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Article

Six-Step Gram-Scale Synthesis of the Human Immunodeficiency Virus Integrase Inhibitor Dolutegravir Sodium

Jule-Philipp Dietz, Tobias Lucas, Jonathan Groß, Sebastian Seitel, Jan Brauer, Dorota Ferenc, B. Frank Gupton, and Till Opatz*

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ABSTRACT: A short and practical synthesis for preparing the active pharmaceutical ingredient dolutegravir sodium was developed. The convergent strategy starts from (*R*)-3-amino-1-butanol and establishes the BC ring system in a 76% isolated yield over four steps. Ring A was constructed by a one-pot 1,4-addition to diethyl-(2*E*/*Z*)-2-(ethoxymethylidene)-3-oxobutandioate and subsequent $MgBr_2 \cdot OEt_2$-mediated regioselective cyclization. Amide formation with 2,4-difluorobenzylamine was either performed from the free carboxylic acid or through aminolysis of the corresponding ethyl ester. Final salt formation afforded dolutegravir sodium in a 48−51% isolated yield (HPLC purity of 99.7−99.9%) over six linear steps.

KEYWORDS: *dolutegravir sodium, active pharmaceutical ingredient, antivirals, integrase inhibitors, carbamoyl pyridones*

INTRODUCTION

Infection with the human immunodeficiency virus (HIV) has become controllable in recent years due to enormous progress in development of highly active drugs, which are given for antiretroviral therapy (ART).[1] ART requires the administration of at least three different antiviral drugs to suppress the development of resistances.[2] Differentiated by the target enzyme, there are several classes of HIV-inhibiting drugs. The class of integrase strand transfer inhibitors (INSTIs) interferes with the HIV integrase enzyme and prevents it from inserting viral DNA into the human genome. INSTIs have been introduced in 2007 with the launch of raltegravir (**1**) followed by elvitegravir (**2**, 2012), dolutegravir (**3**, 2013), bictegravir (**4**, 2018), and cabotegravir (**5**, 2021)[3] (Scheme 1).[4]

The last three compounds exhibit a high similarity in their molecular structures, assigning them to the group of carbamoyl pyridine INSTIs. Dolutegravir (**3**), usually administered orally as its sodium salt, was recently recommended by the World Health Organization for first-line treatment of HIV initiating ART.[5] As a consequence, the demand of this important medication could further rise.

All synthetic approaches to **3** that have been published before 2019 have been carefully reviewed.[4,6] Since then, four more routes were disclosed.[7−10] Most of the published syntheses follow a similar strategy, which is represented here by the hitherto most efficient approach from Micro Labs (2016)[11] (Scheme 2). The highly functionalized pyridone **7** (ring A) is constructed first, which then undergoes cyclization with (*R*)-3-amino-1-butanol (**13**) to construct rings B and C. Deprotection of the usually protected enol and treatment with sodium hydroxide furnish dolutegravir sodium (**15**). The seven-step synthesis by Micro Labs afforded **15** in a 35% overall yield. This retained synthesis concept could be attributed to the late-stage introduction of the expensive amino alcohol **13**.

Since the discovery of dolutegravir and the emerging demand of (*R*)-3-amino-1-butanol (**13**), more efficient syntheses of this crucial building block have been developed with the consequence of a decreasing market price.[12] Thus, an earlier introduction of **13** could add additional value by bringing more diversification to the synthetic portfolio of dolutegravir. Extending the scope of industrially applicable synthetic routes should encourage generic manufacturing to ensure global supply of this important drug.

Here, a new synthetic route should be investigated by taking **13** as a starting material (Scheme 3). By using commodity chemicals, the ring system BC (**16**) should be constructed first. Next, **16** should be reacted with readily accessible diethyl-(2*E*/*Z*)-2-(ethoxymethylidene)-3-oxobutandioate (**17**) to install ring A. Amide coupling with 2,4-difluorobenzylamine and salt formation follow in the last step. All in all, an industrially feasible synthesis route was intended to be developed.

RESULTS AND DISCUSSION

Synthesis of the Ring System BC. The synthesis of amine **16** has already been described twice in the patent literature. Lerner et al. reported a five-step synthesis starting from ethyl bromoactetate (**19**) furnishing amine **16** in a 24% overall yield.[13] The crucial cyclization with amino alcohol **13** gave only a 55% yield under microwave conditions, and

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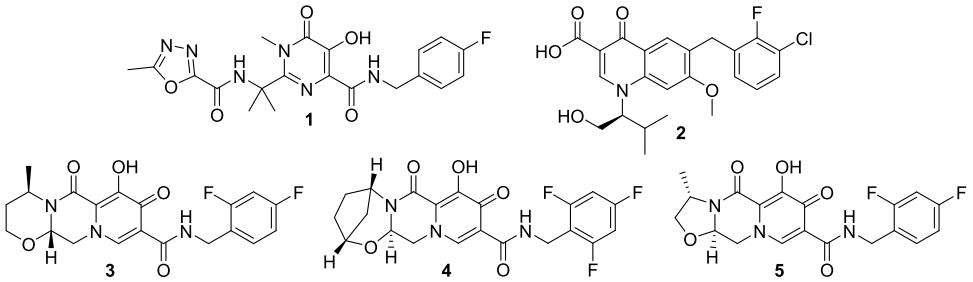
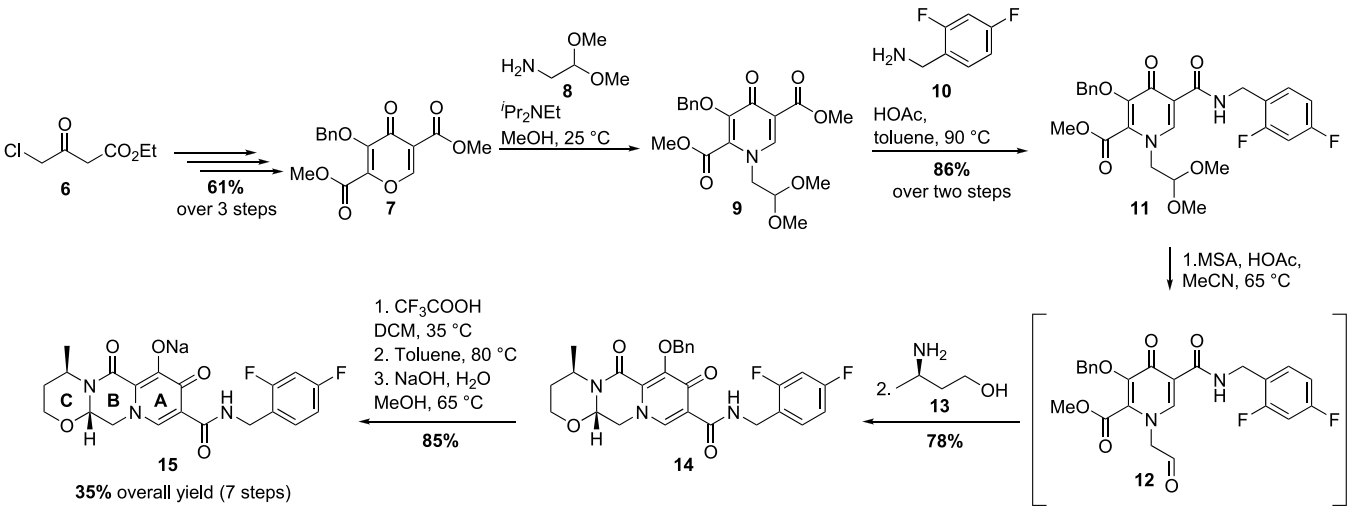
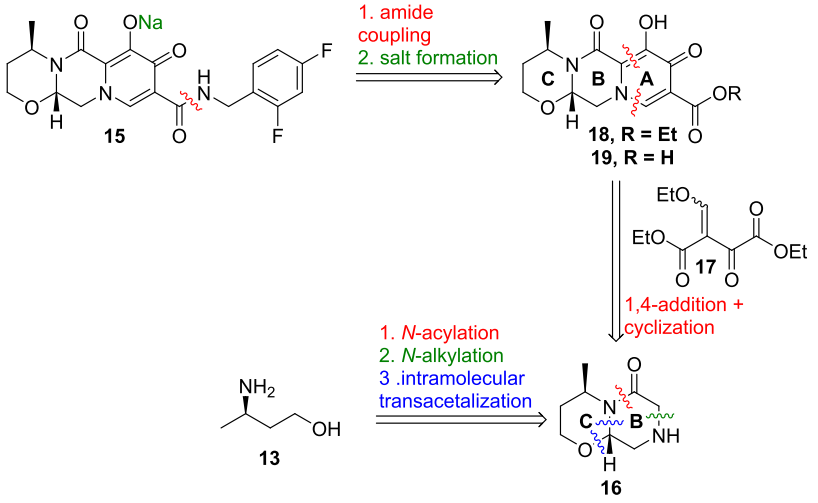
Scheme 1. Structures of Raltegravir (1), Elvitegravir (2), Dolutegravir (3), Bictegravir (4), and Cabotegravir (5)



Scheme 2. Synthesis of Dolutegravir by Micro Labs (2016)



Scheme 3. Retrosynthetic Strategy toward Dolutegravir (15)



chromatographic steps were required. During our synthetic work, a patent from the Virginia Commonwealth University was disclosed, which describes a similar but more efficient approach toward **16**.[10] The methyl ester of intermediate **24** was prepared in three steps from methyl glycinate hydrochloride (**23**) in a 66% yield. The cyclization step was performed in a toluene/methanol/acetic acid mixture and achieved a 71% yield after column chromatography. Removing the Cbz group at higher hydrogen pressure gave slightly better results, and amine **16** was finally obtained in 44% over five steps. The synthesis was even performed on a multigram scale but required two chromatographic steps (Scheme 4).

A potentially shorter approach could be achieved by first *N*-acylating **13** with chloroacetyl chloride (**25**) (Scheme 5). Subsequent alkylation with aminoacetaldehyde dimethyl acetal (**8**) would furnish the acyclic precursor **27**. Acid-catalyzed intramolecular transacetalization would in turn afford the desired amine **16**.

When the acylation of **13** was performed under standard conditions (NEt_3, DCM, 0 °C), a mixture of desired **26** and *N*,*O*-bis-acylated compound **28** was obtained (Scheme 6).

