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Original article

Synthesis and pharmacokinetic profile of highly deuterated brecanavir analogs

Emile J. Velthuisen*, Todd M. Baughman, Brian A. Johns, David P. Temelkoff, Jason G. Weatherhead

GlaxoSmithKline Research & Development, Infectious Diseases Therapeutic Area Unit, Five Moore Drive, Research Triangle Park, NC 27709, USA

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ABSTRACT

Several highly deuterated analogs of the HIV-1 protease inhibitor brecanavir have been prepared to study the effect of deuterium upon metabolic stability. The sites for deuterium incorporation were initially chosen to maximize the potential for a kinetic isotope effect; locations where C–H bond breaking is the rate limiting step. The analogs have been profiled in both *in vitro* and *in vivo* pharmacokinetic studies and the result will be described herein.

1. Introduction

HIV continues to be one of the leading causes of death worldwide. In 2009, there were an estimated 33.3 million people living with HIV/AIDS with 2.6 million new infections and 1.8 million deaths [1]. The overall rates of morbidity and mortality have been decreasing in part due to the advent of highly active antiretroviral therapy (HAART), but despite this progress the treatment of HIV remains problematic due to drug resistance, tolerance, and toxicity.

Protease represents one of three essential enzymes required for replication of HIV and is an important target for antiretroviral therapy. However, the current generation of protease inhibitors (PIs) is susceptible to drug resistance [2,3] and require co-administration with a pharmacokinetic booster to inhibit the metabolism of the parent drug by cytochrome P450-3A4 [4,5].

In 2006, GlaxoSmithKline disclosed the discovery of brecanavir (Fig. 1), a tyrosyl peptidomimetic that demonstrated low nanomolar activity against both wild-type and PI-resistant HIV [6,7]. In clinical studies the drug was safe and well tolerated but poor solubility and first pass metabolism were challenges uncovered during the studies. The metabolic profile was significantly improved by co-administration with the pharmacoenhancer ritonavir. However, due to potential side effects and drug–drug interactions it would be favorable to design brecanavir analogs that had a superior metabolic profile without the aid of a pharmacoenhancer.

One strategy to improve the ADME properties of brecanavir is to incorporate deuterium in place of hydrogen where C–H bond breaking is the rate limiting step during metabolism. Although the use of stable isotope labeled compounds has been important for the study of drug metabolism and organ toxicity [8,9], only recently has deuterium been incorporated into molecules with the specific aim of treating disease [10,11].

Deuterium is a naturally occurring, non-radioactive, and stable isotope of hydrogen. Whereas hydrogen contains a single proton and electron, deuterium also contains an additional neutron in its nucleus effectively doubling the atomic mass of the atom. Because deuterium is isotopic to hydrogen there is no difference in the pharmacological activity of the molecule. However, due to the increased mass, a significant kinetic isotope effect (KIE) is observed between these two isotopes ranging from one to seven [12].

Abbreviations: ADME, absorption, distribution, metabolism, excretion; AIBN, azobisisobutylnitrile; AIDS, acquired immunodeficiency syndrome; DIPEA, diisopropyl ethyl amine; DMP, Dess–Martin periodinane; DMPK, drug metabolism and pharmacokinetics; DNAUC, dose-normalized area under the curve; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; IPA, isopropyl alcohol; KIE, kinetic isotope effect; LDA, lithium diisopropylamide; NBS, *N*-bromosuccinimide; PI, protease inhibitor; PNP, *p*-nitrophenyl; TBDMSCl, *t*-butyldimethylsilyl chloride; TFA, trifluoroacetic acid; TMEDA, tetramethylethylenediamine.

* Corresponding author.
E-mail address: emile.j.velthuisen@gsk.com (E.J. Velthuisen).

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Although several reports suggest a considerable ADME advantage was gained by the incorporation of deuterium into a drug-like molecule it was unclear from the onset of this investigation if the KIE would be enough to have a noticeable impact upon the *in vivo* pharmacokinetics [11].

In designing brecanavir analogs it would be important to understand where metabolism occurs so that deuterium can be strategically incorporated to maximize the primary isotope effect. Characterization of the major metabolites of brecanavir in human and rat hepatocytes indicated that glucuronidation, *O*-dealkylation, and mono-oxidation were predominant (Fig. 1) [13]. Because the primary isotope effect would likely have little influence upon glucuronidation the sites for mono-oxidation were initially targeted for deuterium incorporation.

Due to the extensive amount of oxidation in brecanavir it seemed beneficial to initially incorporate the maximum amount of deuterium into an analog and then profile the *in vivo* pharmacokinetics to observe any potential benefit. Due to the modular nature of the synthesis, it would then be feasible to pinpoint any metabolic soft spots by selectively reverting deuterium back to hydrogen. To avoid ambiguous or partial deuterium incorporation, readily exchangeable hydrogen atoms would be avoided.

2. Results and discussion

2.1. Chemistry

Synthesis of the d_1-tyrosine core of brecanavir started with a Kowalski ester homologation [14] of tyrosine **2** [15] to afford chloromethyl ketone **3** (Scheme 1). A stereoselective sodium borodeuteride reduction, followed by ethanolic KOH mediated cyclization provided the desired oxirane **5** in high yield. The isobutylamine-d_9 coupling partner was then prepared by formation of the mixed anhydride of commercially available isobutyric acid-d_7 **6**, aminolysis with ammonia gas and subsequent reduction with lithium aluminum deuteride. Synthesis of deuterated 1,3-benzodioxole-5-sulfonyl chloride **11** was accomplished by alkylation of catechol **9** with chloroiodomethane-d_2 and subsequent chlorosulfonylation. With these three components synthesized it was now possible to construct the key amino alcohol-d_{12} core of brecanavir. Epoxide **5** was stereoselectively opened with isobutylamine-d_9 **8** and the resulting amine was treated with deuterated 1,3-benzodioxole **11** give sulfonamide **12**. Following removal of the *tert*-butyloxycarbonyl protecting group under acidic conditions our attention turned to the synthesis of the remaining thiazole and bisfuran fragments.

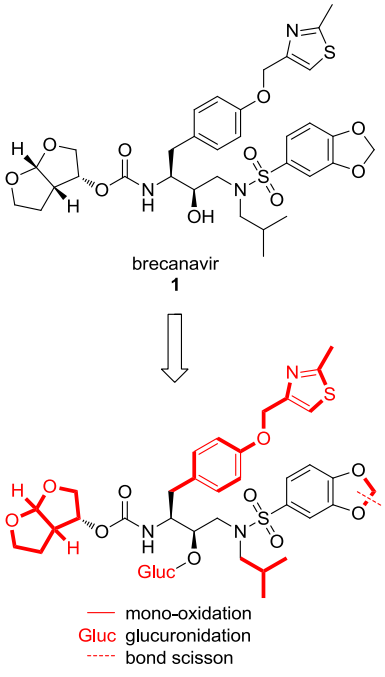


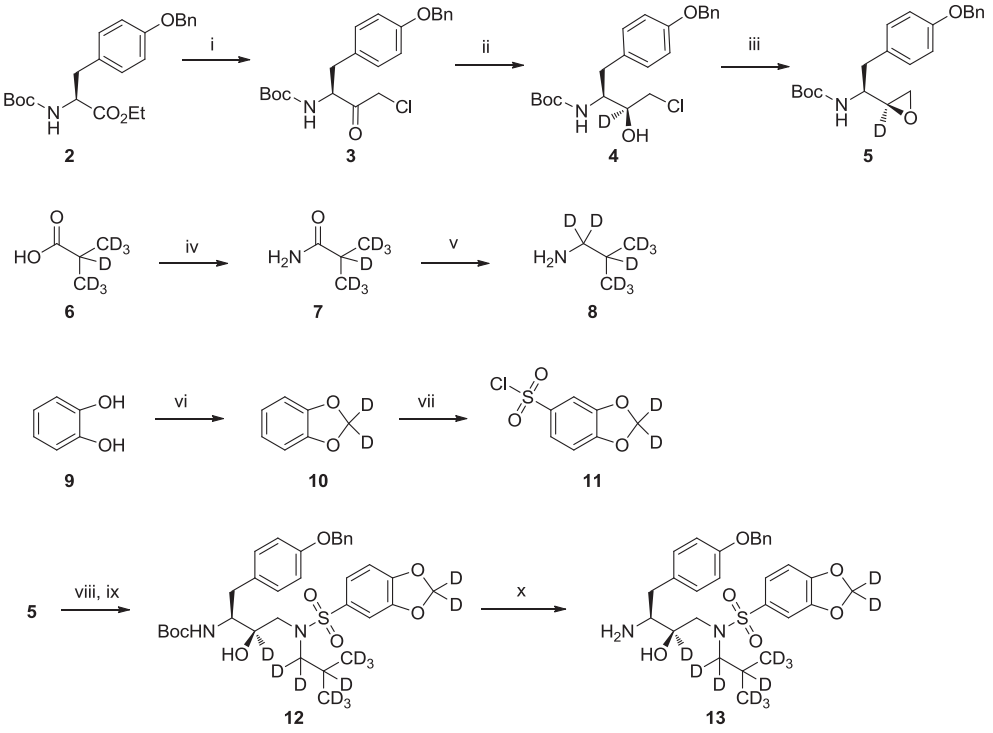
Fig. 1. Structure of the protease inhibitor brecanavir and the major sites of metabolism in human and rat hepatocytes.

