 equiv), NaSH (27.9 mg, 0.498 mmol, 1.5 equiv), malononitrile (24.1 mg, 0.365 mmol, 1.1 equiv), and L-proline (38.2 mg, 0.332 mmol, 1.0 equiv) under an inert atmosphere at 23 °C, and the mixture was stirred at 23 °C for 2 h. When the reaction was complete it was quenched with sat. aq $Na_2S_2O_3$ and sat. aq $NaHCO_3$, and the mixture was extracted with EtOAc (×3). The combined organic layers were washed sequentially with H_2O and brine, dried (Na_2SO_4), and concentrated in vacuo. The residual crude mixture was redissolved in CH_2Cl_2 at 23 °C, silica gel (3 g) was added, and the mixture was stirred for 4 h. The solvent was then removed in vacuo and the crude residue was purified by flash column chromatography on an ISCO machine [silica gel, heptane–EtOAc (0–50%)] to give the cyano derivative **19** as a yellow solid; yield: 21 mg (20%, 0.071).
^{1}H NMR (500 MHz, $CDCl_3$): δ = 17.36–7.28 (m, 4 H), 7.30–7.22 (m, 1 H), 4.59 (s, 2 H), 3.66 (s, 2 H), 3.39 (t, *J* = 2.0 Hz, 2 H), 2.51 (d, *J* = 2.2 Hz, 2 H), 1.23 (s, 6 H). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 160.56, 139.92, 129.78, 128.52 (2 C), 128.36 (2 C), 126.91, 118.51, 100.45, 86.83, 53.94, 53.05, 46.93, 37.46, 24.21 (2 C). HRMS (ESI): *m/z* [M + H]$^+$ calcd for $C_{17}H_{20}N_3S$: 298.1372; found: 298.1374.