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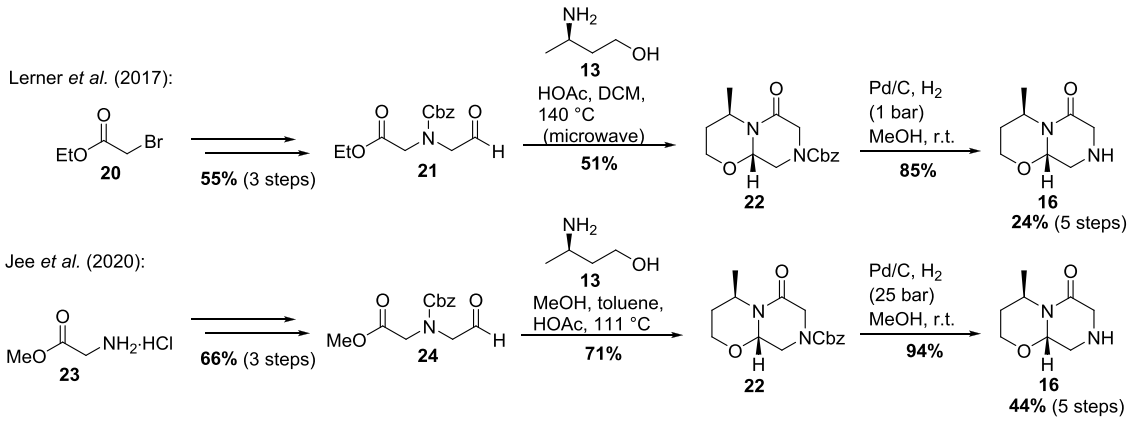
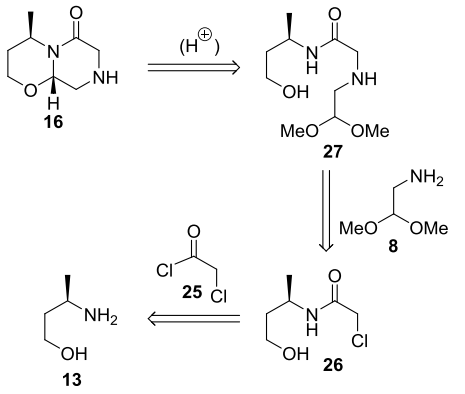
Scheme 4. Known Syntheses of Amine 16



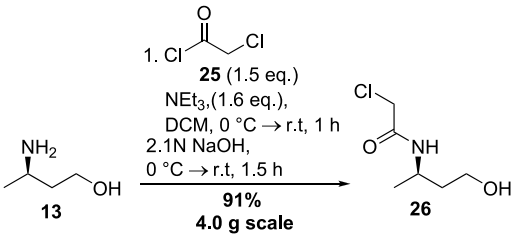
Scheme 5. Retrosynthetic Proposal toward Amine 16



Nevertheless, **28** could be easily saponified to **26** by just adding an aqueous base to the crude reaction mixture. After extractive workup, only a 40% isolated yield of **26** was obtained. It turned out that large amounts of **26** had remained in the aqueous phase. When water was removed in vacuo and the salty residue was suspended in ethyl acetate, a further 40% of **26** could be isolated.

Due to the high polarity of **26**, the whole process was adjusted by using continuous extraction. After complete saponification of **28**, which was followed by GC−MS, the organic phase was separated and additionally extracted with water. The combined aqueous phases were neutralized with an acid, transferred into a Kutscher-Steudel apparatus, and continuously extracted for 72 h with ethyl acetate. This procedure enabled the isolation of **26** in a 91% isolated yield (Scheme 7). It should be noted that **26** showed high purity according to ^{1}H NMR and no further purification step was required. As an ambient-pressure distillation is involved in this continuous extraction, apparatus improvements can likely accelerate the procedure.

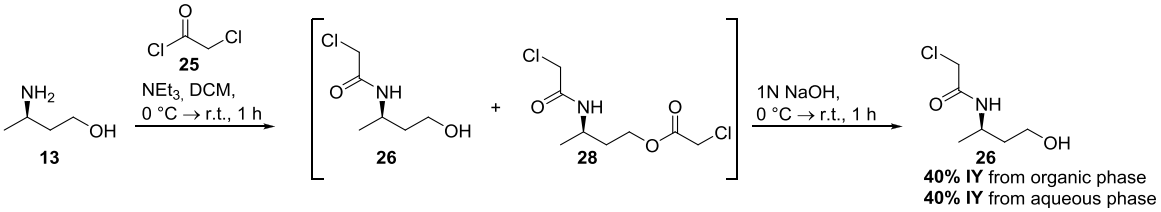
Scheme 7. *N*-Acylation of 13 with Chloroacetyl Chloride



In the next step, **26** should be used for alkylating aminoacetaldehyde dimethyl acetal (**8**) to form the secondary amine **27**. The reaction was performed either by heating in toluene or by stirring at r.t. in DCM in the presence of sodium iodide (Scheme 8). After aqueous workup, only 33−45% of crude **27** could be isolated. As already reported for the previous reaction, a significant quantity of **27** remained in the aqueous phase, which could not be separated from excess amine **8**. Through LC−MS, one main impurity could be identified as the tertiary amine **29**. To circumvent its formation and to solve the water solubility issue, another strategy was chosen.

By converting primary amine **8** into a secondary and more lipophilic amine first, subsequent alkylation would lead to a tertiary and better extractable amine. Benzylation of **8** proved to be a good option as it can be easily reverted by hydrogenation. *N*-Benzyl-2,2-dimethoxyethylamine (**30**) was prepared in two different ways (Scheme 9). When heating chloroacetaldehyde-dimethylacetal (**31**) with an excess of benzylamine (**32**) in toluene, 77% of **30** was isolated after distillation. According to a procedure from Luu et al., **30** could also be prepared through reductive amination from stochiometric amounts of benzaldehyde (**33**) and aminoacetaldehyde dimethyl acetal (**8**).[14] The latter method furnished **30** in a 97% isolated yield.

Scheme 6. *N*-Acylation of 3-(*R*)-Amino-1-butanol (13)



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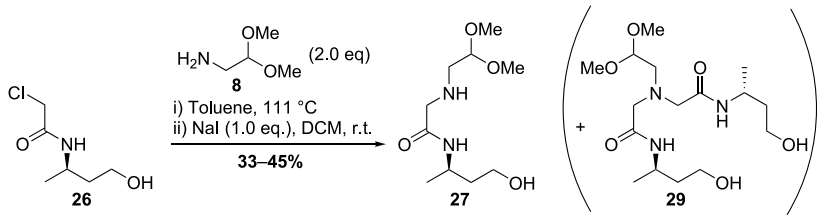
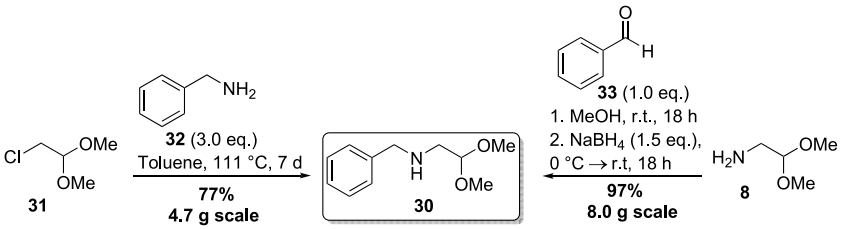
Scheme 8. *N*-Alkylation of Aminoacetaldehyde Dimethyl Acetal (8) with 26

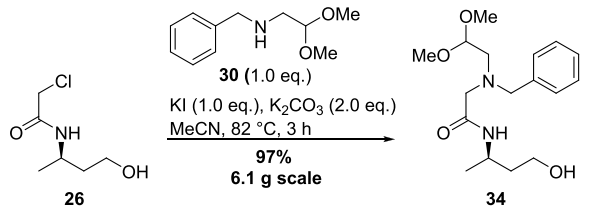


Scheme 9. Synthesis of *N*-Benzyl-2,2-dimethoxyethylamine (30) by *N*-Alkylation or Reductive Amination



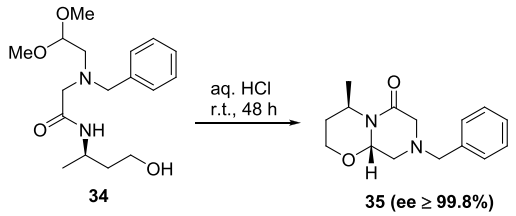
Using secondary amine **30** instead of primary amine **8** for the alkylation gave much better results (Scheme 10). The reaction was performed in acetonitrile (MeCN) in the presence of potassium iodide (KI) and potassium carbonate (K_2CO_3). Complete conversion of **26** was observed after 24 h at r.t. or after 3 h when heating to reflux. For workup, MeCN was removed and an extraction from water/ethyl acetate furnished **34** as a slightly brownish oil. Again, the reaction proceeded very cleanly, and no further purification of the product was necessary.

Scheme 10. Synthesis of Tertiary Amine 34 from 26 and 30 by Heating in MeCN in the Presence of KI and K_2CO_3



34 cyclized cleanly in aqueous hydrochloric acid to the desired diastereomer of oxazinone **35**. At least 6 N HCl was necessary to achieve complete conversion after 48 h at r.t. (Table 1). Only small amounts of byproducts were detected by LC–MS. As the undesired diastereomer with opposite configuration at the acetalic center was not isolated and characterized, diastereoselectivity can only be assumed based on the second largest HPLC peak to amount at least to 44:1 (for details, see the Supporting Information). When the reaction was performed on an 11.5 g scale, crude **35** was isolated in a 92% yield as a brown oil, which only contained slight impurities. For the workup, the reaction mixture was neutralized with sodium hydroxide and extracted with ethyl acetate. The stereoconfiguration of **35** was verified by VCD spectroscopy and comparison of the experimental data with calculations using density functional theory (for more details, see the Supporting Information). The enantiomeric excess was determined by chiral HPLC. Racemic **35** was prepared as a reference material in the same way starting from *rac*-3-amino-1-butanol (*rac*-**35**).

Table 1. Intramolecular Transacetalization of 34

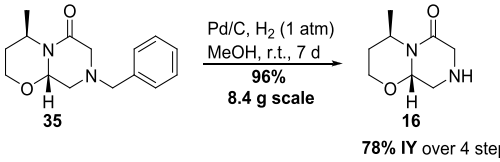


entry	solvent	conversion [%][a]	IY [%]
1	1 N HCl	4	-
2	3 N HCl	34	-
3[b]	6 N HCl	100	92

[a]Determined by LC–MS and UV absorption at 254 nm. [b]11.5 g scale.