Synthesis of the thiazole-d_2 fragment of brecanavir was accomplished by treatment of 2-methylthiazole **14** with lithium aluminum deuteride followed by chlorination with phosphorus oxychloride (Scheme 2). Unfortunately, the synthesis of a more highly deuterated thiazole fragment proved problematic. Upon treatment of known thioamide-d_3 [16,17] with ethyl bromopyruvate in ethanol, thiazole-d_3 **18** was isolated in 60% yield but with only 33% deuterium incorporation based upon integration of the ^{1}H NMR spectrum. To better understand the source of deuterium–hydrogen exchange the same reaction was performed using methanol-d_4 as solvent. In this second experiment, the percent deuterium incorporation was held constant following formation of the thiazole but deuterium–hydrogen exchange of the methyl group was observed once again following reduction of the ester with lithium aluminum deuteride. Unfortunately, the facile nature of deuterium–hydrogen exchange of 2-methyl thiazole precluded this site for the incorporation of deuterium.

Synthesis of the bisfuran-d_5 started with a lithium aluminum deuteride reduction of dimethyl-D-tartrate acetonide (Scheme 3) [17]. Deprotection of the acetonide with Amberlyst-15 afforded symmetrical tetraol **22** in which the primary alcohols were selectively protected as *tert*-butyl dimethyl silyl ethers. Subsequent cleavage of diol **23** with sodium periodate revealed acetaldehyde-d_2 **24**. A lithium aluminum deuteride reduction of 4-benzyloxybutanoate **25** followed by orthogonal silyl protection of the resulting alcohol provided **27**. Hydrogenolysis of the *O*-benzyl ether and oxidation of the resulting alcohol gave aldehyde **28**. The penultimate precursor to the bisfuran was assembled by an L-proline catalyzed mixed aldol of acetaldehyde-d_2 **24** and 4-siloxybutanal **29** where *syn* product **31** was isolated as the major diastereomer in a 7:1 ratio [18]. Following an acid catalyzed silyl deprotection/cyclization cascade, bisfuran **32** was isolated in 30% yield. To incorporate an additional deuterium into the bisfuran unit, the secondary alcohol was first oxidized to ketone **33** using Dess–Martin periodinane and subsequently reduced with lithium aluminum deuteride. Approach of the deuteride from the convex face of the molecule afforded a single alcohol isomer that was enzymatically resolved and then activated as the *p*-nitrophenyl carbonate **35**.

In addition to the bisfuran-d_5 moiety, a complementary bisfuran-d_2 unit was constructed that contained a deuterium at the bridgehead acetal carbon. Synthesis of this fragment commenced with a directed lithiation of dihydrofuran **36** and quenching with D_2O (Scheme 4). The dihyrdofuran was then brominated with NBS and the resulting oxocarbenium ion trapped with propargyl alcohol to provide furan **39**. A radical mediated 5-exo-dig cyclization of the alkynlfuran followed by ozonolysis of the resulting methylene afforded ketone **40**. The second deuterium was then introduced by lithium aluminum deuteride reduction of ketone **41** followed by acetylation of the resulting secondary alcohol. The racemic bisfuran-d_2 was then enzymatically resolved using Chiro-Clec PC to give chiral bisfuran **43** as a single isomer. Following removal of the acetyl group and activation of the chiral secondary alcohol as a p-nitrophenyl carbonate, the bisfuran-d_2 was ready for incorporation into the brecanavir-d_{12} core.

The bisfuran subunits were introduced by acylation of amino alcohol **13** with the respective PNP-carbonate derivative of alcohols **35** and **44**. To avoid any competitive alkylation of the secondary



Reagents and Conditions: (i) ICH_2Cl, LDA, THF, -78° C, (80%); (ii) $NaBD_4$, EtOH, (63%); (iii) KOH, EtOH, (99%); (iv) $EtCO_2Cl$, Et_3N, DCM; NH_3 (g), (99%); (v) $LiAlD_4$, (99%); (vi) chloroiodomethane-d_2, Cs_2CO_3, DMF, 110 °C, (96%); (vii) SO_3·DMF; $(COCl)_2$, DMF, (50%); (viii) IPA, **8**; (ix) **11**, DIPEA, (65% two steps); (x) TFA, DCM, (99%).

Scheme 1. Synthesis of the brecanavir-d_{12} core.

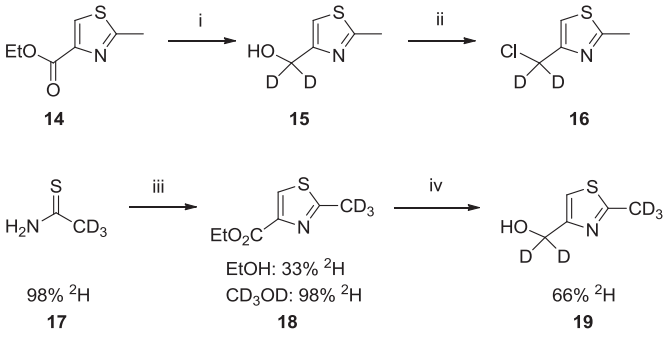
alcohol in the brecanavir core it was protected as an acetonide using dimethoxypropane and TsOH in DCM. Hydrogenolysis of the *O*-benzyl ether afforded the phenol that was subsequently alkylated with thiazole-d_2 **16**. Following deprotection of the acetonide under acidic conditions, brecanavir-d_{19} **52** and brecanavir-d_{16} **53** were isolated (Scheme 5).

2.2. In vivo DMPK

To better understand if there was any metabolic benefit to incorporating deuterium into brecanavir, compounds **52** and **53** were dosed intravenously in rats. We decided to forego the oral arm of the *in vivo* experiment because the metabolic advantage should manifest in a lower overall clearance for the analog if deuterium had any affect.

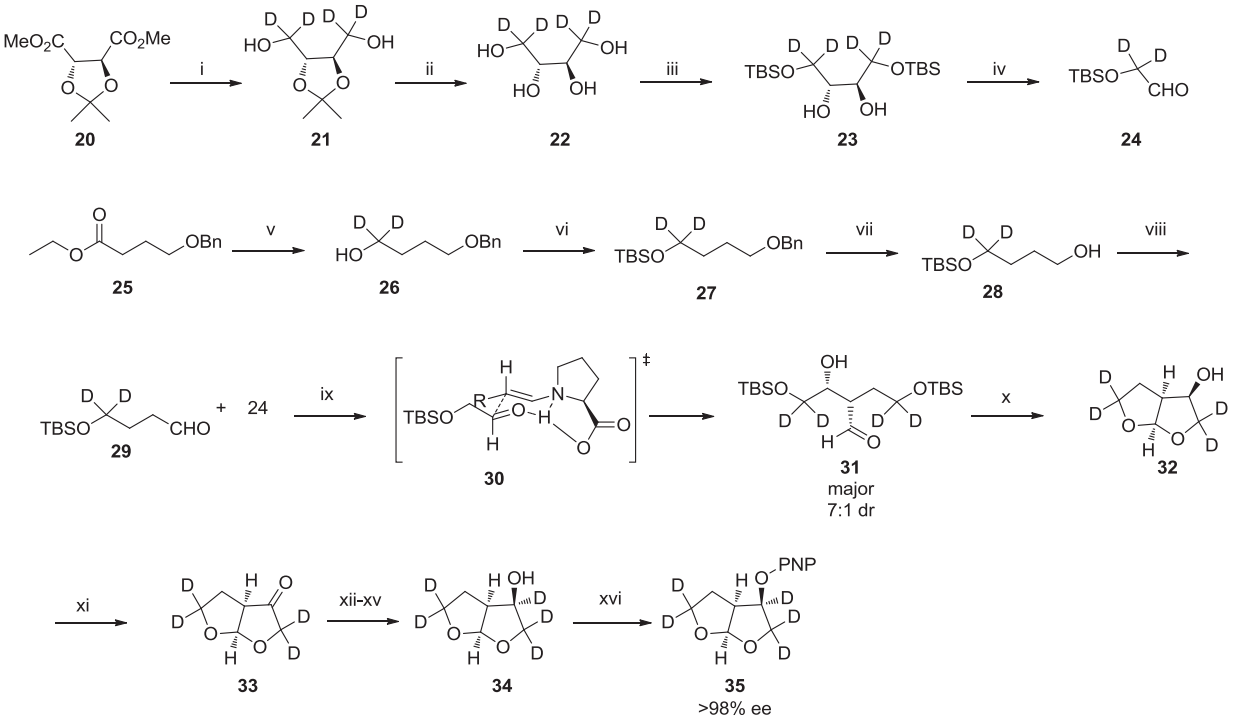
When dosed intravenously in rats, brecanavir was a high clearance compound with a short half-life and low exposure (Table 1). Upon co-dosing with the pharmacokinetic enhancer ritonavir (RTV), the *in vivo* profile was significantly improved. Clearance was reduce by 12-fold, and both the $t_{1/2}$ and exposure was increased by approximately 11-fold. Unfortunately both the brecanavir-d_{19} and -d_{16} analogs had nearly identical intravenous *in vivo* profiles as unboosted **1**.

To help reconcile any metabolic differences between brecanavir-d_{16} **53** and brecanavir **1** the major metabolites were characterized following incubation with human and rat hepatocytes (Fig. 2). Interestingly, the overall metabolic profile appears to have changed with the incorporation of deuterium but the net result of high *in vivo* clearance remains the same. In rats, carbamate bond cleavage of the brecanavir-d_{16} **53** carbonate is now apparent as is



Reagents and Conditions: (i) $LiAlD_4$, THF, (66%); (ii) $POCl_3$, THF, (97%); (iii) Ethyl bromopyruvate, solvent, (60%); (iv) $LiAlD_4$, THF (99%)

Scheme 2. Synthesis of the thiazole-d_2 fragment **16**.

