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Research Article

Isotope labeled 'HEA Moiety' in the synthesis of labeled HIV-protease inhibitors – Part 1

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Summary

[(S)-1′-((*N*-*tert*-Butyloxycarbonyl)amino)-2S-[$^2H_5$]phenyl-ethyl]oxirane ***11***, made from [$^2H_5$]-bromobenzene, was transformed into the HIV-protease inhibitors [$^2H_5$]-DPH 153893 and [$^2H_5$]-DPH 140662. Both compounds are members of the hydroxyethylamine class of protease inhibitors (HIV-PIs). The method of synthesis is applicable to members of this class and the HEE group of HIV-PIs. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: protease inhibitor; anti-HIV; AIDS; [$^2H_5$]-HEA isostere

Introduction

DPH 153893 and DPH 140662, like amprenavir[1] and saquinavir,[2] are members of the hydroxyethylamine (HEA) isostere class of HIV protease inhibitors (HIV-PIs).[3] The two compounds are highly potent HIV-aspartyl protease inhibitors with an average IC90 of 4–8 nM against wild-type viruses. Both were selected for pre-clinical evaluation as potential new generation anti-HIV drugs.[4]

Aspects of the studies involved LC-MS analysis and the stable isotope-labeled forms of DPH 153893 and DPH 140662 were needed as internal standards. We wanted to make the penta-deuterated DPH 153893 and DPH 140662 molecules in which the [$^2H_5$] is situated at the core as the [phenyl ring-$^2H_5$]-HEA. We preferred to make the penta-deuterated analog based on the calculation that the molecular weight is sufficiently increased, such that overlap with other ions due to isotopic mass distribution is avoided.

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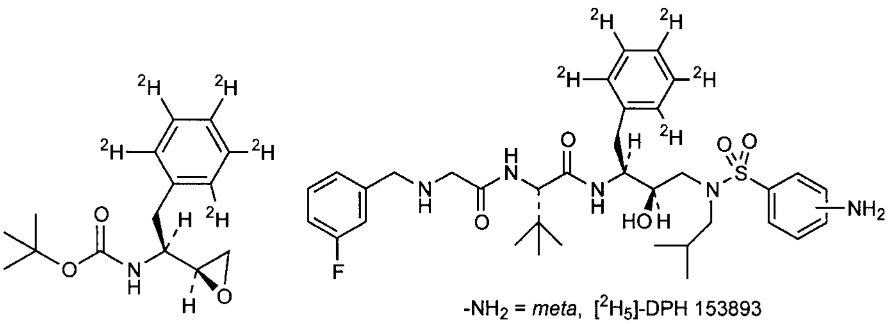
Since both compounds possess the HEA core structure, we planned to prepare DPH 153893 and DPH 140662 from a common labeled precursor bearing the elements of the [phenyl ring-$^2H_5$]-HEA moiety. We believe that the phenyl group of the HEA sub-unit is metabolically stable, since metabolites have not been reported.[5] Similarly, these metabolites are absent from our internal study[6] of DPH 153893 or DPH 140662. As a result, we were encouraged to explore the current synthesis with the prospect that a protease inhibitor containing the [phenyl ring-$^2H_5$]-HEA moiety might, in addition to our initial reason for synthesis, be suitable for *in vivo* investigation. By routine reactions we made the required [$^2H_5$]-intermediate from [$^2H_5$]-bromobenzene and transformed this compound into [$^2H_5$]-DPH 153893 and [$^2H_5$]-DPH 140662.