The benzyl group was subsequently removed by hydrogenation (Scheme 11), and crude amine **16** was obtained in a 96% yield (78% IY over four steps) as a brown-orange oil, which solidified after a while to a beige and free-flowing solid. According to LC–MS and ^{1}H NMR, minor impurities could be detected in the crude material (see the corresponding spectra in the Supporting Information). Crude **16** was used for the next step, and no further purification efforts were investigated.

Scheme 11. Debenzylation of 35 by Hydrogenation



Construction of Ring A. Jee et al. also reported a synthesis route to dolutegravir starting from the ring system BC (**16**) (Scheme 12).[10] Intermediate **39**, which contains the crucial tricarbonyl moiety, was installed in two steps in a 89% yield. Ring A was constructed by regioselective cyclization using magnesium bromide ethyl etherate ($MgBr_2$·OEt_2) and triethylamine (65% yield of **40**). Subsequent amide coupling afforded the desired dolutegravir **3** in a 67% yield. The route depicts the

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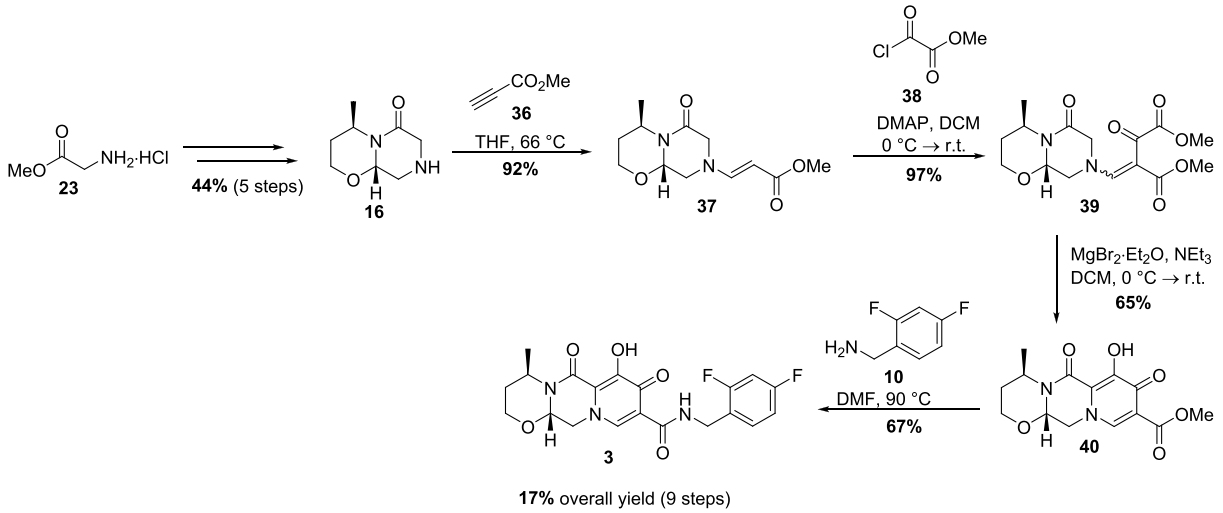
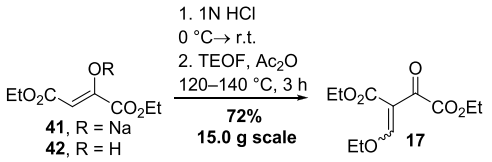
Scheme 12. Synthesis of Dolutegravir (3) by Jee et al.



first one reported where the ring system AB was constructed first. The overall yield was only 17%, and five chromatographic steps were required.

Enaminone **39** should be also accessible in a single step by 1,4-addition from the corresponding enol ether **17**. According to a procedure of Jones,[15] **17** was prepared from the commercially available diethyl oxalacetate sodium salt (in two steps) (Scheme 13). The salt **41** had to be acidified first to obtain diethyl oxalacetate (**42**), which was then condensed with triethyl orthoformate (TEOF) in the presence of acetic anhydride (Ac_2O). The reaction could be performed in a distillation apparatus, and **17** was directly distilled out of the reaction mixture resulting in a 72% yield over two steps.

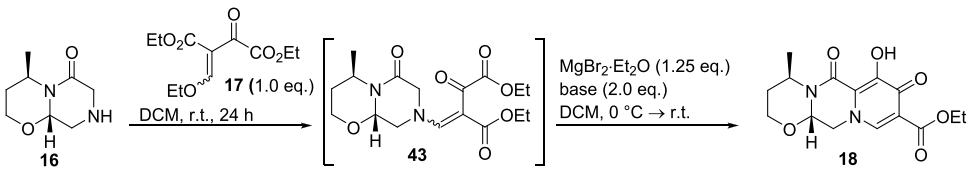
Scheme 13. Synthesis of Enol Ether 17 by Condensation of 42 with TEOF and Ac_2O



Amine **16** readily underwent 1,4-addition to **43** in several solvents (DCM, MeCN, EtOH, THF, and toluene). The formation of only one geometrical isomer of **43** could be detected by NMR and LC−MS (for more details, see the Supporting Information). Regarding the regioselective cyclization, it turned out that using strong bases like KO^{t}Bu, NaOEt, or NaH predominantly led to the formation of a product mixture. Applying the conditions of Jee et al., using a combination of magnesium bromide ethyl etherate ($MgBr_2$·OEt_2) and triethylamine (NEt_3) looked more promising, and predominant conversion to ester **18** (81 area % (254 nm), 35 area % (315 nm)) could be observed (entry 1, Table 2).[10] Nevertheless, there was still significant byproduct formation according to the HPLC trace at 315 nm and ^{1}H NMR. According to LC−MS data (m/z = 351), it is assumed that the main byproduct could be pyrrole **45** (Figure 1), which could form in an undesired 5-exo cyclization with the keto group adjacent to the ester group.

Furthermore, the reported workup method, which consisted of dissolving the crude reaction mixture in sat. $NaHCO_3$ solution followed by extraction, led to formation of barely soluble magnesium salts, which complicated the extraction.

Table 2. Screening Conditions for Building Up Ring A



			area % **18**[a]	
entry	base	t [h]	254 nm	315 nm
1	NEt_3	2	81	35
2	DIPEA	2	91	44
3	2,6-lutidine	24	90	37
4	*N*,*N*-dimethylaniline	24	48	9
5	pyridine	2	67	22
		65	91	71
6	pyridine[b]	65	94	84

[a]Determined by LC−MS and UV absorption. [b]Three equivalents were used.

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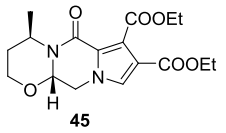


Figure 1. Proposed structure of byproduct **45**.

Additionally, ester **18** was partly saponified under these conditions.

For optimization, bases other than NEt_3 were investigated first. When diisopropylethylamine (DIPEA) was used, conversion slightly improved to 91 area % (254 nm) and 44 area % (315 nm) (entry 2, Table 2). Using 2,6-lutidine as a base resulted in a similar result to NEt_3 (entry 3, Table 2), while *N*,*N*-dimethylaniline gave only low conversion (entry 4, Table 2). With pyridine, the reaction proceeded much slower but also cleaner (91 area % (254 nm) and 71 area % (315 nm) conversion after 65 h) (entry 5, Table 2). Increasing the equivalents of pyridine (3.0) additionally improved the conversion (entry 6, Table 2).

For the workup, the reaction mixture was cooled, quenched with 1 N HCl, and extracted with DCM. When the reaction was performed on a 2 g scale, a 95% isolated yield of crude **18** was obtained. The orange-reddish solid showed purities of 94 area % (254 nm) and 87 area % (315 nm) (Scheme 14).

Crude **18** could be washed with ethyl acetate to remove impurities, but as some material was lost in this step, the crude material was used for the next step. As an alternative procedure for purification, it turned out that saponification furnished acid **19** in a pure form. A simple one-pot procedure was developed by adding aqueous 1 N NaOH to the DCM extract of ester **18**. After stirring the two-phase mixture overnight, **18** was completely saponified to acid **19**. Impurities stayed in the organic phase, and acid **19** precipitated out of the aqueous phase after acidification. Filtration and drying afterward afforded **19** as a colorless solid in a 72% yield over three steps (Scheme 15).

While investigating the regioselective cyclization, it was also considered to replace $MgBr_2$ OEt_2 by cheaper and more widely available $MgCl_2$. From all tested conditions, only heating in MeCN showed promising results (for more details, see the Supporting Information). Conversion to ester **18** was usually lower (85 area % (254 nm), 73 area % (315 nm)). As also partly saponification to acid **19** was observed, it appeared more reasonable to drive the reaction completely toward **18**. After removing MeCN, workup and saponification were performed as mentioned above to furnish a 53% isolated yield of **19** (Scheme 16).

Amide Coupling. Aminolysis of an ethyl ester moiety with 2,4-difluorobenzylamine by heating in toluene in the presence of acetic acid has already been reported in the literature for other dolutegravir building blocks.[11,16,17] The toluene/acetic acid conditions showed to be appropriate also for ester **18** and were thus optimized (for more details, see the Supporting Information). After heating overnight in the presence of an excess of amine and acetic acid (both 2.5 equiv), full conversion to dolutegravir (**3**) was detected by LC−MS. It turned out be more efficient when the aqueous workup was omitted. After complete conversion of ester **18**, all volatiles were removed in vacuo, and the residue was dissolved in hot EtOH and treated with sodium hydroxide (NaOH). After filtration and washing, the filtered salt was heated again in EtOH and hot-filtered to increase the purity. After drying, dolutegravir sodium (DTG-Na, **15**) was obtained in a 70% isolated yield (HPLC purity of 99.7% (254 nm)) (Scheme 17).