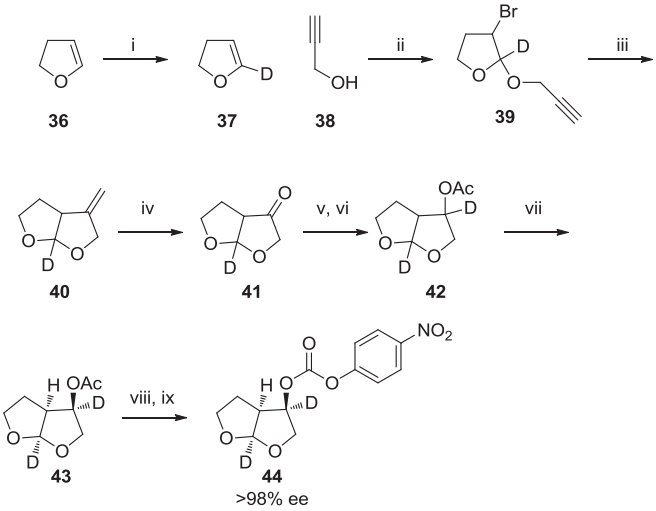


Reagents and conditions: (i) $LiAlD_4$, THF, (45%); (ii) Amberlyst-15, DCM, (84%); (iii) TBSCl, imidazole, DMF, (47%); (iv) $NaIO_4$, (50%); (v) $LiAlD_4$, THF (85%); (vi) TBSCl, imidazole, DMF (97%); (vii) H_2, Pd/C, EtOAc (91%); (viii) $(COCl)_2$, DMSO, Et_3N, DCM (99%); (ix) L-Proline, (cat.); (x) HCl, THF, (30% over 2-steps); (xi) Dess-Martin, $NaHCO_3$, DCM, (78%); (xii) $LiAlD_4$, THF; (xiii) Ac_2O, DMAP, Na_2CO_3, THF/H_2O; (xiv) ChiroCLEC PC; (xv) K_2CO_3, MeOH; (xvi) PNPCOCl, Pyridine, DCM (42% from **33**).

Scheme 3. Synthesis of the bisfuran-d_5 **35**.

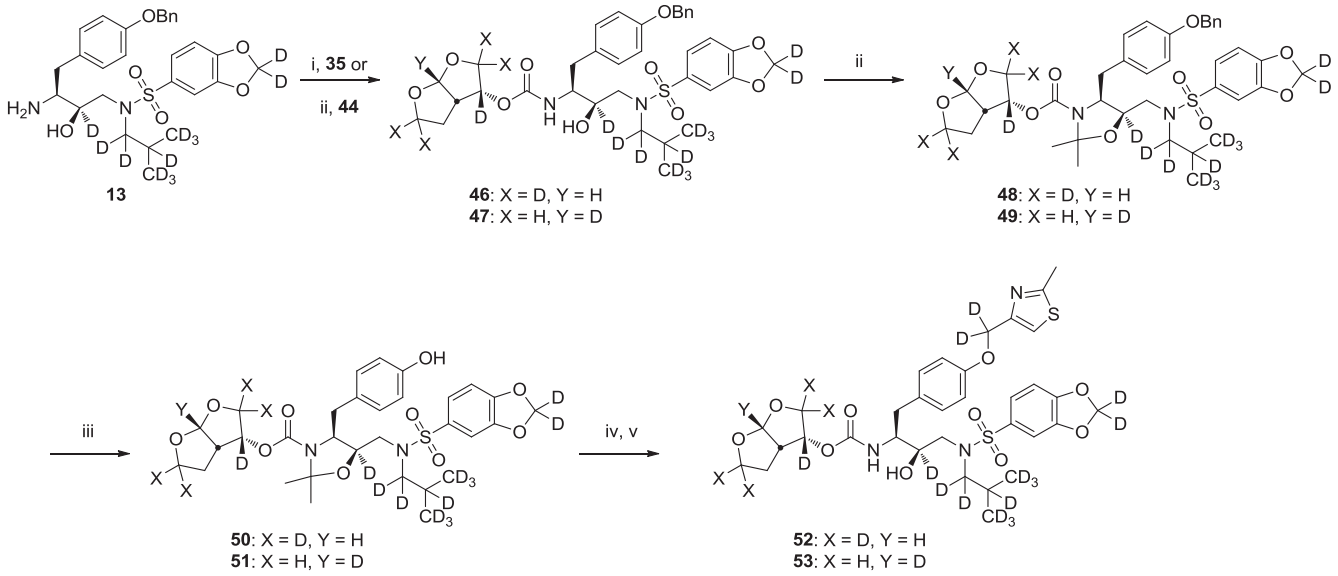
dehydrogenation of the core and glucuronidation of the *O*-dealkylated benzodioxane. In addition, mono-oxidation of the fully deuterated isobutyl amine-d_9 side chain was observed in both rats and humans as was replacement of a single deuterium with a hydroxyl group in the bisfuran unit. Surprisingly, these latter products suggest the KIE of deuterium was not sufficient to overcome the bond breaking step involved with oxidation. In comparison, humans only had a single unique metabolite not found in rats; bond scission of the benzodioxane. Other common metabolites include glucuronidation of the secondary alcohol and oxidation of the phenoxymethyl thiazole side chain.

Although further deuterated analogs could be made, no significant KIE was observed at the key metabolic sites identified in human and rat hepatocytes. It's possible that the *in vivo* rat



Reagents and conditions: (i) a) nBuLi/TMEDA, b) D_2O; (ii) NBS, DCM, 0 °C; (iii) Bu_3SnH, AIBN (26% three steps); (iv) O_3, DCM/MeOH (99%); (v) $LiAlD_4$; (vi) Ac_2O, Na_2CO_3, DMAP, (54% over 2 steps); (vii) ChiroCLEC PC, (39%); (viii) K_2CO_3, MeOH; (ix) PNPCOCl, Pyr, DCM, (73% two steps).

Scheme 4. Synthesis of bisfuran-d_2 **44**.



Reagents and conditions: (i) Hunig's Base, DCM, (**46** = 78%, **47** = 61%); (ii) dimethoxypropane, TsOH, DCM, (**48** = 81%, **49** = 68%); (iii) Pd/C, H_2, EtOAc; (iv) **16**, Cs_2CO_3, (v) HCl (**52** = 66% three steps; **53** = 35% three steps).

Scheme 5. Final assembly of brecanavir-d_{19} **52** and -d_{16} **53**.

pharmacokinetic profile is not reflective of humans. There were several key differences in the metabolite profiles between the two species and perhaps a higher species would more closely resemble that of humans. However, until a better model system exists, or we better understand the human metabolism of brecanavir, it becomes difficult to justify further work on deuterated brecanavir analogs despite the potential benefit this would offer patients.

3. Conclusion

In conclusion, several highly deuterated analogs of the protease inhibitor brecanavir have been synthesized and evaluated for improvements in metabolic stability. However, when dosed intravenously in rats they had a nearly identical *in vivo* profile as unboosted **1**. Interestingly, the metabolic profile appears to have shifted with the incorporation of deuterium but the net result of high clearance remains the same. Also worthy of note is that several locations that contained deuterium were metabolized suggesting the KIE was not sufficient to overcome oxidation under all circumstances.

4. Experimental section

4.1. Chemistry

All commercially available reagents were used without further purification. Column chromatography was carried out on silica gel (70–230 mesh). TLC was conducted on silica gel 250 micron, F254 plates. ^{1}H NMR spectra were recorded at 300 or 400 MHz. Melting points are uncorrected. Purities of test compounds were established by analytical HPLC (C-18 column, 5.0 micron, 0 → 100% CH_3CN (or MeOH)/water with 0.05–0.1% HCOOH (or TFA)) and UV detection with or without mass spectrometer detection. All test compounds showed >95% purity (AUCs by UV detection).

Table 1
Rat *in vivo* pharmacokinetic screening results [19].

Cmpd	Number of deuterium	Clp (mL/min/kg)	$t_{1/2}$ (h)	i.v. DNAUC (ng h/mL/mg)
1	0	65	0.2	66
1 + **RTV**	0	5.4	2.2	774
52	19	59	0.2	59
53	16	64	0.2	64

4.1.1. (S)-tert-butyl (1-(4-(benzyloxy)phenyl)-4-chloro-3-oxobutan-2-yl)carbamate (**3**)

LDA (169 mL, 163 mmol) was added dropwise at −78 °C through a cannula to a mixture of iodochloromethane (9.45 mL, 130 mmol) and ethyl *N*-{[(1,1-dimethylethyl)oxy]carbonyl}-*O*-(phenylmethyl)-L-tyrosinate (prepared as described in *J. Med. Chem.* **1990**, *33*, 1620) also at −78 °C. The resulting dark solution was stirred for one hour at −78 °C, then 50 mL of a 1:1 acetic acid:tetrahydrofuran solution was added slowly and the reaction was warmed to ambient temperature, poured onto a mixture of ice water and ethyl acetate, and the aqueous solution was extracted with ethyl acetate, the organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The brown solid was suspended in cold ether and filtered to give the title compound (3.17 g). The filtrate was concentrated under reduced pressure to give an additional 7.4 g of slightly impure title compound (10.57 g, 80% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 (s, 9H), 2.80–3.21 (m, 2H), 3.87–4.25 (m, 2H), 4.42–4.72 (m, 1H), 5.03 (s, 2H), 6.81–7.17 (m, 4H), 7.29–7.57 (m, 5H); LC/MS (*m*/*z*) $ES^+ = 427$ (M + 23).

