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An improved and robust scale-up process aided with identification and control of critical process impurities in darunavir ethanolate

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Abstract

A robust and safe industrial process, including five isolations and drying steps for widely prescribed anti-HIV (protease inhibitor) drug darunavir ethanolate **2**, has been developed. A salient feature of this process is the development of procedures enabling the efficient synthesis of multi-kilogram quantity of darunavir ethanolate, and process demonstrations through plant scale preparation are offered where darunavir molecule has been prepared with overall > 70% chemical yield and > 99.8% purity without involving any purification procedure(s), with all possible process impurities below than the desired limit (not more than 0.08%) were isolated, synthesized and characterized. The developed process is entirely robust, very efficient and demonstrated up to kilograms scale.

Keywords Darunavir · Safe industrial process · Reduction · Deprotection · Coupling · Critical process impurities

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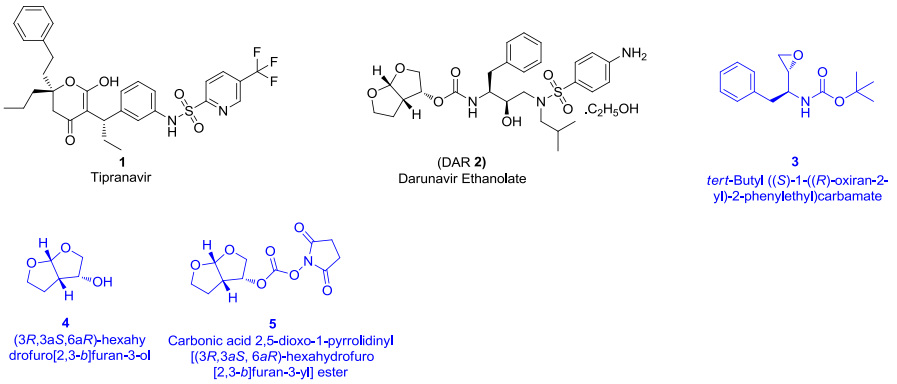


Fig. 1 A second-generation non-peptidic HIV protease inhibitors and raw materials

Introduction

As HIV is unrecoverable and it seems that after cancer, this is the greatest threat to life, millions of AIDS patients from different age group are expecting from scientific community for better treatment.[1] To fulfill this gap, chemists and biologists continued efforts have introduced several classes of potent drugs like highly active antiretroviral therapy (HAART) using more than three drugs ranging from HIV reverse transcriptase to protease inhibitors brought about this remarkable breakthrough [1]. Among this effective blend, HIV protease inhibitors are so essential for HAART that the Food and Drug Administration (FDA) has approved different protease inhibitors and clinically used for the treatment of HIV/AIDS such as amprenavir, indinavir, saquinavir, nelfinavir and ritonavir.[2,3]

Recently, FDA has approved few more HIV protease inhibitors including tipranavir (**1**) and darunavir ethanolate (DAR **2**) focused on drug-resistant HIV. The development of tipranavir and darunavir ethanolate, a second-generation non-peptidic HIV protease inhibitors [2], with marked improved resistance profiles, has opened a new perspective on the treatment of antiretroviral therapy (ART) [3] experienced HIV patients with poor viral load control (Fig. 1).

Whereas, DAR **2** (brand name Prezista, formerly known as TMC114) which is in second-generation protease inhibitor class has been fully recommended by Office of AIDS Research Advisory Council (OARAC) for the treatment of HIV in adults and children 6 years of age and older [4]. It was approved by the FDA on June 23, 2006 to overcome the problems with early protease inhibitor like severe side effects and drug toxicities [5], require a high therapeutic dose, expensive to afford and show a disturbing susceptibility to drug-resistant mutations; a second-generation protease

[1] http://www.cancer.org/acs/groups/cid/documents/webcontent/002295-pdf.pdf.
[2] https://aidsinfo.nih.gov/education-materials/fact-sheets/21/58/fda-approved-hiv-medicines.
[3] http://emedicine.medscape.com/article/1533218-overview#a2.

inhibitor DAR **2** is discovered. Furthermore, DAR **2** is the first drug which is more efficacious and cost-effective than other protease inhibitors.

A strong demand for DAR **2** led to the development of several synthetic schemes for its synthesis [6–13] involving the transformation of *tert*-Butyl[(*S*)-1-((*R*)-oxiran-2-yl)-2-phenylethyl]carbamate **3** as starting material in most of the synthetic protocols to DAR **2**, albeit involving multistep procedures using reagents such as palladium, sodium azide and trifluoroacetic acid (TFA) which are known to be potentially hazardous at the commercial scales besides being environmentally degrading along with having process drawbacks such as tedious workup, excessive effluent generation, low isolated yields, arduous isolation/purification procedures, formation of stagnant impurities/by-products that are stiff to eliminate even after multiple purification. Search for a practically simpler cost-effective method led Tibotec Pharmaceuticals Limited [14] to design a protocol by involving existing **3** as the key starting material, employing simple addition, substitution, reduction, deprotection and coupling strategies as key steps in the construction of the darunavir skeleton.

The scale-up process for the this protocol has been achieved by converting **3** into 4-amino-*N*-((2*R*,3*R*)-3-amino-2-hydroxy-4-phenylbutyl)-*N*-isobutylbenzene sulfonamide **9** in multistep procedures by involving the use of reagents such as palladium metal and concentrated hydrochloric acid, and thus, it was made to participate in a coupling with **4**, and providing DAR **2** in two further steps. Although this protocol provides ready access to **2** via isolated four to five intermediates, it is too long in addition to have some environmental and toxicological drawbacks. The major disadvantages of this route are the use of potentially hazardous reagents in addition to generation of large amounts of effluents besides having low isolated yields and high production cost.