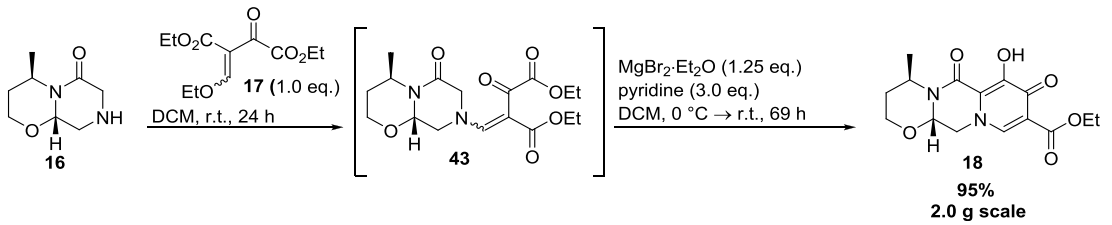
The amide coupling of acid **19** with amine **10** has been reported in the patent literature. By using the expensive coupling reagent HATU (1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate), *N*-methylpyrrolidine, and DMF as the solvent, **15** was isolated in a 55% yield after purification by preparative HPLC.[18] The reaction has also been described for preparing bictegravir (**4**), but 1,1-carbonyldiimidazole (CDI) was used instead as a coupling reagent in this case.[19] Following this protocol, acid **19** was activated with CDI by stirring for 2 h in dimethyl carbonate (DMC) at 80 °C. Amine **10** was added at r.t., and clean conversion to **3** was detected by LC−MS after 2 h. Aqueous workup afforded crude dolutegravir (**3**), which was converted to the sodium salt as mentioned above giving **15** in a 94% isolated yield showing an HPLC purity of 99.9% (254 nm) (Scheme 18).

When only 1.7 equiv of CDI were used, incomplete conversion was observed. As a consequence, the final product contained more nonremovable traces of acid **19** or ester **18**. The reaction was once performed in a smaller scale (0.3 g) with skipping the aqueous workup the same as reported for aminolysis of ester **18**. A similar isolated yield (91%) was obtained, but **15** was slightly less pure (99.7%, 254 nm).

CONCLUSIONS

A practical synthesis route to dolutegravir sodium starting from (*R*)-3-amino-1-butanol (**13**) was introduced (Scheme 19). First, a new four-step and highly yielding synthesis to the ring system BC containing amine **16** was developed. It is noteworthy that the reaction sequence proceeded without significant byproduct formation and no cost-intensive purification steps had to be used. The regioselective cyclization to ring A was carefully optimized and significantly improved. Furthermore, efficient access to acid **19** was demonstrated. Ester **18** was isolated in a higher yield admittedly but showed a lower purity than acid **19**. Both compounds could be transformed to desired DTG-Na in similar overall yields. Conversion of ester **18** with 2,4-difluorobenzylamine required harsher conditions but less expensive reagents (toluene and

Scheme 14. Synthesis of Ester 18 by One-Pot 1,4-Addition of Amines 16 with 17 Followed by Regioselective Cyclization



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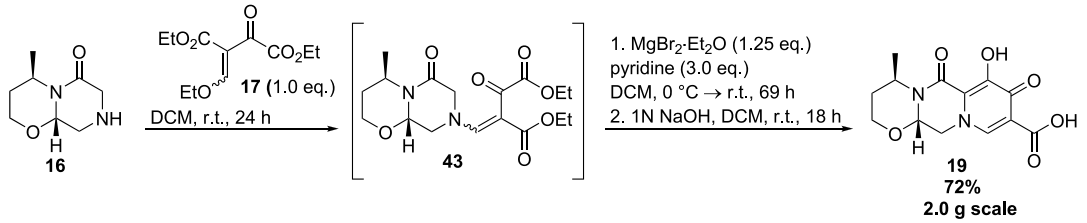
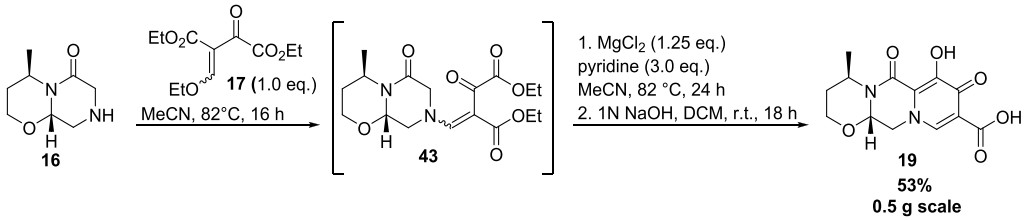
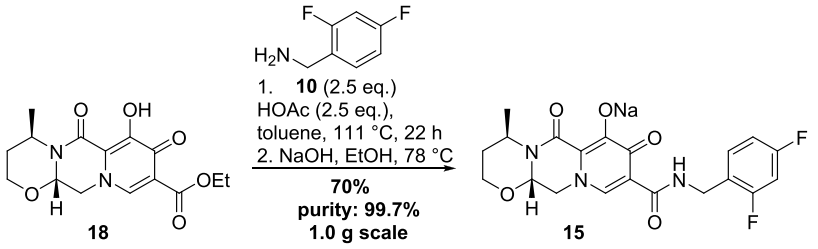
Scheme 15. Synthesis of Acid 19 by One-Pot 1,4-Addition of Amines 16 to 43 Followed by $MgBr_2$-Mediated Regioselective Cyclization and Saponification



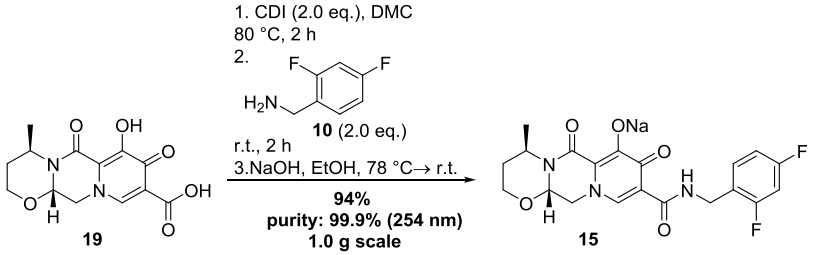
Scheme 16. Synthesis of Acid 19 by One-Pot 1,4-Addition of Amines 16 to 43 Followed by $MgCl_2$-Mediated Regioselective Cyclization and Saponification



Scheme 17. Synthesis of DTG-Na (15) by Aminolysis of Crude Ester 18 and Subsequent Salt Formation



Scheme 18. Synthesis of DTG-Na (15) by Amide Coupling of Acid 19 and Subsequent Salt Formation



acetic acid), while acid **19** showed a cleaner conversion under milder conditions by using CDI as a coupling reagent. Ultimately, both transformations represent attractive routes and enable new synthetic access to DTG-Na.

■ EXPERIMENTAL SECTION

All employed chemicals were commercially available and used without prior purification except 2,4-difluorobenzylamine, which was distilled and stored over a nitrogen atmosphere. Anhydrous solvents were taken from a solvent purification system and under a nitrogen atmosphere. Oven-dried glassware was dried in an oven at 150 °C overnight, assembled while still hot, cooled to room temperature, and then purged with nitrogen. NMR spectra were recorded on a Bruker Avance-III HD instrument (^{1}H NMR, 300 MHz; ^{13}C NMR, 75 MHz) or a Bruker Avance-III HD instrument (^{1}H NMR, 400 MHz; ^{13}C NMR, 101 MHz; ^{19}F NMR, 377 MHz) with a 5 mm BBFO probe. The chemical shifts δ were expressed in ppm downfield from tetramethylsilane (^{1}H NMR and ^{13}C NMR). Deuterated solvents ($CDCl_3$ and DMSO-d_6) served as an internal reference. The reported signal splittings were abbreviated as follows: s_b = broad singlet, s = singlet, d = doublet, and t = triplet. Coupling constants J are reported in Hz. ESI-MS spectra were recorded on a 1260-series Infinity II HPLC system (Agilent Technologies) with a binary pump and an integrated diode array detector coupled to an LC/MSD Infinitylab LC/MSD (G6125B LC/MSD) mass spectrometer. For high-resolution (HR) mass spectra, an Agilent 6545 Q-TOF spectrometer and a suitable external calibrant were used. Analytical HPLC was carried out with an Agilent 1260 Infinity system equipped with a binary pump, a diode array detector, and an LC/MSD InfinityLab LC/MSD (G6125B LC/MSD) mass spectrometer. An Ascentis Express C18 column (2.7 μm, 2.1 mm × 30 mm, 40 °C) or an ACE C18 PFP column (3 μm, 4.6 mm × 150 mm, 40 °C) with gradient elution using acetonitrile/water (+0.1% formic acid) and a flow rate of 1.0

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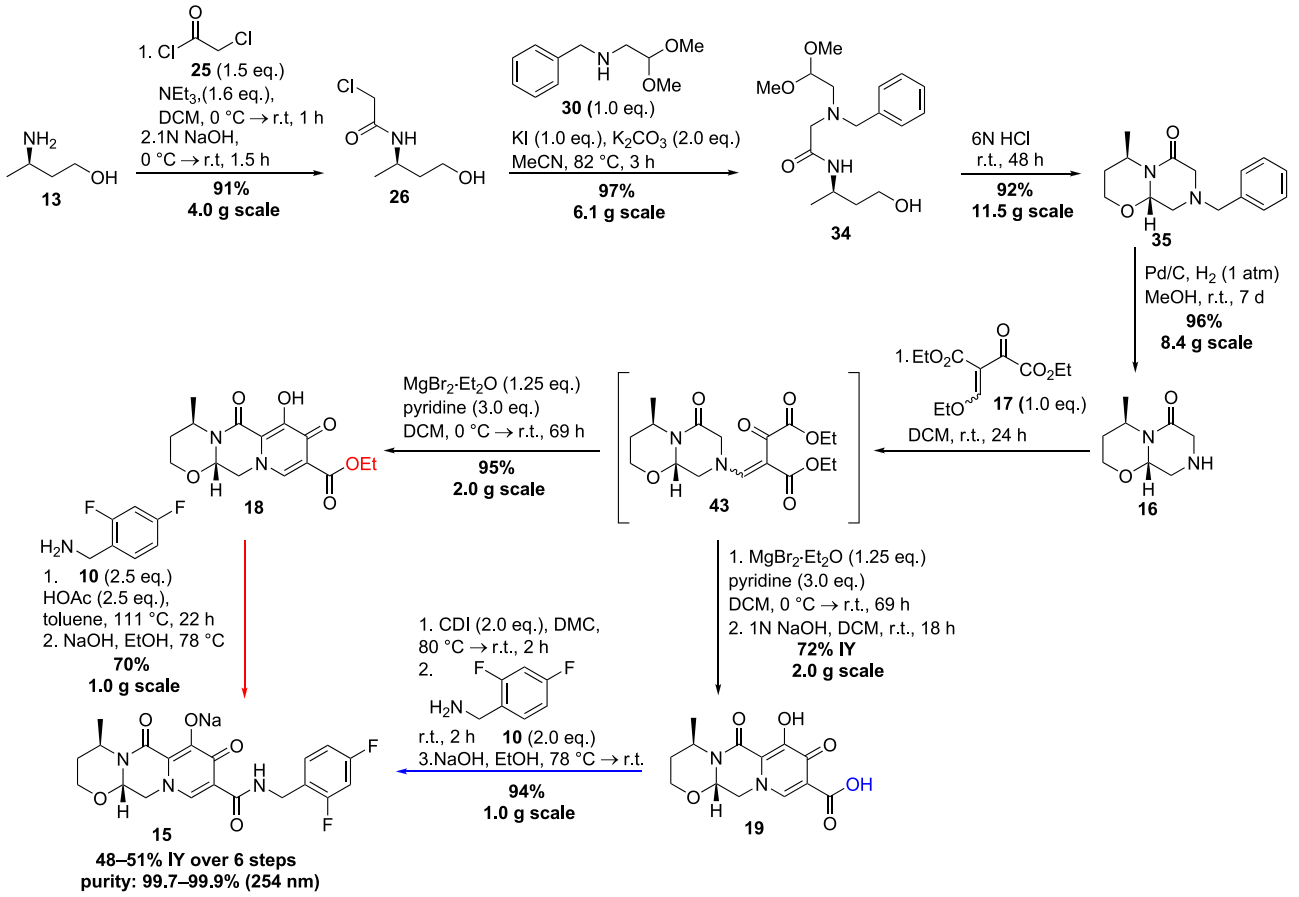
Scheme 19. Here Reported Synthesis of DTG-Na (15)