Results and discussion

In the synthetic literature on anti-HIV protease inhibitors, unlabeled (1S, 2S)-(1-oxiranyl-2-[$^2H_5$]phenylethyl)-carbamic acid *tert*-butyl ester **11** is extensively used as an intermediate[3,7] for synthesis of aspartyl protease inhibitors. We considered **11** to be an attractive key intermediate from which to make [$^2H_5$]-protease inhibitors. Compound **11**, Figure 1, has the epoxide functionality from which to build [$^2H_5$]-DPH 153893 and [$^2H_5$]-DPH 140662. In addition, **11** provides the [phenyl ring-$^2H_5$]-HEA isostere core of both inhibitors. Unlike previous work,[7] the present approach does not draw on any chiral pools of reagents, such as optically pure amino acids or derivatives, to construct **11**. Therefore, constructing the [$^2H_5$]-labeled carbon skeleton from [$^2H_5$]-bromobenzene **1** with the desired 1S, 2S-configuration in **11** was a challenging task.

To address this point, we implemented a facially selective syn-aldol condensation reaction with S-4-benzyl-(3-[$^2H_5$]phenylpropionyl)-oxazolidin-2-one **5**. [$^2H_5$]-Bromobenzene was used to prepare compound **5**, and the



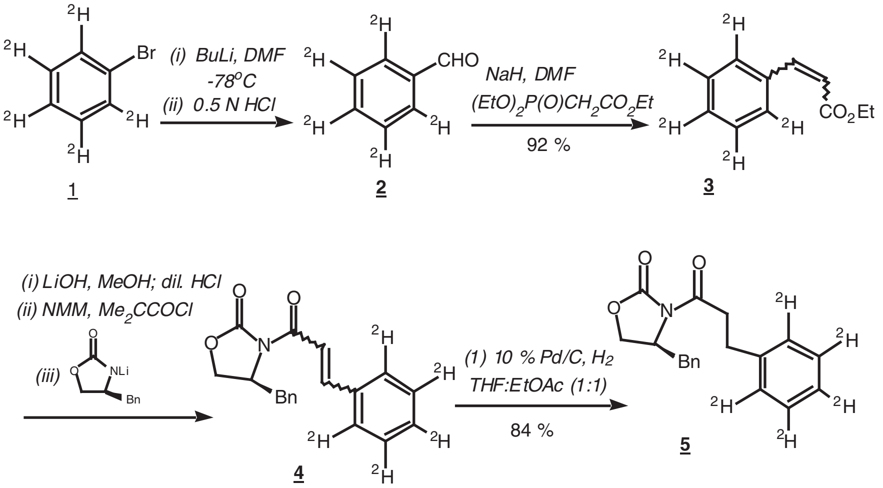
Intermediate **11**

-NH2 = *meta*, [$^2H_5$]-DPH 153893

-NH2 = *para*, [$^2H_5$]-DPH 140662

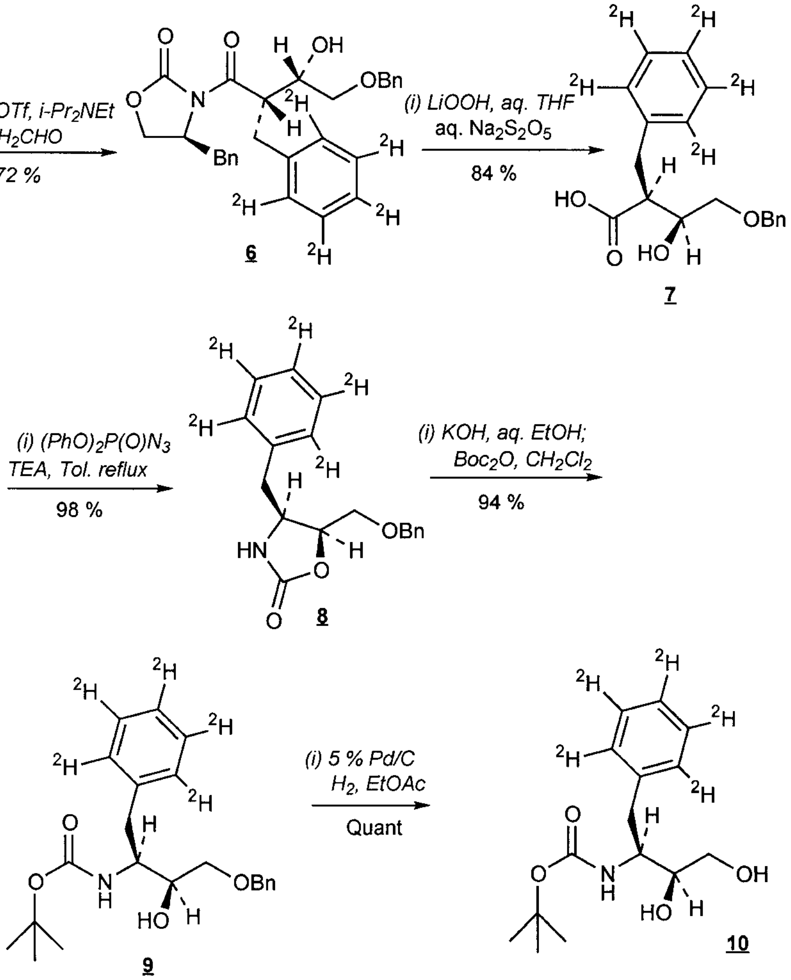
Figure 1. Structures of [$^2H_5$]-epoxide intermediate, [$^2H_5$]-DPH 153893 and [$^2H_5$]-DPH 140662