A perusal of these synthetic processes disclosed in the existing studies [6–14] made it clear that the only one synthetic route [14] could result into the synthesis of DAR **2** successfully at commercial level till now. Most of these synthetic protocols have made their academic value instead of commercial aspects besides involving use of fancy reagents, more effluent generation along with low isolated yield. The established commercial viable protocol has also some major disadvantages such as use of potentially hazardous reagents [6], generation of large amounts of effluents along with the formation of process/degradation impurities [7], which could not be washed out in purifications at mid or at the end of the process, struggling to afford DAR **2** with stringent quality profile (any unspecified impurity: not more than 0.08% w/w) as API [15].

Therefore, evaluation of these reported synthetic strategies for DAR **2** preparation besides understanding of their advantages/disadvantages in consideration of commercial aspect such as process robustness and low cost driven us to work on the synthetic strategy disclosed in US Patent 6,248,775 B1 [14] and 7,700,645 B2 [16]. Though route is an improved one, still more improvements are required to get the right quality of the API consistently. Thus, process was studied thoroughly during the laboratory development stage and introduced some major modifications to encounter the shortcomings of the listed process [15, 19] for making DAR **2** process plant friendly and commercially viable. We envisioned that an efficient route with optimized process to the robust level for DAR **2** without involving hazardous

reagents should make economic sense besides giving a new practical industrial method within the acceptable environmental and toxicological concerns [17]. We further justified that the new method would be more appealing if it can employ the same commercially available starting material; **3** as listed in most of the synthetic routes. In this contribution, we report the design and technical aspects of our new improved process, which enables the commercial production of **2** from **3**.

Experimental

Materials

All materials were purchased from commercial suppliers. Unless specified otherwise, all reagents and solvents were used as supplied by manufacturers. Melting points were determined by open air capillary with Buchi M-565 and are uncorrected. IR spectra of samples were recorded on Shimadzu IR Affinity-I FT-IR spectrophotometer. ^{1}H NMR spectra (400 MHz) and ^{13}C NMR spectra (100 MHz) were recorded in $CDCl_3$, DMSO-d6, on Bruker 400 MHz NMR (ASCEND, 5 mm PABBO) instrument, and mass spectra were determined on Velos Pro ion trap mass spectrophotometer (Thermo Scientific). TLC was done on Merck TLC silica gel 60_{F254} plates using mobile phase MeOH/DCM (8:2 and 9:1).

Synthetic procedure

Preparation of *tert*-butyl((1*S*,2*R*)-1-benzyl-2-hydroxy-3-[isobutyl[(-4-nitrophenyl) sulfonyl]-amino]propyl]carbamate (7)

A mixture of isobutyl amine (1000 mL, 9.981 mol), IPA (200 mL, 1.0 volume) and 200 g (0.759 mol) of **3** was stirred for 12–14 h at 20–30 °C. After completion of reaction (TLC), volatiles were removed under vacuum. Water (1000 mL) was added to the residue followed by stirring for 2 h, and subsequent filtration resulted to afford intermediate **6**, after drying at 50–60 °C in air oven over a period of 10–12 h. Dried solid was then mixed with DCM (740 mL), TEA (115 mL, 0.835 mol) at 15–25 °C followed by slow addition of 4-nitrobenzenesulfonyl chloride solution (168 g, 0.759 mol, in 300 mL of DCM) at 15–25 °C. Resulted reaction mixture was stirred for 1–2 h at 15–25 °C. After reaction completion (TLC), volatiles were evaporated under vacuum completely. Water (1500 mL) and sodium hydroxide solution (200 mL; 0.700 mol) was added to distilled residue followed by stirring for 2 h. The resulting aqueous slurry was filtered, washed with water and dried in air oven at 55–60 °C over a period of 10–12 h to yield the intermediate **7**. (367 g, 93%): *Rf* (**3**–**6** = 0.50 and **6**–**7** = 0.80); ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.81–0.85 (m, 6H, –2C<u>H</u>$_3$), 1.24 (s, 9H, –3C<u>H</u>$_3$), 1.94–1.99 (m, 1H, –C<u>H</u>), 2.91–2.96 (m, 2H, –C<u>H</u>$_2$), 3.04–3.17 and 3.42–3.50 (m, 5H, –2C<u>H</u>$_2$, –C<u>H</u>(OH)), 4.93 (d, J = 6.4 Hz, 1H, –C<u>H</u>), 6.64 (d, J = 8.8 Hz, 1H, –N<u>H</u>), 7.24–7.11 (m, 5H, arom-<u>H</u>), 8.04 (d, J = 8.4 Hz, 2H, arom-<u>H</u>), 8.36 (d, J = 8.4 Hz, 2H, arom-<u>H</u>); ^{13}C NMR (100 MHz, DMSO-d_6) δ 19.5,

19.7, 25.7, 28.0, 35.3, 51.1, 55.0, 71.1, 77.4, 124.2, 125.5, 127.7, 128.4, 129.0, 139.3, 145.4, 149.3, 155.1; IR (KBr, cm^{-1}) 3562, 3363, 2960, 1697; MS (ES+) *m/z*: 522.23 (MH+).