4.1.2. tert-butyl ((2S,3S)-1-(4-(benzyloxy)phenyl)-4-chloro-3-hydroxybutan-2-yl)carbamate-d_1 (**4**)

Sodium borodeuteride (1.64 g, 39 mmol) was added in portions to a suspension of 1,1-dimethylethyl [(1*S*)-3-chloro-2-oxo-1-({4-[(phenylmethyl) oxy]phenyl}methyl)propyl]carbamate (3.16 g, 7.82 mmol) in 60 mL of a 4:1 ethanol:tetrahydrofuran solution at −78 °C. The mixture was warmed to 0 °C and stirred 1 h. The suspension was poured onto ice, placed in a 0 °C bath, acidified with 1 N HCl to a pH ~2 and filtered to collect a brown solid. The filtrate was extracted with ethyl acetate, dried over sodium sulfate and combined with the isolated solid. The brown solid was suspended in ethyl acetate (10 vol.), heated to reflux and stirred for 15 min. The hot solution was filtered and allowed to cool to ambient temperature and aged overnight. The mixture was filtered and the

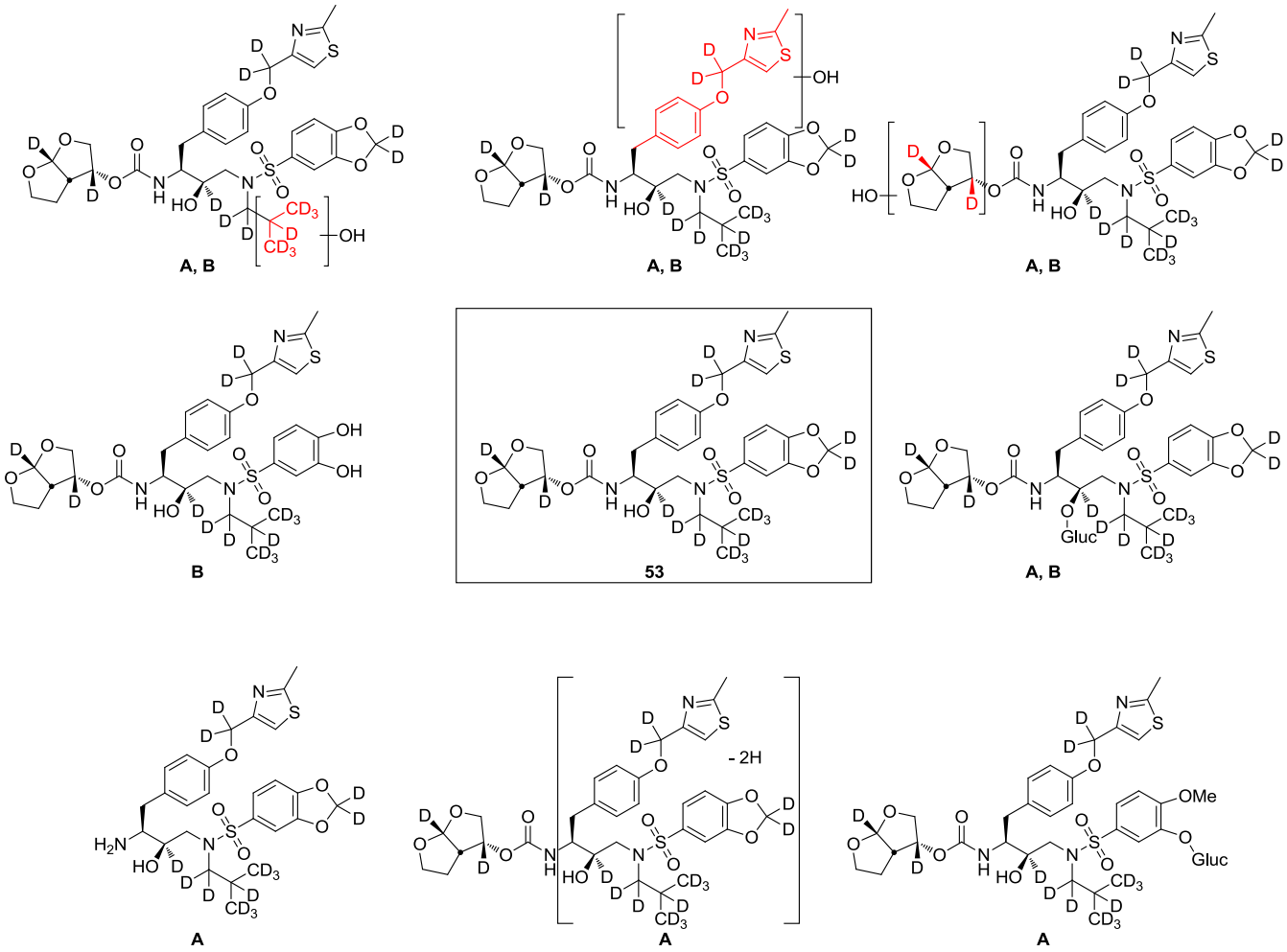


Fig. 2. Major metabolites of brecanavir-d_{16} **53** in rat and human hepatocytes. Bonds/atoms in red designate possible sites of oxidation. A = metabolite identified in rats, B = metabolite identified in humans. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

filter cake was washed with cold ethyl acetate. A second crop was isolated in a similar manner to give the title compound as a white solid in >10:1 d.r. (2 g, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 (s, 9H), 2.71–3.16 (m, 3H), 3.43–3.71 (m, 2H), 3.74–3.94 (m, 1H), 4.40–4.80 (m, 1H), 5.03 (s, 2H), 6.91 (d, J = 8.6 Hz, 2H), 7.12 (d, J = 8.5 Hz, 2H), 7.27–7.49 (m, 5H); LC/MS (m/z) ES$^+$ = 429 (M + 23).

*4.1.3. tert-butyl ((S)-2-(4-(benzyloxy)phenyl)-1-((S)-oxiran-2-yl) ethyl)carbamate-d_1 (**5**)*

Potassium hydroxide (0.82 mL, 1.5 eq, 1 N) was added to a suspension of 1,1-dimethylethyl [(1*S*,2*S*)-3-chloro-2-hydroxy-1-({4-[(phenylmethyl)oxy]phenyl}methyl)propyl]-d_1-carbamate (221 mg, 0.543 mmol) in ethanol (8 mL) and the suspension was stirred for 1 h at ambient temperature. The yellow solution was concentrated under reduced pressure, diluted with water, the aqueous solution was extracted with dichloromethane and the organics were dried over sodium sulfate, filtered and concentrated under reduced pressure to give the title compound as a light yellow solid (200 mg, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (s, 9H), 2.67–2.99 (m, 4H), 3.39–3.74 (m, 2H), 4.41–4.59 (m, 1H), 4.95–5.11 (m, 2H), 6.86–6.98 (m, 2H), 7.06–7.17 (m, 2H), 7.20–7.51 (m, 5H); LC/MS (m/z) ES$^+$ = 393 (M + 23).

*4.1.4. 2-methyl-1-propanamine-d_9 (**8**)*

An ice cold solution of 2-methylpropanoic acid-d_7 (1.0 mL, 9.96 mmol) and triethylamine (1.666 mL, 11.96 mmol) in DCM (50 mL) was treated with ethyl chloroformate (1.053 mL, 10.96 mmol). After stirring for one hour a white suspension had formed. Ammonia gas was bubbled through the mixture for 45 min to give a thick suspension. The mixture was poured into water and the layers were partitioned and separated. Solid NaCl was added and the mixture was extracted with $CHCl_3$/IPA (3:1). The combined extracts were dried over Na_2SO_4, filtered and concentrated to give the crude compound **7** as a white solid. The crude product was dissolved in THF (20 mL) and cooled to 0 °C. Lithium aluminum deuteride (14.95 mL, 14.95 mmol, 1 M in THF) was slowly added and the mixture was heated at 50 °C for 2 h. The mixture was cooled to ambient temperature and then to 0 °C. To the mixture was slowly added water (630 μL) and then stirred for 5 min. The mixture was then treated with 15% NaOH (630 μL) and stirred for 5 min. Lastly, water (1.9 mL) was added, the mixture was diluted with diethyl ether and then stirred at ambient temperature for 30 min. The suspension was filtered over Celite. The filtrate was treated with HCl (*g*) for 5 min and the mixture was concentrated. The crude residue was azeotroped with toluene and MeOH to give 2-methyl-1-propanamine-d_9 as a white solid (1.19 g, quantitative yield). ^{2}H NMR (61 MHz, $CDCl_3$) δ ppm 0.69–1.15 (m, 6D), 1.88–2.19 (m, 1D), 2.66–3.00 (m, 2D); Rf = 0.15 (80:19:1 DCM/MeOH/NH_4OH).

*4.1.5. 1,3-benzodioxole-d_2 (**10**)*

A suspension of catechol (5.0 g, 45.4 mmol) and Cs_2CO_3 (22.19 g, 68.1 mmol) in *N*,*N*-dimethylformamide (DMF) (108 mL)

was treated with chloroiodomethane-d_2 (4.96 mL, 68.1 mmol). The resulting suspension was heated to 110 °C. After 1 h, the reaction mixture was cooled to ambient temperature and filtered through a pad of Celite and rinsed with diethyl ether. The filtrate was diluted with water and extracted with diethyl ether. The combined organic layers were washed with water, brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was then diluted with Et_2O and the solids were filtered off. The filtrate was concentrated to afford 5.39 g (96%) as a brown oil: ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 6.84$ (s, 5H).