sequence of routine reactions that was employed is shown in Scheme 1. Thus, following a lithium-halogen exchange reaction with [$^{2}H_{5}$]-bromobenzene 1, DMF was added and acid workup provided the aldehyde, 2. A Wittig-type homologation protocol[8] with compound 2 furnished 3-[$^{2}H_{5}$]phenylacrylic acid ethyl ester, 3. The carboxylic acid made by the hydrolysis of 3 was activated as the mixed pivaloyl anhydride, and reacted with (S)-(-)-4-benzyl-*N*-lithio-2-oxazolidinone, according to a literature procedure,[9] to form (S)-4-benzyl-3-(3-[$^{2}H_{5}$]phenylacryloyl)-oxazolidin-2-one, 4. Compound 4 was saturated by subjecting it to Pd/C catalyzed hydrogenation to furnish (S)-4-benzyl-3-(3-[$^{2}H_{5}$]phenylpropionyl)-oxazolidin-2-one, 5.



Scheme 1. Conversion of [$^{2}H_{5}$]-bromobenzene into the acyl oxazolidinone 5 to enable the asymmetric synthesis of 11

To complete the required carbon skeleton and simultaneously achieve the desired (S, S)-configuration, compound 5 was condensed with (benzyloxy) acetaldehyde,[10] as shown in Scheme 2. The combination of dibutylboron triflate and diisopropylethylamine reagent resulted in the highly diastereofacially selective syn-aldol condensation reaction yielding 6. In the manner described in the literature,[11] lithium peroxide was used to cleave the amide bond in compound 6 to obtain (2S,3S)-[(2-[$^{2}H_{5}$]benzyl-4-(benzyloxy)-3-hydroxybutyric acid, 7, in 84% yield. Compound 7 was refluxed in a solution of toluene containing diphenylphosphoryl azide and triethylamine to produce 8, an oxazolidinone. Formation of compound 8 is explained by the intramolecular cyclization reaction of the intermediate isocyanate with the participation of the neighboring hydroxy group, as described in the literature.[10] The deprotection of 8 and the formation of (1S,2S)-1-[*N*-*tert*-butyloxycarbonyl)amino]-1-[$^{2}5H_{5}$]benzyl-3-(benzyloxy)propan-2-ol, 9, was