Preparation of 4-Amino-*N*-((2*R*,3*S*)-3-amino-2-hydroxy-4-phenylbutyl)-*N*-isobutylbenzene sulfonamide (**9**)

Mixture of **7** (300 g, 0.575 mol), wet Raney nickel (60 g, 20% w/w) and methanol (1500 mL) was taken in stainless steel autoclave at 20–25 °C under hydrogen gas pressure (5.0 kg). Resulted reaction slurry was stirred for 3–4 h. After reaction completion (TLC), the reaction mixture was filtered through hyflo bed and washed with methanol (300 mL). Filtrate was evaporated under vacuum followed by degassing for 30 min at 40–45 °C. Ethyl acetate (600 mL) and aqueous HCl (254 mL, 2.299 mol; 30–35%) was added to the distilled residue and stirred at 20–25 °C for over a period of 4–5 h. After reaction completion (TLC), reaction mass was filtered and wet solid was suck dried for 30–40 min. A mixture of suck dried solid and water (2400 mL) was treated with sodium hydroxide solution (1500 mL, 1.725 mol) at 10–20 °C over a period of 2–3 h. The resulting heterogeneous slurry was filtered, washed with water and dried in air oven for 8–10 h at 45–50 °C to afford intermediate **9**. (192 g, 85.3%): *Rf* (**7**–**8** = 0.32 and **8**–**9** = 0.21); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 (dd, J = 8 Hz and 12 Hz, 6H, –2C$\underline{H}_3$), 1.85–1.90 (m, 1H, –C$\underline{H}$), 2.44–2.52 (m, 2H, –C$\underline{H}_2$), 2.78–2.85 (m, 1H, –C$\underline{H}_2$), 2.98–3.02 (m, 2H, –2C$\underline{H}_2$), 3.09–3.19 (m; 2H, –C$\underline{H}_2$), 3.24–3.32 (m, 1H, –C$\underline{H}$(NH_2)), 3.55 (br-s, 1H, –O$\underline{H}$), 3.70–3.75 (m, 1H, –C$\underline{H}$), 4.17(s, 2H, arom-N$\underline{H}_2$), 6.68 (d, J = 4.0 Hz, 2H, -arom-$\underline{H}$), 7.20–7.34 (m, 5H, arom-$\underline{H}$), 7.60 (d, J = 8.0 Hz, 2H, arom-$\underline{H}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.9, 20.2, 27.2, 39.4, 52.7, 55.7, 58.7, 73.3, 114.1, 126.4, 126.5, 128.6, 129.3, 129.5, 138.9, 150.6; IR (KBr, cm^{-1}) 3452, 3348, 3240, 2951, 1649, 1598, 1321, 1147; MS (ES+) *m/z*: 392.20 (MH+).

Preparation of darunavir (**10**)

Mixture of **9** (100 g, 0.254 mol) and DMSO (300 mL) was cooled to 15 °C followed by addition of **5** as solution (72.7 g; 0.266 mol in 300 mL of DMSO) at 15–30 °C. DMSO reaction mass was thus stirred for 1–2 h. After reaction completion (TLC), ammonium hydroxide (2.1 g, 0.16 mol) was added to reaction mass followed by water addition and subsequent stirring for 1.0 h. Aqueous slurry was thus filtered, washed with water and treated wet solid with IPA to afford, after drying for 10–12 h at 50–55 °C under vacuum **10** having HPLC Purity > 99.80% with dimer-2 impurity **13** and oxazolidinone impurity **14** to the level of not more than 0.08% (for characterization data of these, see Supporting Information): (132 g, 94%), *Rf* = 0.85; mp 72–74 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.78 (d, J = 6.8 Hz, 3H, –C$\underline{H}_3$), 0.84 (d, J = 6.4 Hz, 3H, –C$\underline{H}_3$), 1.03 (d, 3H, IPA-C$\underline{H}_3$), 1.26 (m, 1H, –C$\underline{H}_2$), 1.40 (m, 1H, –C$\underline{H}_2$), 1.93 (m, 1H, –C$\underline{H}$), 2.46–2.49 (m, 2H, –C$\underline{H}_2$), 2.52–2.68 (m, 2H, –C$\underline{H}_2$), 2.75–2.77 (m, 1H, –C$\underline{H}_2$), 2.93–3.04 (m, 2H, –C$\underline{H}$), 3.26–3.28 (m, 1H,

–CH), 3.56–3.62 (m, 4H, –C<u>H</u>$_2$), 3.63–3.72 (m, 1H, –C<u>H</u>–OH), 4.30 (d, 1H, IPA), 4.83–4.84 (m, 1H, –C<u>H</u>–O), 4.94 (d, $J = 6.0$ Hz, 1H, –C<u>H</u>), 5.50 (d, $J = 5.2$ Hz, 1H, –C<u>H</u>)), 5.94 (s, 2H, arom-N<u>H</u>$_2$), 6.59 (d, $J = 8.8$ Hz, 2H, arom-<u>H</u>), 7.12–7.13 (m, 1H, –CON<u>H</u>), 7.19–7.23 (m, 5H, arom-<u>H</u>), 7.37 (d, $J = 8.4$ Hz, 2H, arom-<u>H</u>); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 20.0, 25.4, 25.5, 26.3, 35.1, 45.0, 52.7, 55.8, 57.2, 62.0, 68.8, 70.3, 72.3, 72.7, 108.7, 112.6, 123.6, 125.6, 127.8, 128.9, 129.1, 139.4, 152.6, 155.1; IR (KBr, cm^{-1}) 3419, 3344, 2962, 2904, 1705; MS (ES+) *m*/*z*: 548.27 (MH+).