4.1.6. 1,3-benzodioxole-5-sulfonyl chloride-d_2 (**11**)

A suspension of SO_3–DMF complex (7.51 g, 49.1 mmol) in 1,2-dichloroethane (DCE) (43.4 mL) was treated dropwise with a solution of 1,3-benzodioxole-d_2 (5.39 g, 43.4 mmol) in 1,2-dichloroethane (43.4 mL) and heated to 75 °C. After 18 h, the reaction mixture was cooled to room temperature and treated dropwise with oxalyl chloride (4.15 mL, 49.1 mmol) and heated to 65 °C. After 2 h, the reaction mixture was quenched by the slow addition of water (5 mL). The layers were partitioned and the combined organics were washed with water, brine, dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was purified by ISCO (0–30% EtOAc–hexanes) to afford 1,3-benzodioxole-5-sulfonyl chloride-d_2 (4.86 g, 21.83 mmol, 50% yield) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 7.63$ (dd, 1H), 7.42 (d, $J = 2.0$ Hz, 1H), 6.96 (d, $J = 8.4$ Hz, 1H); ES − MS: 203 ((M − Cl) + OH).

4.1.7. tert-butyl ((2S,3R)-1-(4-(benzyloxy)phenyl)-3-hydroxy-4-(N-isobutylbenzo[d][1,3]dioxole-5-sulfonamido)butan-2-yl)carbamate-d_{12} (**12**)

2-methyl-1-propanamine-d_9, (15.55 mL, 6.84 mmol, 0.44 M in THF) was added to 1,1-dimethylethyl ((1*S*)-1-[(2*S*)-2-oxiranyl]-2-{4-[(phenylmethyl)oxy]phenyl}ethyl)carbamate-d_1 (507 mg, 1.369 mmol) and heated to 50 °C for 48 h and then concentrated. The light brown solid was dissolved in dichloromethane (20 mL) and triethylamine (0.382 mL, 2.74 mmol) was added. The mixture was cooled to 0 °C and 1,3-benzodioxole-5-sulfonyl chloride-d_2 (366 mg, 1.642 mmol) was added. The mixture was stirred at ambient temperature for 50 min. Water was added and the mixture was extracted with DCM. The extracts were washed with saturated $NaHCO_3$, dried over Na_2SO_4, filtered and concentrated. The residue was purified by ISCO (0–50% EtOAc/hex) to give the title compound as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.37 (s, 9H), 2.83–2.97 (m, 2H), 3.01–3.10 (m, 2H), 3.67–3.75 (m, 1H), 3.87 (br. s., 1H), 4.61–4.67 (m, 1H), 5.05 (s, 2H), 6.83–6.97 (m, 3H), 7.11–7.21 (m, 3H), 7.30–7.48 (m, 6H); ES + MS: 629 (M + 1).

4.1.8. N-((2R,3S)-3-amino-4-(4-(benzyloxy)phenyl)-2-hydroxybutyl)-N-isobutylbenzo[d][1,3]dioxole-5-sulfonamide-d_{12} (**13**)

A solution of *tert*-butyl ((2*S*,3*R*)-1-(4-(benzyloxy)phenyl)-3-hydroxy-4-(N-isobutylbenzo[d][1,3]dioxole-5-sulfonamido)butan-2-yl)carbamate-d_{12} (0.08 g, 0.13 mmol) was dissolved in DCM (2 mL) and treated with TFA (0.5 mL). After 30 min, the reaction mixture was concentrated *in vacuo* to afford the title compound (0.07 g, 100%) that was used immediately without further purification. ES + MS: 539.32.

4.1.9. (2-methyl-1,3-thiazol-4-yl)methanol-d_2 (**15**)

To a −78 °C solution of lithium aluminum deuteride (33.3 mL, 33.3 mmol) in THF (39.7 mL) was slowly added ethyl 2-methyl-1,3-thiazole-4-carboxylate (10 g, 58.4 mmol) in THF (43.7 mL). Following the addition, the mixture was allowed to warm to ambient temperature over a 2 h period. The reaction mixture was then quenched by the slow addition of 1.2 mL H_2O followed by 10 min of stirring and then 1.2 mL 15% NaOH, followed by 10 min stirring and finally 3.6 mL of H_2O. The resulting suspension was filtered through a pad of Celite and concentrated to afford (2-methyl-1,3-thiazol-4-yl) methanol-d_2 (5.05 g, 38.5 mmol, 66% yield) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 7.03$ (s, 1H), 2.72 (s, 3H).

4.1.10. 4-(chloromethyl)-2-methyl-1,3-thiazole hydrochloride-d_2 (**16**)

A solution of (2-methyl-1,3-thiazol-4-yl)methanol-d_2 (6.0 g, 45.7 mmol) in dichloromethane (229 mL) was treated with thionyl chloride (9.98 mL, 137 mmol). After 30 min, the reaction mixture was concentrated *in vacuo* to afford 4-(chloromethyl)-2-methyl-1,3-thiazole hydrochloride-d_2 (8.26 g, 44.4 mmol, 97% yield) as a brown solid: ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 7.57$ (s, 1H), 2.65 (s, 3H).

4.1.11. (4R,5R)-dimethyl 2,2-dimethyl-1,3-dioxolane-4,5-dicarboxylate (**20**)

(2*R*,3*R*)-dimethyl 2,3-dihydroxysuccinate (8.0 g, 44.9 mmol) and *p*-toluene sulfonic acid (100 mg, 0.526 mmol) were dissolved in 1,1-dimethoxypropane (35 mL, 285 mmol) and the mixture was heated at 85 °C for 5 h. Additional *p*-toluene sulfonic acid (134 mg) was added and the reaction was heated at 95 °C overnight. The reaction was allowed to cool to room temperature, diluted with a solution of sodium bicarbonate, and the aqueous layer was extracted with diethyl ether. The organic layer was dried over sodium sulfate and concentrated under reduced pressure to a red oil. High vacuum distillation gave (4*R*,5*R*)-dimethyl 2,2-dimethyl-1,3-dioxolane-4,5-dicarboxylate (8.34 g, 85%) as a clear yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 4.79 (s, 2H), 3.81 (s, 6H), 1.47 (s, 6H).

4.1.12. ((4S,5S)-2,2-dimethyl-1,3-dioxolane-4,5-diyl)dimethanol-d_4 (**21**)

(4*R*,5*R*)-dimethyl 2,2-dimethyl-1,3-dioxolane-4,5-dicarboxylate (4.35 g, 19.94 mmol) was dissolved in diethyl ether and cooled to 0 °C. Lithium aluminum deuteride (20.33 mL, 20.33 mmol, 1 M in diethyl ether) was added dropwise to form a thick white suspension. The reaction was heated to reflux for 2 h and then cooled in an ice water bath. Water (0.7 mL), 1 N NaOH (0.7 mL) and water (2.3 mL) were successively added and the mixture was stirred 15 min, filtered through Celite and the filtrate was carefully concentrated to give (4*S*,5*S*)-2,2-dimethyl-1,3-dioxolane-4,5-diyl) dimethanol-d_4 (1.47 g, 45%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 3.98 (s, 2H), 1.98 (s, 2H, 1.41 (s, 6H).

4.1.13. (2S,3S)-butane-1,2,3,4-tetraol-d_4 (**22**)

((4*S*,5*S*)-2,2-dimethyl-1,3-dioxolane-4,5-diyl)dimethanol (2.96 g, 17.81 mmol) was dissolved in a 10% methanol/water solution (37 mL) and Amberlyst-15 (59 mg) was added and the reaction was heated at 60 °C overnight. The mixture was cooled to room temperature, filtered and the filtrate was concentrated to give (2*S*,3*S*)-butane-1,2,3,4-tetraol-d_4 (1.89 g, 84%) as a colorless residue. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 4.63 (s, 2H).

4.1.14. (6S,7S)-2,2,3,3,10,10,11,11-octamethyl-4,9-dioxa-3,10-disiladodecane-6,7-diol-d_4 (**23**)

(2*S*,3*S*)-butane-1,2,3,4-tetraol-d_4 (148 mg, 1.17 mmol) and imidazole (176 mg, 2.58 mmol) were dissolved in DMF (2 mL) and the mixture was cooled to 0 °C. TBDMSCl (354 mg, 2.35 mmol) was added dropwise as a solution in DMF (5 mL). When the addition was complete, the ice bath was removed and the reaction was stirred at room temperature overnight. The mixture was diluted with water, the aqueous layer was extracted with diethyl ether, and the organic layer was washed with water. The organic layer was dried over sodium sulfate and concentrated to give (6*S*,7*S*)-2,2,3,3,10,10,11,11-octamethyl-4,9-dioxa-3,10-disiladodecane-6,7-

diol-d_4 (195 mg, 47%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 3.65 (m, 2H), 2.82 (m, 2H), 0.87 (s, 18H), 0.05 (s, 12H).

*4.1.15. 2-((tert-Butyldimethylsilyl)oxy)acetaldehyde-d_2 (**24**)*

Sodium periodate (7.08 g, 33.1 mmol) was added to a mixture of (6*S*,7*S*)-2,2,3,3,10,10,11,11-octamethyl-4,9-dioxa-3,10-disiladodecane-6,7-diol-d_4 (5.87 g, 16.55 mmol) in DCM (41 mL), THF (16 mL) and water (41 mL). After stirring at room temperature overnight, additional sodium periodate (7.2 g) was added until the reaction was complete by TLC (9:1 hexanes:ethyl acetate). The mixture was diluted with pentane, extracted aqueous layer with a 9:1 pentane:DCM solution, and washed organic layer with water and brine. The combined organic layer was dried over sodium sulfate and carefully concentrated under reduced pressure to afford 2-((*tert*-Butyldimethylsilyl)oxy)acetaldehyde-d_2 (4.21 g, 50%) as a colorless oil after distillation. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.69 (s, 1H), 0.92 (s, 9H), 0.09 (s, 6H).