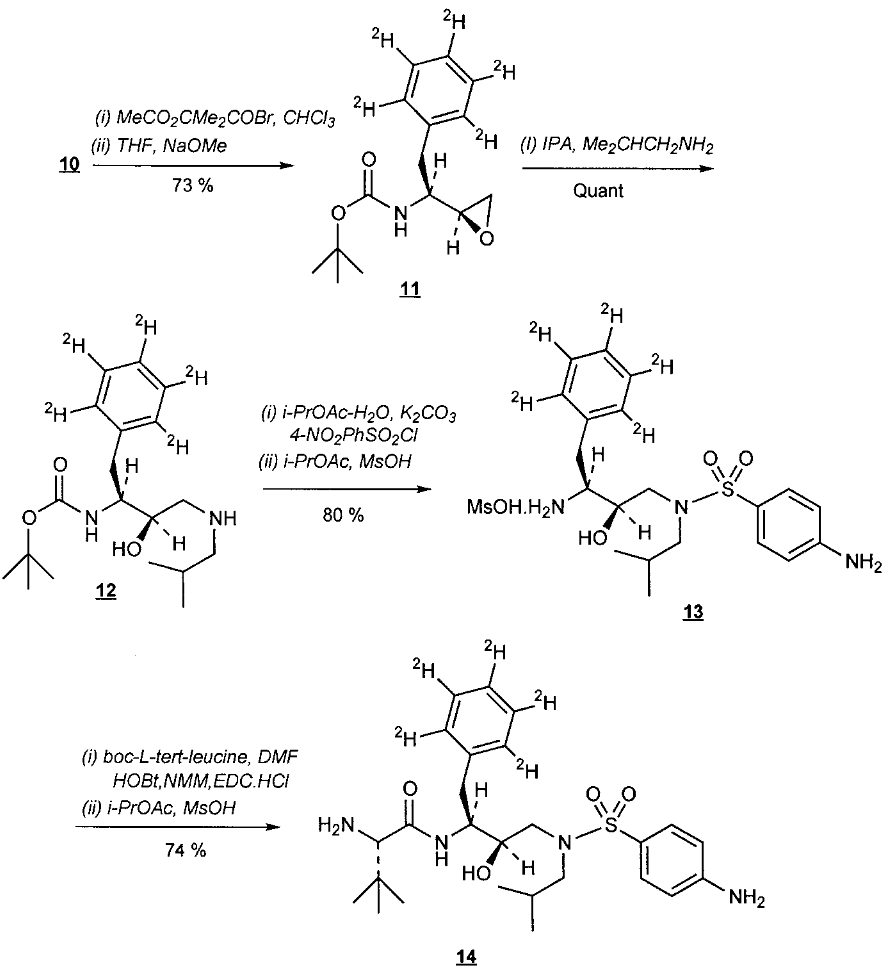


Scheme 2. Sequence of reactions to create desired (S,S)-configuration

achieved in a one-pot procedure. Compound 5 was subsequently hydrogenated under Pd/C catalysis to yield 10 (see Scheme 2).

The diol grouping in 10 was converted to the epoxide ring by a modified literature protocol[12] to give 11 (Scheme 3). Specifically, compound 10 was treated with 1-(bromocarbonyl)-1-methylethyl acetate in ethanol-free chloroform at 0°C, converting it to an intermediate bromohydrin. The bromohydrin was cyclized by treatment with a solution of sodium methoxide in THF to provide (1S, 2S)-(1-oxiranyl-2-[$^2H_5$]phenyl-ethyl)-carbamic acid *tert*-butyl ester, 11, in 73% yield.