Preparation of darunavir ethanolate (**2**)

Mixture of **10** (120 g, 0.219 mol) and ethanol (700 mL) was heated to 75–80 °C to make it clear solution. Ethanolic clear solution thus obtained was cooled gradually to 0–5 °C and stirred for 1–2 h followed by filtration and washing of filtered solid with chilled ethanol, dried for 10–12 h at 50–55 °C under vacuum to afford the targeted darunavir ethanolate API (**2**) having HPLC Purity > 99.80% with dime-2 impurity **13** and oxazolidinone impurity **19** to the level of not more than 0.08% (for characterization data of these, see Supporting Information): (117 g, 90%), mp 99–101 °C; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 0.78 (d, $J = 6.8$ Hz, 3H, –C<u>H</u>$_3$), 0.84 (d, $J = 6.4$ Hz, 3H, –CH$_3$), 1.05 (t, $J = 6.8$, 3H, Ethanol-C<u>H</u>$_3$), 1.26 (m, 1H, –C<u>H</u>$_2$), 1.42 (m, 1H, –C<u>H</u>$_2$), 1.83 (m, 1H, –C<u>H</u>$_2$), 2.46–2.65 (m, 4H, –C<u>H</u>$_2$ and –C<u>H</u>) 2.93–3.01 and 2.91 (m, 2H, –C<u>H</u>$_2$), 3.26–3.45 (m, 3H, Ethanol-C<u>H</u>$_2$, and –C<u>H</u>$_2$), 3.56–3.63 (m, 4H, –2C<u>H</u>$_2$), 3.71–3.372 (m, 1H, –C<u>H</u>), 3.84–3.88 (m, 1H, –C<u>H</u>), 4.31 (t, $J = 4.8$ Hz, 1H, Ethanol-O<u>H</u>), 4.84 (d, $J = 8.0$ Hz, 1H, –C<u>H</u>), 4.94 (d, $J = 6.4$ Hz, 1H, –CHO<u>H</u>), 5.51 (d, $J = 5.2$ Hz, 1H, –C<u>H</u>), 5.94 (s, 2H, arom-N<u>H</u>$_2$), 6.60 (d, $J = 8.8$ Hz, 2H, arom-<u>H</u>), 7.12–7.23 (m, 6H, –N<u>H</u> and arom-<u>H</u>), 7.38 (d, 2H, arom-<u>H</u>); ^{13}C NMR (100 MHz, CDCl$_3$) δ 18.4, 20.0, 25.4, 26.3, 35.1, 45.0, 52.7, 55.8, 56.0, 57.3, 68.8, 70.3, 72.3, 72.8, 108.8, 112.6, 123.7, 125.6, 127.8, 128.9, 129.2, 139.4, 152.6, 155.1; IR (KBr, cm^{-1}) 3466, 3437, 3360, 2964, 1707, 1597, 1313; MS (ES+) *m*/*z*: 592.18 (MH−).

tert-Butyl[(1*S*,2*R*)-1-benzyl-2-hydroxy-3-(isobutylamino) propyl]-*N*-[(1*S*,2*R*)-1-benzyl-2-hydroxypropyl]carbamate (Dimer-1 impurity; **11**)

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 0.87 (dd, $J = 6.4$ Hz and 17.2 Hz, 3H, –C<u>H</u>$_3$), 1.25 (s, 18H, –C(C<u>H</u>$_3$)$_3$), 1.68–1.76 (m, 1H, –C<u>H</u>), 2.18–2.20 (m, 2H, –C<u>H</u>$_2$), 2.50–2.57 (m, 4H, –C<u>H</u>$_2$), 3.03 (d, 2H, –C<u>H</u>$_2$), 3.49 (br-s, 4H, –C<u>H</u>$_2$ and –C<u>H</u>), 4.68 (s, 2H, –N<u>H</u>), 6.63 (d, 2H, –N<u>H</u>), 7.12–7.25 (m, 10H, arom-<u>H</u>); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 20.7, 20.9, 25.8, 28.1, 36.1, 55.0, 58.7, 63.9, 70.3, 77.2, 125.5, 127.8, 129.1, 139.6, 155.2; IR (KBr, cm^{-1}): 3356, 1683, 1529; MS (ES+) *m*/*z*: 600.65 (MH+).

tert-Butyl-*N*-[(2*S*,3*R*)-4-[(4-(*N*-hydroxylamino)phenyl)sulfonyl-(2-methylpropyl) amino]-3-hydroxy-1-phenylbutan-2-yl-]carbamate (*N*-hydroxyl impurity; **12**)

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 0.82 (dd, $J = 7.2$ Hz and 3.6 Hz, 6H, –C<u>H</u>$_3$), 1.40 (s, 9H, –C(C<u>H</u>$_3$)$_3$), 1.91–2.08 (m, 1H, –C<u>H</u>), 2.67–2.77 (m, 2H, –C<u>H</u>$_2$), 2.91–3.00

(m, 2H, –C$\underline{H}_2$), 3.30 (br-s, 1H, –C$\underline{H}_2$), 3.48–3.52 (m, 1H, –C$\underline{H}_2$), 3.61 (d, J = 5.6 Hz, 1H, C$\underline{H}$OH), 4.94 (d, J = 6.4 Hz, 1H, –C$\underline{H}$), 6.68 (d, J = 9.2 Hz, 1H, –N$\underline{H}$), 6.87 (d, J = 8.8 Hz, 2H, arom-$\underline{H}$), 7.14 (d, J = 6.8 Hz, 1H, arom-$\underline{H}$), 7.18–7.24 (m, 4H, -arom-$\underline{H}$), 7.55 (d, J = 8.8 Hz, 2H, arom-$\underline{H}$), 8.66 (s, 1H, NHO$\underline{H}$), 8.97 (s, 1H, –N$\underline{H}$OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 19.9, 20.0, 26.2, 28.1, 35.2, 52.4, 55.1, 56.7, 72.4, 77.3, 111.3, 125.5, 127.6, 127.8, 128.3, 129.1, 139.6, 155.2; IR (KBr, cm^{-1}): 3363, 1693, 1598, 1332; MS (ES+) *m/z*: 508.09 (MH+).