mL/min was used. Chiral HPLC was performed on a 1260-series Infinity II HPLC system (Agilent Technologies) in a normal phase and isocratic mode with EtOH/*n*-hexane as the mobile phase. A Daicel Chiralpak IF-3 column (3 μm, 4.6 mm × 250 mm, 40 °C) was used for enantiomeric excess determination. Gas chromatography was performed on an Agilent 8890 gas chromatograph equipped with a 5977 GC/MS detector. An Agilent Technologies HP 5MS UI column (30 m × 0.25 mm × 0.25 μm) as a stationary phase with helium as a carrier gas and a flow rate of 1.2 mL/min was used. The following parameters were used: an inlet temperature of 250 °C, a transfer line temperature of 250 °C, an ion source temperature of 230 °C, an MS-quadrupole temperature of 150 °C, and an initial oven temperature of 40 °C for 2 min with a temperature ramp of 50 °C/min to 320 °C over 5.6 min followed by 7.4 min holding. IR spectroscopy was conducted on a Bruker Tensor 27 FTIR spectrometer using a diamond ATR unit. Thin-layer chromatography was performed on Merck F_{254} silica gel plates. Spots were visualized with UV light (λ = 254 nm) or stained with appropriate reagents. Melting points are uncorrected and were taken by using a Krüss KSP1N digital melting point apparatus. Optical rotations were measured on a PerkinElmer 241 MC polarimeter.

4-Hydroxybutan-2-one Oxime, 44. According to a modified procedure by Budidet et al.,[20] a solution of 4-hydroxybutan-2-one (95%, 5.0 mL, 55 mmol, 1.0 equiv) in EtOH (60 mL) was cooled in an ice bath. Hydroxylamine hydrochloride (4.6 g, 66.0 mmol, 1.2 equiv) was added, and the pH was adjusted to 6 by slow addition of aq. sodium hydroxide solution (40 wt %). The colorless suspension was stirred for 4 h at r.t. (complete conversion detected by GC–MS) before it was filtered. The solvent was removed in vacuo at 40 °C, and the residue was suspended in EtOAc (50 mL). After drying over Na_2SO_4, all volatiles were removed in vacuo at 40 °C to obtain **44** as a mixture of anti/syn isomers (5.60 g, 54.3 mmol, 99%) as a colorless viscous oil. *M* ($C_4H_9NO_2$) = 103.12 g/mol. R_f (SiO_2) = 0.21 (EtOAc), stained with a ninhydrin reagent. IR (ATR): ν = 3249, 2889, 1660, 1427, 1370, 1261, 1050 cm^{-1}. ^1H NMR, COSY (400 MHz, DMSO-d_6): δ = 10.26/10.17 (s, 1H, −NOH), 4.62–4.56/4.55–4.48 (m, 1H, −OH), 3.59–3.50 (m, 2H, H-4), 2.41/2.25 (t, ^3J = 6.8 Hz, H-3), 1.78/1.73 (s, 3H, H-1) ppm. ^{13}C NMR, HSQC, HMBC (100 MHz, DMSO-d_6): δ = 154.1/153.8 (C-2), 58.4/57.3 (C-4), 38.8/32.1 (C-3), 20.2/13.5 (C-1) ppm. GC–MS: *m*/*z* = 58.1 (100%). ESI-HRMS: calcd for $[C_4H_9NO_2 + H]^+$, *m*/*z* = 104.0706; found, *m*/*z* = 104.0703.

***rac*-3-Aminobutan-1-ol, *rac*-13.** According to a modified procedure by Budidet et al.,[20] a suspension of 4-hydroxy-2-butanone oxime (**44**, 8.60 g, 83.4 mmol) and Raney nickel (10 wt %) in MeOH (70 mL) was hydrogenated in an autoclave for 28 h (10 bar H_2, 45 °C) (reaction control by TLC). The suspension was suction-filtered over celite, and the celite cake was washed several times with MeOH. All volatiles were removed in vacuo at 40 °C in order to obtain *rac*-**13** as a colorless oil (6.91 g, 77.6 mmol, 93%), which was used for the next step without further purification. *M* ($C_4H_{11}NO$) = 89.14 g/mol. T_b = 81–83 °C (22 mbar); lit. 95–97 °C (28 mbar).[21] R_f (SiO_2) = 0.19 (EtOAc:MeOH:NEt_3 = 2:1:1), stained with ninhydrin reagent. IR (ATR): ν = 3347, 3280, 3183, 2957, 2924, 2870, 1599, 1455, 1375, 1062 cm^{-1}. ^1H NMR, COSY

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(400 MHz, $CDCl_3$): δ = 3.84−3.72 (m, 2H, H-1), 3.17−3.04 (m, 1H, H-3), 2.69 (s_B, 3H, −OH and $-NH_2$), 1.66−1.57 (m, 1H, H_a-2), 1.53−1.42 (m, 1H, H_b-2), 1.13 (d, ^3J = 6.4 Hz, 3H, H-4) ppm. ^{13}C NMR, HMBC, HSQC (101 MHz, $CDCl_3$): δ = 62.4 (C-1), 48.0 (C-3), 39.5 (C-2), 25.8 (C-4) ppm. ESI-HRMS: calcd for $[M + H]^+$, m/z = 90.0913; found, m/z = 90.0913. The spectrometric data are consistent with literature values.[22]

(*R*)-2-Chloro-*N*-(4-hydroxybutan-2-yl)acetamide, 26. In an oven-dried Schlenk flask, **13** (4.00 g, 44.9 mmol, 1.0 equiv) was dissolved in DCM (80 mL). NEt_3 (10.0 mL, 71.8 mmol, 1.6 equiv) was added, and the solution was cooled in an ice bath. Chloroacetyl chloride (**25**, 5.3 mL, 67 mmol, 1.5 equiv) was added dropwise over 10 min, cooling was removed, and the dark red-brown solution stirred for 1 h at r.t. (complete consumption of **13** detected by TLC). While cooling, first water (64 mL) and then 3 N NaOH (32 mL) were added to the reaction mixture. The cooling bath was removed, and the two-phasic mixture was stirred vigorously for 90 min at r.t. (complete saponification to **26** detected by GC−MS). The organic phase was separated and extracted with water (3 × 40 mL). The combined aqueous phases were cooled and adjusted to pH = 7−8 using conc. HCl. The solution was transferred into a Kutscher-Steudel apparatus and extracted continuously with EtOAc for 72 h. The orange organic phase was dried over Na_2SO_4 and filtered, and the solvent was removed in vacuo at 40 °C. **26** was obtained as an orange-brown viscous oil (6.73 g, 40.6 mmol, 91%) and used for the next step without any further purification. M ($C_6H_{12}ClNO_2$) = 165.63 g/mol. $[\alpha]_{589}^{20}$= −32.7 ($CHCl_3$, c = 10 mg/mL). R_f (SiO_2) = 0.30 (EtOAc), stained with ninhydrin reagent. IR (ATR): ν = 3280, 2936. 1651, 1543, 1056 cm^{-1}. ^{1}H NMR, COSY (300 MHz, $CDCl_3$): δ = 6.68 (s_B, 1H, -NH-), 4.27−4.12 (m, 1H, H-2′), 3.71−3.54 (m, 2H, H-4′), 3.01 (s_B, 1H, -OH), 1.93−1.80 (m, 1H, H_a-3′), 1.53−1.41 (m, 1H, H_b-3′), 1.26 (d, ^3J = 1.3 Hz, 3H, H-1′) ppm. ^{13}C NMR, HMBC, HSQC (75 MHz, $CDCl_3$): δ = 166.6 (C-1), 58.9 (C-4′), 43.2 (C-2′), 42.6 (C-2), 39.5 (C-3′), 20.9 (C-1′) ppm. GC−MS: m/z = 120.1 (100%). ESI-HRMS: calcd for $[M + H]^+$, m/z = 166.0629; found, m/z = 166.0633.