*4.1.16. 4-[(phenylmethyl)oxy]-1-butanol-d_2 (**26**)*

Ethyl 4-[(phenylmethyl)oxy]butanoate (8.23 g, 45.7 mmol) was dissolved in THF, cooled to 0 °C and a solution of lithium aluminum deuteride (1 M diethyl ether, 22.84 mL) was added dropwise. After stirring 20 min, water (0.84 mL), 1 N sodium hydroxide (0.84 mL), and water (2.5 mL) were successively added and the mixture was vigorously stirred for 10-min and filtered through a pad of Celite. The filtrate was concentrated under reduced pressure to give 4-[(phenylmethyl)oxy]-1-butanol-d_2 (6 g, 85%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.67 (m, 6 H), 2.11 (s, 1H), 3.50 (m, 2H), 4.50 (s, 2H), 7.00–7.50 (s, 5H).

*4.1.17. (4-(benzyloxy)butoxy)(tert-butyl)dimethylsilane-d_2 (**27**)*

TBDMSCl (5.39 g, 35.7 mmol) was added to a mixture of 4-[(phenylmethyl)oxy]-1-butanol-d_2 (5 g, 27.5 mmol), imidazole (2.43 g, 35.7 mmol), and *N*,*N*-dimethylformamide (20 mL) and the mixture was stirred 2 h at room temperature. Water was added, the aqueous layer was extracted with diethyl ether, and the combined organics were washed with brine, dried over sodium sulfate and concentrated under reduced pressure to give (4-(benzyloxy) butoxy)(*tert*-butyl)dimethylsilane-d_2 (7.94 g, 97%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.03 (s, 6H), 0.88 (s, 9H), 1.50–1.80 (m, 4H), 3.40–3.54 (m, 2H), 4.49 (s, 2H), 7.00–7.50 (m, 5H).

*4.1.18. 4-{[(1,1-dimethylethyl)(dimethyl)silyl]oxy}-1-butanol-d_2 (**28**)*

A mixture of (1,1-dimethylethyl)(dimethyl)({4-[(phenylmethyl) oxy]butyl}oxy)silane-d_2 (7.94 g, 26.8 mmol), Pd/C (812 mg) and methanol (10 mL) was stirred under an atmosphere of H_2 (40 psi) for 12 h. Acetic acid (4 mL) was added and the mixture was stirred under an atmosphere of H_2 (50 psi) for 12 h. The suspension was filtered through Celite and the filtrate was concentrated under reduced pressure to give 4-{[(1,1-dimethylethyl)(dimethyl)silyl]oxy}-1-butanol-d_2 (5.03 g, 91%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.05 (s, 6H), 0.88 (s, 9H), 1.51–1.73 (m, 4H), 3.63 (s, 2H).

*4.1.19. 4-{[(1,1-dimethylethyl)(dimethyl)silyl]oxy}butanal-d_2 (**29**)*

A −78 °C solution of oxalyl chloride (0.964 mL, 11.01 mmol) in dichloromethane (30 mL) was treated slowly with DMSO (1.56 mL, 22 mmol) and stirred for 10 min. A solution of 4-{[(1,1-dimethylethyl)(dimethyl)silyl]oxy}-1-butanol-d_2 (1.5 g, 7.34 mmol) in dichloromethane (20 mL) was added slowly and the mix was stirred 15 min at −78 °C. Triethylamine (3.07 mL, 22 mmol) was added slowly and the mix was stirred 10 min at −78 °C and allowed to warm to room temperature and stirred 30 min. Saturated sodium bicarbonate solution and brine were added, the aqueous layer was extracted with dichloromethane, the organics were washed with brine, dried over sodium sulfate and concentrated under reduced pressure to give 4-{[(1,1-dimethylethyl)(dimethyl)silyl]oxy}butanal-d_2 (1.49 g, 100%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.01 (s, 6H), 0.85 (s, 9H), 1.76–1.87 (m, 2H), 2.41–2.53 (m, 2H), 9.68–9.85 (m, 1H).

*4.1.20. (3R,3S)-hexahydrofuro[2,3-b]furan-3-ol-d_4 (**32**)*

A solution of 4-{[(1,1-dimethylethyl)(dimethyl)silyl]oxy}butanal-d_2 (1.21 g, 5.92 mmol) in tetrahydrofuran (5 mL) was cooled to 0 °C and treated with and L-proline (0.136 g, 1.182 mmol) and 2-((*tert*-Butyldimethylsilyl)oxy)acetaldehyde-d_2 (4.2 g, 23 mmol). The reaction was stirred at room temperature for 12 h. Hydrochloric acid (1.4 mL, 1 N) was added, and the mixture was stirred for 48 h. Pyridine (0.23 mL), toluene (6.4 vol.), and water (3.4 mL) were added and the mixture was stirred 10 min and then filtered through a pad of Celite. The organic layer was extracted with water, the aqueous layer was washed with toluene, and the aqueous layer was concentrated under reduce pressure to give a yellow oil. Purification by silica gel chromatography (20–100% ethyl acetate/hexanes) gave (3*R*,3*S*)-hexahydrofuro[2,3-b]furan-3-ol-d_4 (234 mg, 30% for two steps) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.71–1.80 (m, 1H), 1.81–1.91 (m, 1H), 2.28 (m, 1H), 2.83 (m, 1H), 4.43 (m, 1H), 5.68 (m, 1H).

*4.1.21. (3R)-tetrahydrofuro[2,3-b]furan-3(2H)-one-d_4 (**33**)*

DMP (506 mg, 1.19 mmol) was added to a suspension of (3*R*,3a*S*)-hexahydrofuro[2,3-b]furan-3-ol-d_4 (100 mg, 0.745 mmol) and sodium bicarbonate (315 mg, 3.75 mmol) in DCM (3 mL) at 0 °C. The mixture was stirred at room temperature for 2 h. The reaction was diluted with a solution of sodium thiosulfate and a sodium bicarbonate solution was added. The aqueous layer was extracted with DCM. The combined organics were washed with sodium thiosulfate solution and sodium bicarbonate solution and then dried over sodium sulfate and concentrated to a white solid. Purification by silica gel chromatography afforded (3*R*)-tetrahydrofuro [2,3-b]furan-3(2H)-one-d_4 (77 mg, 78%) as a white solid.

*4.1.22. (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl acetate (**34**)*

(3*R*)-tetrahydrofuro[2,3-b]furan-3(2H)-one-d_4 (58 mg, 0.439 mmol) was dissolved in DCM (5 mL) and cooled to 0 °C. Lithium aluminum deuteride (0.198 mmol, 0.2 mL) was added dropwise and the reaction was stirred 10 min. Water (0.01 mL), 1 N NaOH (0.01 mL) and water (0.03 mL) were successively added and the mixture was filtered through Celite and the filtrate was concentrated under reduced pressure. The residue was dissolved in THF (5 mL) and cooled to 0 °C. Sodium carbonate (116 mg, 1.1 mmol), and DMAP (0.5 mg) were added followed by dropwise addition of acetic anhydride (0.06 mL, 0.658 mmol). The reaction was stirred at room temperature for 2 h, filtered, and the filtrate was diluted with 1 N HCl. The aqueous layer was extracted with DCM and the combined organics were dried over sodium sulfate and concentrated to a give (3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-b]furan-3-yl acetate (69 mg, 89%) as a colorless residue. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.74 (m, 1H), 3.05 (m, 1H), 2.11 (s, 3H), 1.99 (m, 1H), 1.88 (m, 1H).

4.1.23. (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl acetate

(155 mg, 0.875 mmol) was dissolved in a sodium hypophosphate solution (pH ~ 4) (1 mL) and 1 N NaOH (0.5 mL) was added to adjust the pH ~ 5. Added two pipet drops of Altus Biologics Chiro-Clec PC, (0.05 g/mL dry weight suspension) and the mixture was heated to 40 °C overnight. The pH was adjusted to ~5 with 1 N NaOH and the mixture was heated at 40 °C for another 7 h at 40 °C. The reaction was cooled to room temperature, filtered and the filtrate was extracted with DCM. The organic layer was washed with water several times and the combined

organic layer was dried over sodium sulfate and concentrated to give 111 mg of a colorless residue. This material was dissolved in MeOH (5 mL) and potassium carbonate (84 mg, 0.608 mmol) was added and the mixture was stirred at room temperature overnight. The reaction was diluted with ethyl acetate and filtered. The filtrate was concentrated to give (3*R*,3a*S*)-hexahydrofuro[2,3-b]furan-3-ol-d_5 (85 mg) which was used directly in the next step without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.61 (m, 1H), 2.77 (m, 1H), 2.24 (m, 1H), 1.77 (m, 1H).

4.1.24. (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl (4-nitrophenyl) carbonate-d_5 (**35**)

(3*R*,3a*S*)-hexahydrofuro[2,3-b]furan-3-ol-d_5 (216 mg, 1.6 mmol) was suspended in DCM (5 mL) and cooled to 0 °C. Pyridine (0.39 mL, 4.79 mmol) and 4-nitrophenylchloroformate (338 mg, 1.68 mmol) were added and the mixture was stirred 2 h letting ice bath expire. The mixture was diluted with water and the aqueous layer was extracted with DCM. The combined organics were washed with a sodium bicarbonate solution, 1 N HCl, and sodium bicarbonate solution successively and then dried over sodium sulfate. Purification by silica gel chromatography afforded (3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-b]furan-3-yl (4-nitrophenyl) carbonate (46% over 2 steps from (3*R*,3a*S*,6a*R*)-Hexahydrofuro[2,3-b]furan-3-yl acetate). Chiral SFC (15% MeOH on an OJH column, at 140 bar, with a temperature of 40 °C and a flow rate of 2 mL/min) of a with racemic sample show >99% purity (see Supporting information for chiral HPLC trace). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.90–2.26 (m, 2H), 3.07–3.25 (m, 1H), 5.68–5.95 (m, 1H), 7.39 (d, J = 9.2 Hz, 2H), 8.30 (d, J = 9.2 Hz, 2H).