_N_-[(1_S_,2_R_)-3-[[(4-Amino(3_R_,3a_S_,6a_R_)-hexahydrofuro(2,3-b)-furan-3-yl-oxycarbonyl](phenyl)sulfonyl](2-methylpropyl)amino]-2-hydroxy-1-(phenylmethyl)propyl] carbamic acid(3_R_,3a_S_,6a_R_)-hexahydrofuro[2,3-b]-furan-3-yl ester (dimer-2 impurity; 13)

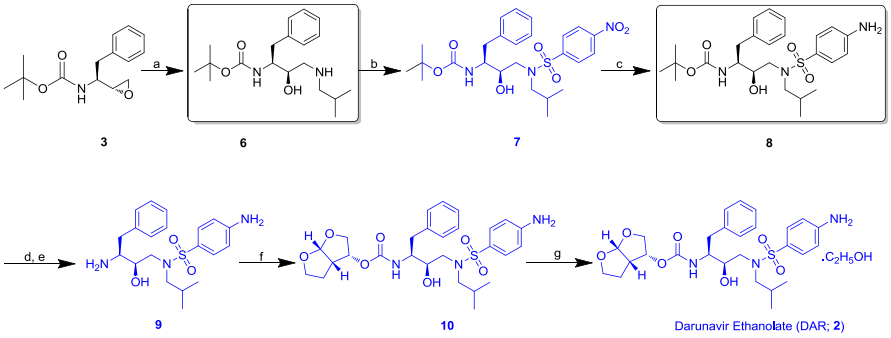
^{1}H NMR (400 MHz, DMSO-d_6) δ 0.82 (d, J = 8.0 Hz, 6H, –C(C$\underline{H}_3$)$_3$), 1.20 (m, 1H, –C$\underline{H}_2$), 1.40 (m, 1H, –C$\underline{H}_2$), 1.80 (m, 1H, –C$\underline{H}_2$), 1.94–1.97 (m, 2H, –C$\underline{H}_2$ and –C$\underline{H}$), 2.74–2.79 (m, 3H, –C$\underline{H}_2$), 2.99–3.06 (m, 3H, –C$\underline{H}_2$ and –C$\underline{H}$), 3.27 (m, 1H, –C$\underline{H}$), 3.56–3.61 (m, 4H, –C$\underline{H}_2$ and –C$\underline{H}$), 3.69–3.73 (m, 2H, –C$\underline{H}_2$), 3.85–3.87 (m, 3H, –C$\underline{H}_2$ and –C$\underline{H}$), 3.96–4.00 (m, 1H, –C$\underline{H}_2$), 4.82–4.84 (m, 1H, –C$\underline{H}_2$), 5.00 (d, J = 8.0 Hz, 1H, –O$\underline{H}$), 5.16 (dd, J = 8 Hz, 1H, –C$\underline{H}$), 5.50 (d, J = 5.6 Hz, 1H, –C$\underline{H}$), 5.63 (d, J = 5.2 Hz, 1H, –C$\underline{H}$), 7.12–7.14 (m, 1H, –N$\underline{H}$), 7.19–7.25 (m, 5H, arom-$\underline{H}$), 7.66 (dd, J = 8 Hz and 24 Hz, 4H, arom-$\underline{H}$), 10.22 (s, 1H, –N$\underline{H}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.5, 26.7, 35.1, 44.6, 44.9, 52.2, 55.8, 56.6, 68.6, 70.0, 70.2, 72.3, 73.3, 108,6, 108.7, 117.7, 125.6, 127.7, 128.2, 129.0, 132.2, 139.3, 142.6, 152.6, 155.0; IR (KBr, cm^{-1}): 3342, 2962, 2875, 1718, 1595, 1315; MS (ES+) *m/z*: 704.40 (MH+).

4-Amino-_N_-(2-methylpropyl)-_N_-[[(4_S_,5_R_)-2-oxo-4-(phenylmethyl)-5-oxazolidinyl] methyl] benzenesulfonamide (oxazolidinone impurity; 14)

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 (d, J = 8.0 Hz, 6H, –C(C$\underline{H}_3$)$_3$), 1.89–193 (m, 1H, –C$\underline{H}$), 2.60–2.65 (m, 1H, –C$\underline{H}_2$), 2.84–2.97 (m, 2H, –C$\underline{H}_2$), 3.06–3.17 (m, 1H, –C$\underline{H}_2$), 3.25–3.34 (m, 1H, –C$\underline{H}_2$), 3.74–3.85 (m, 1H, –C$\underline{H}_2$), 4.06–4.10 (m, 1H, –O–C$\underline{H}$), 4.78–4.87 (m, 1H, –NH–C$\underline{H}$), 5.12 (s, 1H, –N$\underline{H}$), 7.05–7.07 (m, 2H, arom-$\underline{H}$), 7.14–7.18 (m, 2H, arom-$\underline{H}$), 7.21–7.24 (m, 1H, arom-$\underline{H}$), 7.36–7.37 (m, 2H, arom-$\underline{H}$), 7.65 (d, 2H, –arom-$\underline{H}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.9, 20.0, 26.7, 35.9, 48.0, 55.9, 57.0, 78.8, 114.8, 127.3, 128.0, 128.8, 129.1, 129.3, 136.1, 149.6, 157.8; IR (KBr, cm^{-1}): 3367, 2960, 2929, 1753, 1317; MS (ES+) *m/z*: 418.51 (MH+).