***N*-Benzyl-2,2-dimethoxyethylamine, 30.** For method 1, benzylamine (**32**, 12.2 g, 113 mmol, 3.0 equiv) and chloroacetaldehyde-dimethylacetal (**31**, 4.71 g, 38 mmol, 1.0 equiv) were dissolved in toluene (50 mL) and heated to reflux for seven days. The suspension was cooled in an ice bath and filtered, toluene was then removed in vacuo at 40 °C. The residue was distilled under vacuum to afford **30** as a colorless liquid (5.71 g, 29.2 mmol, 77%). For method 2, according to a modified procedure from Luu et al.,[14] to a solution of aminoacetaldehyde dimethyl acetal (**8**, 7.91 g, 75.2 mmol) in dry methanol (300 mL), prepared in an oven-dried Schlenk flask under a nitrogen atmosphere, was added freshly distilled benzaldehyde (**33**, 7.60 mL, 75.2 mmol, 1.0 equiv), and the solution was stirred for 18 h at r.t. While cooling in an ice bath, $NaBH_4$ (4.30 g, 114 mmol, 1.5 equiv) was added portion-wise over 3 min. The ice bath was removed, and the suspension was stirred at r.t. for 18 h. The reaction was quenched by adding sat. $NaHCO_3$ solution (30 mL), and the mixture was extracted with DCM (3 × 60 mL). The combined organic phases were washed once with brine (150 mL), dried over Na_2SO_4, and filtered. After removing all volatiles in vacuo at 40 °C, **30** was obtained as a clear colorless liquid (14.19 g, 72.7 mmol, 97%) and used for the next step without further purification. M ($C_{11}H_{17}NO_2$) = 195.26 g/mol. T_b = 130−138 °C (13 mbar); lit., 147−149 °C (18 mbar).[23] R_f (SiO_2) = 0.35 (EtOAc + 1% NEt_3). IR (ATR): ν = 2934, 2830, 1454, 1192, 1127, 1056 cm^{-1}. ^{1}H NMR, COSY (300 MHz, $CDCl_3$): δ = 7.34−7.20 (m, 5H, Ar-H), 4.49 (t, ^3J = 5.5 Hz, 1H, H-2), 3.81 (s, 2H, $-CH_2Ar$), 3.37 (s, 6H, 2× OCH_3), 2.75 (d, ^3J = 5.5 Hz, 2H, H-1), 1.54 (s_B, 1H, −NH−) ppm. ^{13}C NMR, HMBC, HSQC (75 MHz, $CDCl_3$): δ = 140.3 (Ar-C-1), 128.5 (Ar-C-3 and Ar-C-5), 128.3 (Ar-C-2 and Ar-C-6), 127.1 (Ar-C-4), 104.1 (C-2), 54.1 (2× $-OCH_3$), 54.0 ($-CH_2Ar$), 50.7 (C-1) ppm. ESI-MS: m/z = 196.1 (100%, $[M + H]^+$). The spectroscopic data are consistent with literature values.[14]

(*R*)-2-(Benzyl(2,2-dimethoxyethyl)amino)-*N*-(4-hydroxybutan-2-yl)acetamide, 34. To a solution of **30** (6.11 g, 36.9 mmol, 1 equiv) in MeCN (40 mL) were added K_2CO_3 (10.2 g, 73.8 mmol, 2.0 equiv) and KI (6.13 g, 36.9 mmol, 1.0 equiv) while stirring, followed by a solution of **26** (7.21 g, 36.9 mmol, 1.0 equiv) in MeCN (40 mL) and additional MeCN (130 mL). The suspension was heated to reflux for 3 h (full conversion detected by LC−MS, 254 nm). The solvent was removed in vacuo at 40 °C, and the salt-like residue was suspended in EtOAc (150 mL) and water (210 mL). The mixture was transferred into a separatory funnel, and the organic phase was separated. The aqueous phase was extracted with EtOAc (2 × 100 mL), and the combined organic phases were dried over Na_2SO_4. After removing all volatiles in vacuo at 40 °C, **34** was obtained as a brown oil (11.63 g, 35.8 mmol, 97%). M ($C_{17}H_{28}N_2O_4$) = 324.42 g/mol. $[\alpha]_{589}^{19}$= −29.6 ($CHCl_3$, c = 10 mg/mL). R_f (SiO_2) = 0.32 (EtOAc + 2% NEt_3). IR (ATR): ν = 3324, 2933, 2833, 1649, 1529, 1453, 1120, 1063 cm^{-1}. ^{1}H NMR, COSY (300 MHz, $CDCl_3$): δ = 7.59 (m, 1H, −NH−), 7.38−7.22 (m, 5H, Ar-H), 4.39 (t, ^3J = 5.3 Hz, H-2″), 4.20−4.04 (m, 1H, H-2′), 3.92−3.80 (m, 1H, −OH), 3.72 (s, 2H, $PhCH_2-$), 3.59−3.46 (m, 1H, H_a-4′), 3.35 (s, 3H, $-OCH_3$), 3.33 (s, 3H, $-OCH_3$), 3.33−3.30 (m, 1H, H_b-4′), 3.20 (d, ^4J = 1.8 Hz, 2H, H-2), 2.71 (dd, ^3J = 5.3 Hz, ^4J = 1.1 Hz, 2H, H-1″), 1.89−1.76 (m, 1H, H_a-3′), 1.33−1.24 (m, 1H, H_b-3′), 1.22 (d, ^3J = 6.7 Hz, 3H, H-1′) ppm. ^{13}C NMR, HMBC, HSQC (75 MHz, $CDCl_3$): δ = 172.0 (C═O), 138.0 (Ar-C), 129.0 (Ar-C), 128.7 (Ar-C), 127.8 (Ar-C), 102.9 (C-2″), 60.7 ($PhCH_2-$), 58.6 (C-4′), 58.6 (C-2), 57.3 (C-1″), 54.1 ($-OCH_3$), 53.9 ($-OCH_3$), 41.4 (C-2′), 40.5 (C-3′), 21.2 (C-1′) ppm. ESI-HRMS: calcd for $[M + H]^+$, m/z = 325.2122; found, m/z = 325.2130.

(4*R*,9a*S*)-8-Benzyl-4-methylhexahydro-2*H*,6*H*-pyrazino[2,1-b][1,3]oxazin-6-one, 35. Acetal **34** (11.53 g, 35.5 mmol) was suspended in water (160 mL) and cooled in an ice bath. Conc. HCl (160 mL) was added through a dropping funnel over 15 min while stirring. The cooling bath was removed, and the slightly yellow solution was stirred at r.t. for 48 h (complete conversion detected by LC−MS at 254 nm). The solution was cooled again in an ice bath, and sodium hydroxide pellets (75 g) were added in small portions over 2 h followed by sodium bicarbonate powder (4 g) in order to adjust the pH to 7−8. While approaching the desired pH, **35** started precipitating, resulting in a murky beige suspension. EtOAc (300 mL) was added while stirring, and the mixture was transferred into a separating funnel. The organic phase was separated, and the aqueous phase was extracted with EtOAc (2 × 200 mL). The combined organic phases were dried over Na_2SO_4 and filtered, and the solvent was removed in vacuo at 40 °C. Crude **35** was obtained as a thick orange-brown oil (8.49, 32.6 mmol, 92%, ≥99.8% ee according to chiral HPLC).

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Racemic **35** was synthesized analogously from *rac*-**13**. *M* ($C_{15}H_{20}N_2O_2$) = 260.34 g/mol. $[\alpha]_{589}^{19}$ = −46.1 ($CHCl_3$, *c* = 10 mg/mL). R_f (SiO_2) = 0.32 (EtOAc). IR (ATR): ν = 2969, 2860, 1653, 1455, 1328, 1197, 1095, 1068 cm^{-1}. ^1H NMR, COSY (300 MHz, $CDCl_3$): δ = 7.36−7.23 (m, 5H, Ar-H), 4.97−4.86 (m, 2H, H-4 and H-9a), 3.93−3.81 (m, 2H, H-2), 3.57 (d, ^2J = 13.1 Hz, 1H, $-NCH_aAr$), 3.54 (d, ^2J = 13.1 Hz, 1H, $-NCH_bAr$), 3.24 (dd, ^2J = 16.0 Hz, ^4J = 1.9 Hz, 1H, H_a-7), 3.02 (dd, ^2J = 16.3 Hz, ^4J = 0.8 Hz, 1H, H_b-7), 2.97 (ddd, ^2J = 12.0 Hz, ^3J = 4.9 Hz, ^4J = 1.9 Hz, 1H, H_a-9), 2.44 (ddd, ^2J = 12.0 Hz, ^3J = 6.7 Hz, ^4J = 0.8 Hz, 1H, H_b-9), 2.19−2.04 (m, 1H, H_a-3), 1.43−1.34 (m, 1H, H_b-3), 1.27 (d, ^3J = 7.1 Hz, 4-CH_3) ppm. ^{13}C NMR, HSQC, HMBC (75 MHz, $CDCl_3$): δ = 166.3 (C-6), 136.3 (Ar-H), 129.4 (Ar-H), 128.6 (Ar-H), 127.7 (Ar-H), 79.3 (C-9a), 62.8 (C-2), 61.6 ($-NCH_2Ar$), 57.5 (C-7), 54.3 (C-9), 41.7 (C-4), 29.8 (C-3), 15.9 ($-CH_3$) ppm. ESI-HRMS: calcd for $[M + H]^+$, *m*/*z* = 261.1594; found, *m*/*z* = 261.1598.

(4*R*,9a*S*)-4-Methylhexahydro-2*H*,6*H*-pyrazino[2,1-b]-[1,3]oxazin-6-one, 16. A solution of oxazinone **35** (8.44 g, 324 mmol) in MeOH (120 mL) was degassed for 10 min by purging with nitrogen. Palladium (10% on carbon, 0.85 g) was added and the mixture was purged with hydrogen three times. The mixture stirred under hydrogen atmosphere for seven days (complete conversion detected by LC−MS and UV detection at 254 nm). After purging for 10 min with nitrogen, the mixture was suction-filtered over celite and washed several times with MeOH. All volatiles were removed in vacuo at 40 °C. Crude **16** was obtained as a viscous yellow-orange oil (5.31 g, 31.2 mmol, 96%), which solidified to a slight yellowish solid after a while. **16** was used for the next step without further purification. *M* ($C_8H_{14}N_2O_2$) = 170.21 g/mol. T_m = 56−61 °C; 79−83 °C (rac.). $[\alpha]_{589}^{19}$ = −103.1 ($CHCl_3$, *c* = 10 mg/mL). R_f (SiO_2) = 0.31 (EtOAc + 10% MeOH + 5% NEt_3), stained with ninhydrin reagent. IR (ATR): ν = 3307, 2968, 2865, 1643, 1451, 1324, 1193, 1080, 1064 cm^{-1}. ^1H NMR, COSY (300 MHz, $CDCl_3$): δ = 5.04−4.92 (m, 1H, H-4), 4.85−4.80 (m, 1H, H-9a), 4.00−3.85 (m, 1H, H-2), 3.48 (d, ^2J = 17.3 Hz, 1H, H_a-7), 3.38 (d, ^2J = 17.3 Hz, 1H, H_b-7), 3.14 (dd, ^2J = 13.6 Hz, ^3J = 3.8 Hz 1H, H_a-9), 2.97 (dd, ^2J = 13.6 Hz, ^3J = 4.3 Hz 1H, H_b-9), 2.16−2.01 (m, 1H, (H_a-3), 1.78 (s_B, 1H, −NH−), 1.41−1.32 (m, 1H, H_b-3), 1.26 (t, ^3J = 7.1 Hz, 3H, $-CH_3$) ppm. ^{13}C NMR, HSQC, HMBC (75 MHz, $CDCl_3$): δ = 167.8 (C-6), 78.8 (C-9a), 63.0 (C-2), 50.3 (C-7), 48.1 (C-9), 42.2 (C-4), 29.9 (C-3), 15.9 ($-CH_3$) ppm. ESI-MS: *m*/*z* = 171.1 (100%, $[M + H]^+$). The spectrometric data are consistent with literature values.[10]