4.1.25. 3-methylidenehexahydrofuro[2,3-b]furan-d_1 (**40**)

An ice cold solution of 2,3-dihydrofuran (5.0 mL, 66.1 mmol) in TMEDA (2.0 mL, 13.25 mmol) was treated by dropwise addition of *n*-butyllithium (30.9 mL, 77 mmol, 2.5 M solution in hexanes). The mixture was stirred for 2 h, then cooled to −78 °C and treated dropwise with deuterium oxide (3 mL, 166 mmol). The cooling bath was allowed to expire and the mixture was stirred overnight. The pale yellow solution was filtered over Celite to remove the precipitates, dried over sodium sulfate, filtered and the solution was used crude in the next step. A mixture of *n*-bromosuccinimide (12.95 g, 72.7 mmol) in DCM (40 mL) was cooled to 0 °C and treated with the crude solution of 2,3-dihydrofuran-d_1 and propargyl alcohol (5.76 mL, 98.9 mmol). The ice bath was allowed to expire and the mixture was stirred for 2 h at ambient temperature. The mixture was diluted with DCM and then washed with saturated sodium thiosulfate and saturated sodium bicarbonate. The extracts were dried over sodium sulfate, filtered and concentrated to give a pale yellow oil that was used without further purification. A mixture of tributyltin hydride (21.91 mL, 83 mmol) and AIBN (0.109 g, 0.661 mmol) in toluene (10 mL) was heated to 105 °C and a solution of the crude 3-bromo-2-(2-propyn-1-yloxy)tetrahydrofuran-d_1 in toluene (10 mL) was slowly added. The mixture was heated at 105 °C for 4 h and then stirred at room temperature overnight. The mixture was concentrated and then azeotroped with acetonitrile. The resultant yellow oil was purified on silica gel (0–30% ethyl acetate/hexanes gradient) to give the desired product as a pale yellow oil (2.17 g, 26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.94 (m, J = 12.3, 5.3, 1.6, 1.6 Hz, 1H), 2.19 (tt, J = 11.8, 8.2 Hz, 1H), 3.30 (d, J = 8.4 Hz, 1H), 3.81 (ddd, J = 11.1, 8.6, 5.3 Hz, 1H), 3.96 (td, J = 8.2, 1.6 Hz, 1H), 4.35–4.46 (m, 1H), 4.46–4.57 (m, 1H), 5.06 (dq, J = 12.4, 2.2 Hz, 2H).

4.1.26. tetrahydrofuro[2,3-b]furan-3(2H)-one-d_1 (**41**)

A solution of 3-methylidenehexahydrofuro[2,3-b]furan-d_1 (3.13 g, 24.61 mmol) in DCM (15 mL) and methanol (15 mL) at −78 °C was treated with a steady stream of ozone until starting material was consumed as monitored by TLC. O_2 gas was bubbled through the reaction mixture to evacuate the remaining ozone. Dimethyl sulfide (10.92 mL, 148 mmol) was cooled to 0 °C and slowly transferred to the reaction flask which was held at −78 °C. The mixture was warmed to ambient temperature and then concentrated to give tetrahydrofuro[2,3-b]furan-3(2H)-one-d1 as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.15–2.34 (m, 2H), 2.92–3.08 (m, 1H), 3.69–3.85 (m, 1H), 3.97–4.11 (m, 1H), 4.15 (s, 2H).

4.1.27. hexahydrofuro[2,3-b]furan-3-yl acetate-d_2 (**42**)

To a −78 °C solution of tetrahydrofuro[2,3-b]furan-3(2H)-one-d_1 (3178 mg, 24.61 mmol) in THF (15 mL) was added lithium aluminum deuteride (18 mL, 18.0 mmol, 1 M in THF). The mixture was stirred for 20 min at −78 °C and then warmed to 0 °C for 30 min. At 0 °C, water (760 μL) was added and the mixture was stirred for 5 min. The mixture was then treated with 15% NaOH (760 μL) and stirred for 5 min. Lastly, water (2.3 mL) was added, the mixture was diluted with ether and stirred at ambient temperature for 30 min. The mixture was filtered over Celite and the filtrate was concentrated to a pale yellow oil. The crude alcohol intermediate was dissolved in THF (25 mL) and cooled to 0 °C. Sodium carbonate (7825 mg, 73.8 mmol) and DMAP (50.0 mg, 0.409 mmol) were added, followed by dropwise addition of acetic anhydride (3.48 mL, 36.9 mmol). The mixture was stirred at ambient temperature for 2 h and then filtered. DCM was added and the organic phase was washed with 1 N HCl, then dried over Na_2SO_4, filtered and concentrated. The residue was purified on silica gel (0–50% EtOAc/hexanes gradient) to give hexahydrofuro[2,3-b]furan-3-yl acetate-d_2 as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.82–1.98 (m, 1H), 1.98–2.06 (m, 1H), 2.08–2.14 (m, 3H), 3.06 (d, J = 9.4 Hz, 1H), 3.77 (d, J = 9.6 Hz, 1H), 3.87–3.96 (m, 1H), 3.96–4.04 (m, 1H), 4.08 (d, J = 9.8 Hz, 1H).

4.1.28. (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl acetate-d_2 (**43**)

Hexahydrofuro[2,3-b]furan-3-yl acetate-d_2 (2.30 g, 13.2 mmol) was dissolved in 0.1 M NaH_2PO_4 (7 mL) and the mixture was adjusted to pH ~5 by adding 1.0 N NaOH. Altus Biologics Chiro-Clec PC, 0.05 g/mL dry weight suspension (13.20 mmol) (2 drops) was added and the mixture was heated to 40 °C for 36 h with periodic adjustments to pH 5 by addition of 1 N NaOH. The mixture was cooled and filtered. The filtrate was extracted with DCM and the extracts were washed with water until TLC (1:1 EtOAc/hexanes) indicated no alcohol present in the organic layer. The organic phase was dried over Na_2SO_4, filtered and concentrated. The residue was purified on silica gel (0–50% EtOAc/hexanes gradient) to give (3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-b]furan-3-yl acetate-d_2 as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.80–1.97 (m, 1H), 1.97–2.06 (m, 1H), 2.11 (s, 3H), 3.06 (d, J = 9.4 Hz, 1H), 3.76 (d, J = 9.6 Hz, 1H), 3.91 (ddd, J = 10.2, 8.6, 6.1 Hz, 1H), 3.96–4.03 (m, 1H), 4.08 (d, J = 9.6 Hz, 1H).

4.1.29. (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl (4-nitrophenyl) carbonate-d_2 (**44**)

A solution of (3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-b]furan-3-yl acetate-d_2 (904 mg, 5.19 mmol) in MeOH (15 mL) was treated with potassium carbonate (861 mg, 6.23 mmol) and stirred until the reaction was judged complete by TLC. The mixture was concentrated and the residue was triturated with EtOAc and DCM, filtered and concentrated. ^{1}H NMR indicated reversion to the starting material. The residue was dissolved in MeOH (15 mL), treated with potassium carbonate (10 mg) and then stirred overnight at ambient temperature. The mixture was concentrated to give a pale yellow oil which was used crude in next step. ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 1.89 (m, J = 12.9, 9.9, 9.9, 8.5 Hz, 1H), 2.22–2.39 (m, 1H), 2.86 (d, J = 9.8 Hz, 1H), 3.65 (d, J = 9.2 Hz, 1H), 3.91 (td,

J = 9.3, 6.4 Hz, 1H), 4.00 (m, J = 8.6, 5.6, 2.7, 2.7 Hz, 2H). To an ice cold solution of the crude alcohol in DCM (15 mL) was added pyridine (0.839 mL, 10.38 mmol) followed by 4-nitrophenyl chloroformate (2092 mg, 10.38 mmol). The mixture was stirred at ambient temperature until complete by TLC (several hours). The mixture was diluted with DCM and partitioned with water. The organic phase was washed with 1 N HCl, then saturated $NaHCO_3$, dried over Na_2SO_4, filtered and concentrated. The residue was purified twice on silica gel (0–50% EtOAc/hexanes gradient) to give (3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-b]furan-3-yl (4-nitrophenyl) carbonate-d2 as a white solid (1.13 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.01 (m, J = 13.2, 9.9, 9.9, 8.2 Hz, 1H), 2.13–2.25 (m, 1H), 3.15 (d, J = 9.6 Hz, 1H), 3.85–4.11 (m, 3H), 4.17 (d, J = 10.0 Hz, 1H), 7.35–7.45 (m, 2H), 8.26–8.35 (m, 2H).

*4.1.30. (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl ((2S,3R)-1-(4-(benzyloxy)phenyl)-3-hydroxy-4-(N-isobutylbenzo[d][1,3]dioxole-5-sulfonamido)butan-2-yl)carbamate-d_{17} (**46**)*

Compound **45** (63 mg, 117 mmol) was dissolved in MeCN (5 mL), DIPEA (0.06 mL, 0.34 mmol) was added followed by compound **35** (35 mg, 0.117 mmol) and the mixture was heated at 76 °C overnight. The reaction was concentrated, diluted with sodium bicarbonate solution, and the aqueous layer was extracted with ethyl acetate. The organic layer was dried over sodium sulfate, concentrated and purified by silica gel chromatography to afford the title compound (64 mg, 78%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29–7.47 (m, 6H), 7.05–7.20 (m, 3H), 6.81–6.99 (m, 3H), 5.59–5.74 (m, 1H), 5.03 (m, 2H), 4.89 (m, 1H), 3.82 (m, 1H), 3. 56 (m, 1H), 2.66–3.25 (m, 5H); ES + MS: 700.34.