Results and discussion

It was apparent that, in order to achieve an efficient synthesis, the best option would be to find a suitable reaction conditions that can functionalize epoxide ring of the **3** in such a way so as to install an isobutyl amine moiety in the desired position at room temperature, instead of heating to reflux which is supposed to

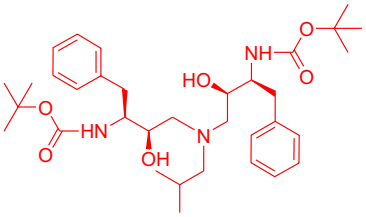


Scheme 1 Improved and robust process[16] for the preparation of DAR (2) starting from 3. Reagents and conditions: (a) isobutyl amine/IPA, 20–30 °C; (b) DCM, TEA, 4-nitrobenzenesulfonyl chloride, 15–30 °C; (c) MeOH, Raney Ni, H_2 gas, 20–30 °C; (d) aqueous HCl, EtOAc, 20–30 °C; (e) water, NaOH solution, 20–30 °C; (f) DMSO, 2,5-dioxopyrrolidin-1-yl((3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-*b*]furan-3-yl) carbonate, 15–30 °C; (g) EtOH, 75–80 °C

result into dimer-1 impurity; **11** formation (which is not disclosed till now and made it know while we developed the process), followed by substitution of secondary amine proton of **6** with 4-nitrobenzenesulfonyl chloride and successive reduction of nitro group to amine without use of palladium metal besides having the process control for formation of *N*-hydroxyl impurity; **12** below than the desired limit (Scheme 1).

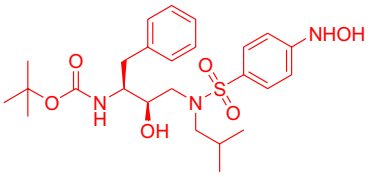
Next, synthetic sequence in the formation of DAR **2** is deprotection of *N*-Boc group which would be more appealing if performed in mild acidic condition at room temperature (RT) rather than refluxing it in strong acidic solution followed by coupling of **4** to aliphatic amine of compound **9,** without formation of critical process impurities such as dimer-2 impurity; **13** and oxazolidinone impurity; **14**, which were not exterminating by any of the purification methods, if formed, leading to the DAR **2** after treating it with ethanol (Fig. 2).

As described in Scheme 1, our synthesis of **2** begins with the addition of the isobutyl amine moiety to epoxide ring of **3** with excess of isobutyl amine reagent (13.15 mol. equiv.) along with IPA (1.0 volume) for 12–14 h at 20–30 °C. The solubility of starting material is slight poor in isobutyl amine, thus warranting the use of 1.0 T of IPA which increases the solubility of starting material. It was found that, at this concentration, a clear solution results as the reaction approaches toward completion followed by slow precipitation of desired compound **6**. TLC examination of this clear solution indicates a clean reaction with partial formation of **6**. A time study of this reaction carried out shows that the reaction is incomplete below 10 h, whereas at a time range of 12–14 h the reaction is completed when temperature is 20–30 °C. After the completion of the reaction (TLC), the volatiles are removed under vacuum followed by chasing of left over solvents traces with water. The resulting viscous material thus obtained is stirred with water at 20–30 °C and affording in separation of solid **6** as crude, which on treatment with IPA at 40–50 °C results to yield **6** in 80–85%. However, detailed study was performed to have a suitable solvent selection



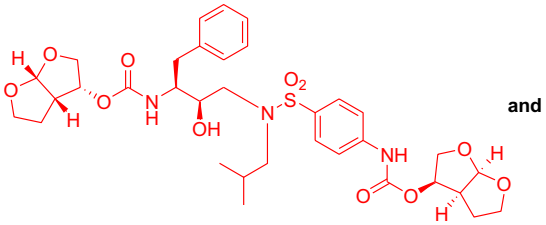
Dimer-1 impurity (**11**)

Process impurity at stage **3** to **6**



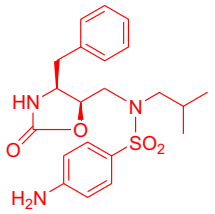
N-Hydroxyl impurity (**12**)

Process impurity at stage **6** to **7**



Dimer-2 Impurity (**13**)

and



Oxazolidinone impurity (**14**)

Critical process Impurities at stage **9** to **10**

Fig. 2 Structures of process impurities formed in stage **3**–**6**, **6**–**7** and **9**–**10** in the process

Table 1 Solvent selection for the formation of **6**

S. No.	Solvent	Isobutyl amine (mol. equiv.)	Yield (%)	Dimer-1 impurity (%)	Remarks
1.	1-Propanol	12–14	94.00	~ 10–15	Impurity formation and incomplete reaction
2.	Toluene	12–14	80.00	~ 13–18	Impurity formation and incomplete reaction
3.	Acetone	12–14	–	~ 10–15	Impurity formation and incomplete reaction
4.	DMSO	12–14	78.00	~ 2–4	Incomplete reaction
5.	MEK	12–14	–	~ 10–15	Impurity formation and incomplete reaction
6.	DMF	12–14	91	~ 10–15	Impurity formation and incomplete reaction
7.	IPA	12–14	94.67	~ 1.09	–
8.	DMA	12–14	88	~ 10–15	Impurity formation and incomplete reaction
9.	Isobutyl amine[11]	24–26	93	1.3	–

Table 2 Screening of solvent for the formation of **7**

S. No.	Solvent	4-Nitrobenzenesulfonyl chloride (mol. equiv.)	Base	Yield (%)	Remarks
1.	Acetone[11]	1.1	$NaHCO_3$	55–60	Incomplete reaction
2.	Acetone	1.1	TEA	80–85	Thick reaction mass
3.	EtOAc	1.1	TEA	80–85	Thick reaction mass
4.	Toluene	1.1	TEA	70–75	Thick reaction mass
5.	DCM	1.1	TEA	93–96	Clear reaction mass
6.	MEK	1.1	TEA	90–95	Thick reaction mass
7.	MIBK[18]	1.1	TEA	85–90	Free flowing reaction mass
8.	DMF	1.1	TEA	55–60	Clear reaction mass

along with quantity of isobutyl amine as a reagent for the formation of **6**. The results are summarized in Table 1.