Diethyl-(2*E*/*Z*)-2-(ethoxymethylidene)-3-oxobutan-dioate, 17. A diethyl oxalacetate sodium salt (**41**, 95%, 15.0 g, 67.8 mmol) was weighed into an Erlenmeyer flask and suspended in EtOAc (90 mL). The suspension was cooled in an ice bath, and 1 N HCl (86 mL) was added while stirring. After all of the salt was dissolved, the biphasic murky mixture was transferred into a separatory funnel. The organic phase was separated, and the aqueous phase was extracted with EtOAc (2 × 45 mL). The combined organic phases were dried over Na_2SO_4 and filtered, and the solvent was removed in vacuo at 30 °C. To the orange-brown oily residue (13.1 g) were added triethyl orthoformate (20.7 mL, 122 mmol, 1.8 equiv) and acetic anhydride (17.9 mL, 190 mmol, 2.8 equiv). The flask was equipped with a distillation apparatus, and the solution was heated for 1 h to 120 °C, for 1 h to 130 °C, and for 1 h to 140 °C, while a colorless clear liquid was distilled off. After cooling down, distillation under high vacuum afforded **17** as a yellow clear liquid (12.0 g, 49.2 mmol, 72%). *M* ($C_{11}H_{16}O_6$) = 244.24 g/mol. T_b = 116−120 °C (0.45 mbar); lit., 155−160 °C (1.3 mbar).[15] R_f (SiO_2) = 0.15 and 0.81 (EtOAc). IR (ATR): ν = 2987, 2937, 1359, 1256, 1177, 1019 cm^{-1}. ^1H NMR, COSY (300 MHz, $CDCl_3$): δ = 7.90 (s, 1H, =CH−), 7.88 (s, 1H, =CH′−), 4.40−4.32 (m, 2H, $=CHOCH_2-$), 4.40−4.32 (m, 2H, $=CHOCH'_2-$), 4.32−4.27 (m, 2H, O=C-4-OCH_2-), 4.32−4.27 (m, 2H, O=C-4′-OCH_2-), 4.27−4.18 (m, 2H, O=C-1-OCH_2-), 4.27−4.18 (m, 2H, O=C-1′-OCH_2-), 1.44 (t, ^3J = 7.2 Hz, 3H, $=CHOCH_2CH_3$), 1.43 (t, ^3J = 7.2 Hz, 3H=$CHOCH_2CH'_3$), 1.36 (t, ^3J = 7.2 Hz, 3H, O=C-4-OCH_2CH_3), 1.35 (t, ^3J = 7.2 Hz, 3H, O=C-4-$OCH_2CH'_3$), 1.28 (t, ^3J = 7.2 Hz, 3H, O=C-1-OCH_2CH_3), 1.27 (t, ^3J = 7.2 Hz, 3H, O=C-1-$OCH_2CH'_3$) ppm. ^{13}C NMR, HSQC, HMBC (75 MHz, $CDCl_3$): δ = 185.2 (C-3), 183.2 (C-3′), 170.1 (=CH−), 170.0 (=CH′−), 165.2 (C-1), 164.2 (C-4), 164.0 (C-4′), 163.5 (C-1′), 109.6 (C-2), 108.3 (C-2′), 74.7 ($=CH-O-CH_2-$), 74.6 ($=CH-O-C'H_2-$), 62.2 (O=C-1-OCH_2-), 62.0 (O=C-1′-OCH_2-), 61.1 (O=C-4-OCH_2-), 61.0 (O=C-4′-OCH_2-), 15.4 ($=CHOCH_2CH_3$), 15.4 ($=CHOCH_2C'H_3$), 14.3 (O=C-1-OCH_2CH_3), 14.2 (O=C-1′-OCH_2CH_3), 14.1 (O=C-4-OCH_2CH_3), 14.1 (O=C-4′-OCH_2CH_3) ppm. ESI-MS: *m*/*z* = 217.1 (100%, $[M\text{-}Et + H]^+$). Ethyl enol ether hydrolyzes during the LC−MS run to the free enol. The spectrometric data are consistent with literature values.[15]

Ethyl (4*R*,12a*S*)-7-Hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2*H*-pyrido[1′,2′:4,5]pyrazino-[2,1-b][1,3]oxazine-9-carboxylate, 18. Amine **16** (2.00 g, 11.8 mmol, 1.0 equiv) was added in a single portion to a solution of enol ether **17** (2.87 g, 11.8 mmol, 1.0 equiv) in dry DCM (80 mL), which was prepared in an oven-dried Schlenk flask under a nitrogen atmosphere. The yellow-greenish solution was stirred at r.t. for 24 h (full conversion of **17** detected by LC−MS and UV detection at 254/315 nm) before it was cooled in an ice bath. $MgBr_2{\cdot}OEt_2$ (3.79 g, 14.7 mmol, 1.25 equiv) was added all at once under a nitrogen reverse flow, and the suspension was stirred for 10 min before dry pyridine (2.8 mL, 35 mmol, 3.0 equiv) was dripped into the yellowish suspension within 2 min. A clear orange-red solution formed immediately, which was stirred at r.t. for three days (full conversion of **43** detected by LC−MS and UV detection at 254/315 nm). The suspension was cooled in an ice bath, and 1 N HCl (60 mL) was added while stirring. The mixture was transferred into a separatory funnel and vigorously shaken before the organic phase was separated. The aqueous phase was extracted with DCM (2 × 50 mL), combined organic phases were dried over Na_2SO_4 and filtered, and the solvent was removed in vacuo at 40 °C. Crude **18** was obtained as an orange fluffy solid (3.61 g, 11.2 mmol, 95%, HPLC purities of 94% (254 nm) and 87% (315 nm)) and used for the next step without further purification. To obtain a pure material, crude **18** was heated in EtOAc (0.1 g/2 mL) and cooled down to r.t. first then to −24 °C (freezer). The solid was filtered off, washed with ice-cold EtOAc, and dried in vacuo at 40 °C. *M* ($C_{15}H_{18}N_2O_6$) = 322.32 g/mol. T_m = 90−96 °C (sintering to an orange resin) and 104−106 °C (resin melts to a yellow-greenish liquid). $[\alpha]_{589}^{21}$= −35.6 ($CHCl_3$, *c* = 10 mg/mL). R_f (C_{18}-SiO_2) = 0.58 (EtOH:H_2O = 1:1 + 10% HOAc). IR (ATR): ν = 2979, 1725, 1632, 1452, 1282, 1262, 1181, 1090, 1048 cm^{-1}. ^1H NMR, COSY (300 MHz, $CDCl_3$): δ = 12.33 (s_B, 1H, −OH), 7.91 (s, 1H, H-10), 5.40−5.28 (m, 1H, H-

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12a), 5.00−4.87 (m, 1H, H-4), 4.40−4.19 (m, 3H, H_a-12 and $-OCH_2-$), 4.12−3.91 (m 3H, H-2 and H_b-12), 2.29−2.11 (m, 1H, H_a-3), 1.60−1.49 (m, 1H, H_b-3), 1.43 (t, ^3J = 7.1 Hz, 3H, 4-CH_3), 1.33 (t, ^3J = 7.0 Hz, 3H, $-OCH_2CH_3$) ppm. ^{13}C NMR, HMBC, HSQC (75 MHz, $CDCl_3$): δ = 169.8 (C-8), 164.3 (−COOEt), 162.5 (C-6), 156.5 (C-7), 141.5 (C-10), 115.3 (C-6a), 114.8 (C-9), 76.4 (C-12a), 62.8 (C-2), 61.1 ($-OCH_2$), 52.6 (C-12), 44.8 (C-4), 29.5 (C-3), 15.7 (4-CH_3), 14.4 ($-OCH_2CH_3$) ppm. ESI-HRMS: calcd for $[M + H]^+$, m/z = 323.1238; found, m/z = 323.1227.