*4.1.31. (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl [(1S,2R)-3-[(1,3-benzodioxol-5-ylsulfonyl)(2-methylpropyl)amino]-2-hydroxy-1-({4-[(phenylmethyl)oxy]phenyl}methyl)propyl]carbamate-d_{14} (**47**)*

A solution of 1,1-dimethylethyl [(1*S*,2*R*)-3-[(1,3-benzodioxol-5-ylsulfonyl)(2-methylpropyl)amino]-2-hydroxy-1-({4-[(phenylmethyl) oxy]phenyl}methyl)propyl]carbamate-d_{12} (114 mg, 0.178 mmol) in DCM (2 mL) was treated with TFA (0.4 mL). The pink solution was stirred for one hour and then concentrated. The residue was partitioned between EtOAc and saturated $NaHCO_3$. The aqueous phase was extracted with EtOAc. The combined extracts were dried over Na_2SO_4, filtered and concentrated. The crude amine was dissolved in MeCN (2 mL), treated with DIPEA (0.125 mL, 0.714 mmol) and (3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-b]furan-3-yl 4-nitrophenyl carbonate-d_2 (85 mg, 0.286 mmol) and the mixture was heated to 45 °C for 2 h. The mixture was cooled to ambient temperature and then concentrated. The residue was dissolved in EtOAc, washed with saturated $NaHCO_3$ (×3), dried over Na_2SO_4, filtered and concentrated. The residue was purified on silica gel (0–50% EtOAc/hexanes gradient) to give (3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-b]furan-3-yl [(1*S*,2*R*)-3-[(1,3-benzodioxol-5-ylsulfonyl)(2-methylpropyl)amino]-2-hydroxy-1-({4-[(phenylmethyl) oxy]phenyl}methyl)propyl]carbamate-d_{14} as a white foam (76 mg, 61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.42–1.58 (m, 1H), 1.60–1.75 (m, 1H), 2.77 (dd, J = 14.1, 9.4 Hz, 1H), 2.85–3.07 (m, 3H), 3.08–3.21 (m, 1H), 3.58 (s, 1H), 3.66–3.78 (m, 2H), 3.79–3.92 (m, 2H), 3.98 (d, J = 9.8 Hz, 1H), 4.92 (d, J = 9.2 Hz, 1H), 5.03 (s, 2H), 6.90 (dd, J = 8.5, 1.7 Hz, 3H), 7.09–7.22 (m, 3H), 7.29–7.37 (m, 2H), 7.37–7.46 (m, 4H); ES + MS: 697 (M + 1).

*4.1.32. (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl (4S,5R)-5-{[(1,3-benzodioxol-5-ylsulfonyl)(2-methylpropyl)amino]methyl}-2,2-dimethyl-4-({4-[(phenylmethyl)oxy]phenyl}methyl)-1,3-oxazolidine-3-carboxylate-d_{14} (**49**)*

A solution of (3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-b]furan-3-yl [(1*S*,2*R*)-3-[(1,3-benzodioxol-5-ylsulfonyl)(2-methylpropyl)amino]-2-hydroxy-1-({4-[(phenylmethyl)oxy]phenyl}methyl)propyl]carbamate-d_{14} (32 mg, 0.046 mmol) and dimethoxypropane (0.080 mL, 0.65 mmol) in DCM (1 mL) was treated with p-toluenesulfonic acid monohydrate (31 mg, 0.16 mmol) and then heated to 40 °C for 90 min. The mixture was cooled to ambient temperature, saturated $NaHCO_3$ was added and the mixture was extracted with DCM. The extracts were dried over Na_2SO_4, filtered and concentrated and then purified on silica gel (0–50% EtOAc/hexanes gradient) to give (6a*R*)-hexahydrofuro[2,3-b]furan-3-yl (4*S*,5*R*)-5-{[(1,3-benzodioxol-5-ylsulfonyl)(2-methylpropyl)amino]methyl}-2,2-dimethyl-4-({4-[(phenylmethyl)oxy]phenyl}methyl)-1,3-oxazolidine-3-carboxylate-d_{14} as a pale yellow residue (23 mg, 68%). ^{1}H NMR (400 MHz,$CDCl_3$) δ = 7.48–7.37 (m, 4H), 7.37–7.32 (m, 1H), 7.21–7.00 (m, 4H), 6.92 (d, J = 8.6 Hz, 2H), 6.88–6.75 (m, 1H), 5.06 (s, 2H), 4.42–4.06 (m, 1H), 4.03–3.93 (m, 1H), 3.93–3.71 (m, 2H), 3.51–3.23 (m, 2H), 3.09–2.64 (m, 4H), 1.99–1.84 (m, 2H), 1.61–1.54 (m, 3H), 1.53–1.40 (m, 3H); ES + MS: 737 (M + 1).

*4.1.33. (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl (4S,5R)-5-{[(1,3-benzodioxol-5-ylsulfonyl)(2-methylpropyl)amino]methyl}-4-[(4-hydroxyphenyl)methyl]-2,2-dimethyl-1,3-oxazolidine-3-carboxylate-d_{14} (**51**)*

A solution of (3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-b]furan-3-yl (4*S*,5*R*)-5-{[(1,3-benzodioxol-5-ylsulfonyl)(2-methylpropyl)amino] methyl}-2,2-dimethyl-4-({4-[(phenylmethyl)oxy]phenyl}methyl)-1,3-oxazolidine-3-carboxylate-d_{14} (23 mg, 0.031 mmol) in EtOAc (5 mL) was treated with Pd/C, Degussa type (33 mg, 0.031 mmol) and then stirred under a balloon of hydrogen gas for 2 h. The mixture was filtered over Celite and the filtrate was concentrated to give (3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-b]furan-3-yl (4*S*,5*R*)-5-{[(1,3-benzodioxol-5-ylsulfonyl)(2-methylpropyl)amino]methyl}-4-[(4-hydroxyphenyl)methyl]-2,2-dimethyl-1,3-oxazolidine-3-carboxylate-d_{14} as an off-white solid (19 mg, 92%) which was used crude without further purification.

*4.1.34. (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl {(1S,2R)-3-[(1,3-benzodioxol-5-ylsulfonyl)(2-methylpropyl)amino]-2-hydroxy-1-[(4-{[(2-methyl-1,3-thiazol-4-yl)methyl]oxy}phenyl)methyl] propyl}carbamate-d_{19} (**52**)*

The title compound was prepared using the procedures described for compound **53** to give the desired product from compound **13** (54 mg, 0.073 mmol) and thiazole **19** (11 mg, 0.073 mmol) in 66% yield over three steps after purification by reverse phase HPLC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25–1.75 (m, 3H), 2.60–3.23 (m, 7H), 3.50–4.00 (m, 1H), 4.89–5.03 (m, 1H), 5.64 (m, 1H), 6.81–6.95 (m, 3H), 7.07–7.18 (m, 3H), 7.22 (s, 1H), 7.28–7.36 (m, 1H); HRMS cacld for 723.3504 (M + H); found 723.3506.

*4.1.35. (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl {(1S,2R)-3-[(1,3-benzodioxol-5-ylsulfonyl)(2-methylpropyl)amino]-2-hydroxy-1-[(4-{[(2-methyl-1,3-thiazol-4-yl)methyl]oxy}phenyl)methyl] propyl}carbamate-d_{16} (**53**)*

A suspension of (3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-b]furan-3-yl (4*S*,5*R*)-5-{[(1,3-benzodioxol-5-ylsulfonyl)(2-methylpropyl)amino] methyl}-4-[(4-hydroxyphenyl)methyl]-2,2-dimethyl-1,3-oxazolidine-3-carboxylate-d_{14} (18.5 mg, 0.029 mmol) and cesium carbonate (37.3 mg, 0.114 mmol) in DMF (1.5 mL) was treated with 4-(chloromethyl)-2-methyl-1,3-thiazole-d_2 (14.37 mg, 0.077 mmol) and then heated to 70 °C for 4 h. The mixture was cooled to ambient temperature, water was added and the mixture was diluted with ethyl acetate. The phases were separated and the organic phase was washed with water, dried over Na_2SO_4, filtered and concentrated. The residue was dissolved in 1,4-Dioxane (0.5 mL) and then treated with 4 M HCl/dioxane (1 mL, 4.00 mmol) and water (0.2 mL). The mixture was stirred for 4 h and then concentrated. The residue was

purified by RP-HPLC to give (3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-b] furan-3-yl {(1*S*,2*R*)-3-[(1,3-benzodioxol-5-ylsulfonyl)(2-methylpropyl) amino]-2-hydroxy-1-[(4-{[(2-methyl-1,3-thiazol-4-yl)methyl]oxy} phenyl)methyl]propyl}carbamate-d_{16} as a white solid (7.9 mg, 38%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.55–1.75 (m, 2 H), 2.76–2.82 (m, 4H), 2.92–3.03 (m, 3H), 3.12–3.15 (m, 1H), 3.70–3.77 (m, 2H), 3.81–3.91 (m, 2H), 3.99 (d, J = 9.6 Hz, 1H), 4.94 (d, J = 9.2 Hz, 1H), 6.90–6.93 (m, 3H), 7.14–7.18 (m, 3H), 7.23 (s, 1H), 7.33–7.36 (m, 1H); ES + MS: 720 (M + 1).

Conflicts of interest

None.

Appendix A. Supporting information

Supporting information related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.02.001.

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[19] IV doses of 0.25 mg/kg were delivered as a DMSO/solutol/mannitol (2.5/10/87.5) solution formulation. Studies were performed in fed rats. RTV was co-administered at 4 mg/kg.