After having the selection study for desired solvent, dimer-1 impurity; **11** formation (~ 10–15%) was observed on TLC in most of the solvents, while the formation of **6** was executed. The best results could be obtained when IPA was used as solvent in combination with isobutyl amine at proposed reaction conditions or when reaction was performed only in isobutyl amine at 20–30 °C for around 12–14 h resulting to afford > 80% yield of compound **6**.

Thereafter, considering our next strategy for substitution of secondary amine proton of **6** with 4-nitrobenzenesulfonyl chloride, we attempted a reaction of **6** with listed procedures such as using inorganic base ($NaHCO_3$, Na_2CO_3) and acetone/or DCM [13] as solvent to obtain an intermediate **7**. However, a mixture of **6, 7** and 4-nitrobenzenesulfonic acid was obtained due to degradation of 4-nitrobenzenesulfonyl chloride in aqueous basic reaction medium. Reaction of **6** with 4-nitrobenzenesulfonyl chloride in acetone and organic base like TEA was the next to be tried, and it resulted in isolation of the expected **7** in 80–85% yield. Although the yield of **7** in this case was satisfactory, the reproducibility of yield as well as quality was not up to the mark. Therefore, we tried this substitution reaction in different solvents by using preferable organic base (TEA) to have a better reaction profile with desired yield and quality (Table 2).

Surprisingly, in most of the solvents along with TEA as base, reaction was either resulting to form additional by-products or resulting to have a thick reaction mass which was offering constraints in completion of reaction conversion as well as in filtration. The best results could be achieved when reaction for the formation of **7** was executed in DCM/or MIBK as a reaction solvent; however, DCM was preferred over MIBK due to having low boiling point which could be easily evaporated after reaction completion for making isolation of **7** as solid with ease in 90% of isolated yield.

After having the detailed study for the formation of **6** and **7** separately, it was considered that if both stages could be performed in one pot without isolation of **6** would be real assets in the way for manufacturing the robust process for DAR **2**. Therefore, after a few trials, we were able to perform these two chemical transformations such as isobutyl amine addition and substitution of aminic proton of **6** in the

Table 3 Screening of reducing catalyst for the formation of **8**

S. No.	Reducing reagents	8 (%)	7 (%)	Remarks
1.	Zn/NH_4Cl	~85	~7–10	Incomplete reaction and impurities formation
2.	$Zn/HCOONH_4$	~80	~10–15	Incomplete reaction and impurities formation
3.	Zn/NH_2NH_2	~50	~40–50	Incomplete reaction and impurities formation
4.	Fe/CH_3COOH	~70	~20	Incomplete reaction and impurities formation
5.	Pd/C	98.91	ND	–
6.	Raney Ni	98.94	ND	–

synthetic sequence for preparation of compound **7** in tandem without confirming and analyzing the intermediate **6**. Thus, volatiles are removed under vacuum from the reaction mixture after the reaction completion of **6** followed by filtration and drying of filtered solid, without any purification and analysis, is taken up in DCM and TEA and treated with 4-nitrobenzenesulfonyl chloride solution in 2–3 volumes of DCM for 1–2 h at 15–30 °C to afford, after filtration, **7** in 88–93% overall yield from **3**. Combining these two steps offers significant advantages, as both steps necessitate the use of a single reactor at the plant level, thus lowering the reaction time and utility cost besides saving the costs involved in purification and analysis of **6**. Moreover, the overall yield from **3**–**7** is about 15–20% higher by a one-pot procedure.

The formation of compounds **8** from **7** was achieved by treating it with Raney nickel in methanol and stirring the resulting mixture under hydrogen gas pressure for 3–4 h at 20–40 °C. As the reaction proceeds, the temperature keeps on increasing and is controlled at 35–40 °C till the end of the reaction. However, several conventional reducing agents were tried, which are reported for reduction of nitro to amine in literature such as Zn/NH_4Cl [18], $Zn/HCOONH_4$ [19], Zn/NH_2NH_2 [20], Fe/CH_3COOH [21] Pd/C [13, 22, 23] and Raney nickel [24, 25] during laboratory development for achieving the formation of **8** without/or minimum formation of by-products along with complete conversion of **7**–**8** (Table 3).

As noticeable, reduction of nitro to amine was either incomplete or form undesired by-products when reagent(s), other than Pd/C and Raney nickel, were tried for the formation of **8**. Best results for reducing nitro to amine could be obtained, while reaction was performed either with wet Pd/C (10%) or Raney nickel along with methanol. Considering very low cost of Raney nickel over Pd/C was selected as reagent of choice for the formation of **8**. As a result, the metallic catalyst is removed by filtration after reaction completion and is washed with methanol. Excess methanol is evaporated, and the resulting thick oily mass is dissolved in ethyl acetate at 20–30 °C for next chemical transformation. Being a thick oily nature of **8**, isolation of this intermediate was avoided and preferred in situ conversion of **8**–**9** without further any detailed study. After a few tryouts, we were able to perform these two steps such as reduction and the *N*-Boc deprotection without confirming and analyzing the intermediate **8**.

Initial investigation for confirming *N*-Boc deprotection, after having a thick oily residue of **8**, was initiated with known conventional reagent like TFA [26, 27] in

Table 4 Screening of acid for the formation of **9**

S. No.	Acid	Solvent	8 (%)	9 (%)	Yield (%)	Remarks
1.	TFA	DCM	~7–10	~80	70	Incomplete reaction and impurities formation
2.	Conc. HCl	EtOAc	ND	99.88	88	–

DCM as solvent led us with incomplete reaction besides formation of several by-products which were hard to wipe out in the next stage(s). Understanding this, we attempted *N*-Boc deprotection with easily available commercial reagent, i.e., aqueous hydrochloric acid solution (30–35%) [28] along with ethyl acetate as reaction solvent (Table 4).