(4*R*)-7-Hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2*H*-pyrido[1′,2′:4,5]pyrazino[2,1-b][1,3]-oxazine-9-carboxylic Acid, 19. For method 1, a solution of enol ether **17** (2.87 g, 11.8 mmol, 1.0 equiv) in dry DCM (80 mL) was prepared in an oven-dried Schlenk flask under a nitrogen atmosphere. Amine **16** (2.00 g, 11.8 mmol, 1.0 equiv) was added all at once, and the yellow-greenish solution was stirred at r.t. for 24 h (full conversion of **17** detected by LC−MS and UV absorption at 254/315 nm) and then cooled in an ice bath. $MgBr_2{\cdot}OEt_2$ (3.79 g, 14.7 mmol, 1.25 equiv) was added all at once under a nitrogen reverse flow, and the suspension was stirred for 10 min before dry pyridine (2.8 mL, 35 mmol, 3.0 equiv) was dripped into the yellowish suspension within 2 min. A clear orange-red solution formed immediately, which was stirred at r.t. for three days (full conversion of **43** detected by LC−MS and UV detection at 254 nm). The solution suspension was cooled in an ice bath, and 1 N HCl (60 mL) was added while stirring. The mixture was transferred into a separating funnel and vigorously shaken, and the organic phase was separated. The aqueous phase was extracted with DCM (2 × 50 mL), and to the combined organic phases was added 1 N NaOH (60 mL). The two-phase mixture was stirred vigorously at r.t. for 24 h (complete saponification detected by LC−MS and UV detection at 254 nm). The aqueous phase was separated, extracted once with DCM (50 mL), and cooled in an ice bath. The pH was adjusted to 1−2 by slow addition of conc. HCl (5 mL) where a colorless suspension formed. The solid was vacuum-filtered and washed several times with cold water (5 × 3 mL). The colorless solid was dried in air first then at 70 °C in fine vacuum to obtain **19** (2.49 g, 8.46 mmol, 72%). For method 2, a solution of enol ether **17** (0.72 g, 2.94 mmol, 1.0 equiv) in dry MeCN (25 mL) was prepared in an oven-dried Schlenk flask under a nitrogen atmosphere. Amine **16** (0.5 g, 2.94 mmol, 1.0 equiv) was added in a single portion, and the yellow-greenish solution was heated to reflux for 16 h (full conversion of **17** detected by LC−MS and UV absorption at 254/315 nm). The orange solution was cooled to r.t. before anhydrous $MgCl_2$ (0.35 g, 3.67 mmol, 1.25 equiv) was added. The suspension was stirred for 10 min at r.t. before dry pyridine (0.71 mL, 8.81 mmol, 3.0 equiv) was added. The mixture was heated to reflux under a nitrogen atmosphere for 24 h (95% conversion of **43** as judged by LC−MS and UV detection 254 nm). MeCN was removed in vacuo at 40 °C, and to the brownish salty residue was added DCM (15 mL). The suspension was cooled in an ice bath, and 1 N HCl (15 mL) was added while stirring vigorously. The mixture was transferred into a separating funnel and shaken vigorously. The organic phase was separated, and the aqueous phase was extracted with DCM (2 × 10 mL). 1 N NaOH (15 mL) was added to the combined organic phases, and the mixture was stirred vigorously at r.t. for 24 h (complete saponification detected by LC−MS and UV detection at 254 nm). The aqueous phase was separated and extracted once with DCM (10 mL). The aqueous phase was cooled in an ice bath, and conc. HCl was slowly added for acidification to pH = 1−2. A colorless solid precipitated, which was vacuum-filtered and washed with water (4 × 2 mL). The solid was dried in air first then at 70 °C under high vacuum to obtain **19** (0.46 g, 1.56 mmol, 53%). M ($C_{13}H_{14}N_2O_6$) = 294.26 g/mol. T_m = 248−252 °C (decomposition). $[\alpha]_{589}^{21}$= −120.9 (MeCN, c = 10 mg/mL). R_f (C_{18}-SiO_2) = 0.65 (EtOH:H_2O = 2:1 + 20% HOAc). IR (ATR): ν = 1735, 1646, 1618, 1546, 1461, 1440, 1346, 1285, 1095, 1081 cm^{-1}. ^{1}H NMR, COSY (400 MHz, DMSO-d_6): δ = 15.39 (s_B, 1H, −COOH), 12.77 (s_B, 1H, −OH), 8.67 (s, 1H, H-10), 5.52−5.47 (m, 1H, H-12a), 4.84−4.75 (m, 1H, H-4), 4.65 (dd, ^2J = 13.9 Hz, ^3J = 4.6 Hz, 1H, H_a-12), 4.43 (dd, ^2J = 13.9 Hz, ^3J = 5.9 Hz, 1H, H_b-12), 4.09−3.99 (m, 1H, H_a-2), 3.95−3.87 (m, 1H, H_b-2), 2.09−1.97 (m, 1H, H_a-3), 1.60−1.53 (m, 1H, H_b-3), 1.34 (t, ^3J = 7.0 Hz, 3H, $-CH_3$) ppm. ^{13}C NMR, HMBC, HSQC (101 MHz, DMSO-d_6): δ = 172.2 (C-8), 165.4 (−COOH), 161.8 (C-6), 153.6 (C-7), 141.1 (C-10), 118.7 (C-6a), 113.0 (C-9), 76.0 (C-12a), 62.0 (C-2), 51.5 (C-12), 44.9 (C-4), 29.1 (C-3), 15.2 ($-CH_3$) ppm. ESI-HRMS: calcd for $[M + H]^+$, m/z = 295.0925; found, m/z = 295.0929.

Sodium (4*R*,12a*S*)-9-((2,4-Difluorobenzyl)carbamoyl)-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2*H*-pyrido[1′,2′:4,5]pyrazino[2,1-b][1,3]oxazin-7-olate (Dolutegravir Sodium), 15. For method 1, an oven-dried Schlenk flask was charged with ester **18** (94%, 1.00 g, 2.92 mmol, 1.0 equiv), dry toluene (30 mL), acetic acid (0.42 mL, 7.29 mmol, 2.5 equiv), and 2,4-difluorobenzylamine (**10**, 0.87 mL, 7.29 mmol, 2.5 equiv) under a nitrogen atmosphere. The mixture was heated to reflux for 22 h (full conversion of **18** detected by LC−MS and UV detection at 254/315 nm). All volatiles were removed in vacuo at 40 °C, and the residue was dissolved in EtOH (30 mL) by heating to reflux. NaOH (0.13 g, 3.21 mmol, 1.1 equiv) was added, and the solution was heated again to reflux for 2 min during which a beige suspension formed. The mixture was stirred to r.t., filtered, and washed with EtOH (4 × 2 mL). The yellow filter cake was dried in air overnight and transferred into a new flask. EtOH (15 mL) was added, and the suspension was heated again to reflux, hot-filtered, and washed with hot ethanol (4 × 2 mL). The solid was dried in air overnight then for 2 h at 70 °C in fine vacuum to obtain **15** as a faint yellow solid (0.90 g, 2.04 mmol, 70%, purity of 99.7% (254 nm)). For method 2, an oven-dried flask was charged with acid **19** (1.00 g, 3.40 mmol, 1.0 equiv), carbonyl diimidazole (97%, 1.14 g, 6.80 mmol, 2.0 equiv), and dry dimethyl carbonate (30 mL) under a nitrogen atmosphere. The suspension was heated to 80 °C for 2 h during which a nearly clear orange solution formed. After cooling to r.t., 2,4-difluorobenzylamine (**10**, 0.81 mL, 6.80 mmol, 2.0 equiv) was added dropwise within 2 min, and the solution was stirred for 2 h at r.t. (full conversion of **19** detected by LC−MS and UV detection at 254/315 nm). The solvent was removed in vacuo at 40 °C, and the residue was redissolved in DCM (30 mL) and 1 N NaOH (30 mL). After stirring for 18 h at r.t., the colorless suspension was transferred into a separatory funnel. The organic phase was separated, and the aqueous phase was extracted twice with DCM (30 mL). The aqueous phase was cooled in an ice bath, acidified (pH = 1−2) with conc. HCl, and extracted with DCM (3 × 25 mL). The combined organic phases were dried over Na_2SO_4, and all volatiles were removed in vacuo at 40 °C. The colorless foamy residue was dissolved by heating in EtOH (30 mL). NaOH

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(0.15 g, 3.74 mmol, 1.1 equiv) was added to the hot solution, and heating to reflux was continued for 2 min. The suspension was stirred to r.t., filtered, and washed with EtOH (5 × 3 mL). The solid was dried in air overnight then for 2 h at 70 °C under high vacuum to obtain **15** as a faint yellow solid (1.41 g, 3.19 mmol, 94%, purity of 99.9% (254 nm)). M ($C_{20}H_{18}F_2N_3NaO_5$) = 441.37 g/mol. T_m = 314 °C (decomposition); lit., 296 °C.[24] $[\alpha]_{589}^{21}$= −46.4 (DMSO-d_6, c = 10 mg/mL). IR (ATR): ν = 2975, 2913, 1641, 1537, 1504, 1424, 1321, 1274, 1258, 1106, 1093 cm^{-1}. ^1H NMR, COSY (400 MHz, DMSO-d_6): δ = 10.69 (t, ^3J = 6.0 Hz, 1H, −NH−), 7.89 (s, 1H, H-10), 7.39−7.27 (m, 1H, Ar-H-6), 7.25−7.14 (m, 1H, Ar-H-3), 7.06−6.93 (m, 1H, Ar-H-5), 5.22−5.10 (m, 1H, H-12a), 4.87−4.72 (m, 1H, H-4), 4.50 (d, ^3J = 6.0 Hz, 2H, −NHCH_2Ar), 4.36−4.24 (m, 1H, H_a-11), 4.21−4.08 (m, 1H, H_b-11), 4.04−3.87 (m, 1H, H_a-2), 3.86−3.73 (m, 1H, H_b-2), 1.96−1.76 (m, 1H, H_a-3), 1.45−1.29 (m, 1H, H_b-3), 1.23 (t, ^3J = 7.0 Hz, 3H, −CH_3) ppm. ^{13}C NMR, HMBC, HSQC (101 MHz, DMSO-d_6): δ = 177.9 (C-8), 167.0 (C-7), 166.0 (−CONH−), 162.0 (dd, ^1J = 247 Hz, ^3J = 12.3 Hz, Ar-C2), 161.2 (C-6), 158.8 (dd, ^1J = 249 Hz, ^3J = 12.3 Hz, Ar-C4), 134.4 (C-10), 130.5 (dd, ^3J = 9.2 Hz, ^3J = 6.2 Hz, Ar-C6), 115.0 (C-9), 111.3 (dd, ^2J = 20.9 Hz, ^4J = 3.6 Hz, Ar-C5), 108.9 (C-6a), 103.7 (t, ^2J = 25.7 Hz, Ar-C3), 75.6 (C-12a), 62.0 (C-2), 53.1 (C-11), 43.1 (C-4), 35.4 (^3J = 3.7 Hz, −NHCH$_2$Ar), 29.2 (C-3), 15.3 (-CH_3) ppm. ^{19}F NMR (377 MHz, DMSO-d_6): δ = −112.9, −115.2 ppm. ESI-MS: m/z = 418.2 (100%, [M−Na]$^-$). The spectroscopic data are consistent with literature values.[11]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.oprd.1c00139.

Optimization studies, chromatograms, VCD spectroscopy, computational details, and NMR spectra (PDF)

XYZ coordinates of benzyloxazinone **35** (ZIP)

■ AUTHOR INFORMATION

Corresponding Author

Till Opatz − *Department of Chemistry, Johannes Gutenberg University, 55128 Mainz, Germany;* orcid.org/0000-0002-3266-4050; Email: opatz@uni-mainz.de

Authors

Jule-Philipp Dietz − *Department of Chemistry, Johannes Gutenberg University, 55128 Mainz, Germany*

Tobias Lucas − *Department of Chemistry, Johannes Gutenberg University, 55128 Mainz, Germany*

Jonathan Groß − *Department of Chemistry, Johannes Gutenberg University, 55128 Mainz, Germany*

Sebastian Seitel − *Department of Chemistry, Johannes Gutenberg University, 55128 Mainz, Germany*

Jan Brauer − *Department of Chemistry, Johannes Gutenberg University, 55128 Mainz, Germany*

Dorota Ferenc − *Department of Chemistry, Johannes Gutenberg University, 55128 Mainz, Germany*

B. Frank Gupton − *Department of Chemical and Life Sciences Engineering, Virginia Commonwealth University, Richmond, Virginia 23284, United States;* orcid.org/0000-0002-8165-1088

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.oprd.1c00139

Notes

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