Results were quite impressive when **8** was treated with aqueous HCl solution for the formation of **9** with ethyl acetate in place of TFA/DCM reagent/solvent system. Based on quality, experimental output, process and handling ease, aqueous HCl as deprotecting reagent was selected for preparation of **9** and resulted in 85–90% yield by a one-pot procedure. Combining these two steps offers significant advantages, as both steps necessitate the use of a single reactor at the plant level, thus lowering the reaction time and utility cost besides saving the costs involved in isolation of **8**.

Final step of DAR **2** preparation involves the condensation of key pharmaceutically active candidate (3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-*b*]furan-3-ol (**4**) with **9** to structure darunavir (**10**) skeleton, was achieved by treating **9** with **5**, derivative of **4**, in different solvents, in presence/or absence of base [29, 30]. However, during process development, we encountered the formation of very critical process impurities such as dimer-2 impurity; **13** [32] and oxazolidinone impurity; 14 (which is not disclosed in reported routes till now and made it know, while we developed the process), which were tightly entangled to the DAR **2** and were very hard to remove/or minimize/or control even after multiple purification/crystallization procedures. This was understood that this might be due to the strong behavior of darunavir API skeleton to form solvate with every single solvent and reported in the studies also [19, 31, 32]. Because of strong affinity of darunavir to form solvate with almost all solvents, impurities could also be crystallized out as a result of having almost similar structure, from reaction solution, along with darunavir without solubilizing/or washing out in mother liquor, while purifications/crystallizations were tried. Therefore, it was very significant and mandatory to control these impurities formation, below than the desired limit, during reaction conversion rather than going for unpromising purifications. Before to come across for its cause, it was necessary to identify and confirm these impurities, with the aid of LCMS, NMR, mass, etc. (see supporting information).

In next attempt, a number of experiments were performed by varying all process parameters such as molar ratio of **5,** reaction time, temperature and reaction media (DCM, DMF, DMSO, THF and acetone) for the preparation of **10** from **9** (Table 5).

To our delight, desired compound **10** could be obtained in >99.80% purity by reacting **9** with compound **5** (1.05 mol. equiv.) in DMSO at 15–30 °C having

Table 5 Screening of solvent for the formation of **10**

S. No.	Solvent	9 (%)	10 (%)	Dimer-2 impurity; 13 (%)	Oxazolidinone impurity; 14 (%)
1.	DCM	0.10	99.58	0.06	0.20
2.	DMSO[16]	0.01	99.76	0.02	0.06
3.	DMF	27.78	66.37	2.26	1.16
4.	THF	57.10	37.63	0.80	0.04
5.	Acetone	2.96	94.79	1.37	0.10

impurities formation below than desired limit (NMT 0.08%). It was also understood that under these reaction conditions if temperature raises beyond the range of 30 °C, have directly impact on the formation of impurities (**13** and **14**) and temperature less than 15 °C causes freezing of reaction mass, which again suppressed the reaction conversion rate along with increased reaction time, further resulting to have a possibility of impurities formation above than controlled limits. Therefore, condensation of **9** with **5** is achieved by executing reaction in DMSO at 15–30 °C and at the end of the reaction at 15–30 °C, aqueous ammonia solution (0.24 mol. equiv.) is added to the reaction mass for decomposition of left over traces of compound **5**, which could further react with **10** if remains as such, followed by slow addition of water to reaction mass for solid precipitation, stirred and filtered to afford **10** in 93–97% yield.

The optimized synthesis is proven economically rewarding in terms of increased yield, reduced timings as well as workup hassles along with low level of known/unknown impurities below than the controlled limit. The newly developed protocol for DAR **2** has been found to be a robust process as it has achieved reproducible results on commercial scale. As per studies, DAR **2** in dose form is used as in the form of ethanolate solvate. Therefore, no further attempts were made to optimize the ethanolate solvate formation and implemented the same prior art reported process [6, 12, 13] for the formation of DAR **2** at the end could be afforded solid DAR **2** with desirable yield of 85–90% and purity > 99.80% (any unspecified impurity = not more than 0.08% w/w) under considerations of ICH guidelines [33],[4] requirements and its daily in-take dose. It is worth mentioning that in final process step, purification/re-crystallization and such operations were avoided to make this scale-up protocol most efficient, inexpensive, productive and superior in comparison with existing processes/patents.

Conclusion

Starting from the conventional and commercial available epoxide starting material **3**, the newly developed production process for DAR **2** has an overall yield of around 70% and involves five isolation and drying steps, namely **6, 7, 9, 10** and the target

[4] http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm065005.htm.

product **2**. The new process offers distinctive advantages over earlier reported procedures in terms of less effluent generation, avoiding the use of highly expensive reagent like palladium besides having the robust process for controlling the process/degradation by-products below than the desired limit (not more than 0.08%) without any purification procedures as well as significant cost reduction on commercial scale. The structural evolution of critical impurities (**11**–**14**) led to their identification and origin followed by instituting a suitable control strategy that could result to highly pure DAR **2** in a consistent way. To the best of our knowledge, we have used the industrially important Raney nickel for nitro reduction and DMSO as solvent in condensation reaction for the first time in the synthesis of darunavir. The by-products of this process, namely isobutylene, *N*-hydroxysuccinimide, NaCl and $Et_3N{\cdot}HCl$, are water soluble as well as non-toxic. This process is amenable for the large-scale production of DAR **2**.

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Compliance with ethical standards

Conflict of interest The authors declared that they have no conflict of interest.

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