From intermediate 11 we proceeded to make [$^2H_5$]-DPH 153893 and [$^2H_5$]-DPH 140662. At the second step in the reaction sequence isobutylamine was reacted with 11 in hot isopropyl alcohol to give 12. Compound 12 is the last



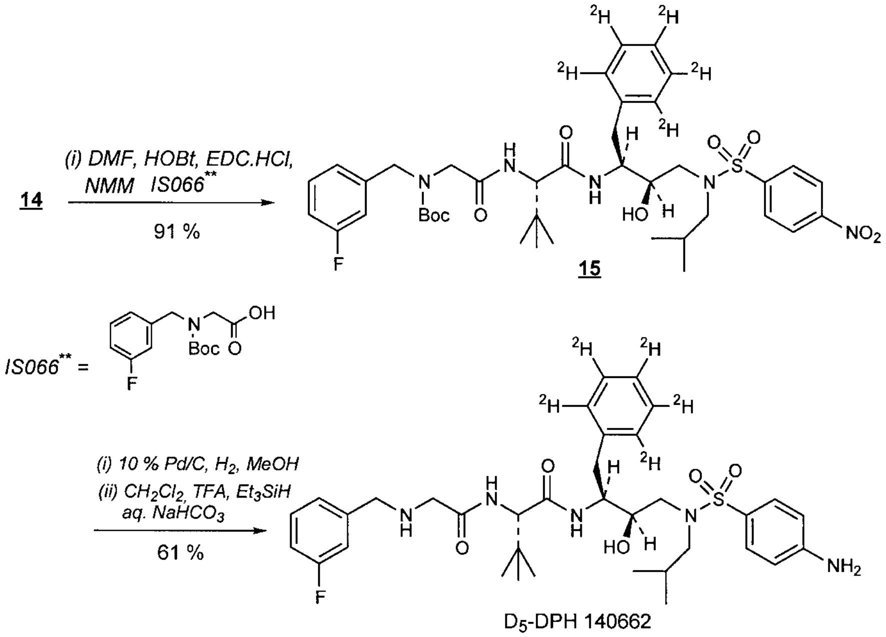
Scheme 3. Elaboration of $[^2H_6]$-epoxide 11 into 14 via compound 12, a common intermediate to $[^2H_6]$-DPH 153893 and $[^2H_5]$-DPH 140662

intermediate common to both synthetic targets, i.e. the reaction step after 12 is the point of divergence of the reaction sequences to DPH 140662 and DPH 153893.

In order to make $[^2H_5]$-DPH 140662, compound 12 was treated with 4-nitrobenzenesulfonyl chloride in the presence of potassium carbonate as acid scavenger. After removal of the Boc group, 13 was obtained as the methanesulfonic acid salt in 80% overall yield over the two steps. By a standard coupling method with EDC.HCl, 4-methylmorpholine and HOBt[13] in DMF, compound 13 and Boc-L-*tert*-leucine formed the Boc protected 14.

We found that the stepwise sequence of coupling Boc-L-*tert*-leucine to make the Boc protected 14, the removal of Boc to yield 14 and the coupling reaction of compound 14 with IS066 (IS066 was generously supplied by the

Department of Chemical Process, DuPont Pharmaceuticals Company, Wilmington, DE) to give 15, as shown in Scheme 4, was the best sequence for making [$^2H_5$]-DPH 140662. An alternative single coupling reaction to DPH 140662 from 13 was complicated by intra-molecular cyclization of the dipeptide reagent under our reaction conditions. Chromatographic purification was required to obtain 15, and then only in poor yield. The single coupling approach was therefore abandoned.



Scheme 4. Conversion of 14 into [$^2H_5$]-DPH 140662

A standard coupling protocol in DMF containing EDC.HCl, HOBt and 4-methylmorpholine as acid scavenger converted 14 to compound 15. After Pd/C catalyzed hydrogenation of 15 to the 4-aminobenzenesulfonamide, the Boc group was removed with TFA-Et_3SiH and the reaction mixture was neutralized with $NaHCO_3$ to provide [$^2H_5$]-DPH 140662 in 61% yield.

By substituting 3-nitrobenzenesulfonyl chloride for 4-nitrobenzenesulfonyl chloride in Scheme 3, and following a sequence of reactions analogous to Schemes 3 and 4, we accomplished the synthesis of [$^2H_5$]-DPH 153893 in comparable yield.

Conclusion

Two HIV protease inhibitors were specifically labeled with [$^2H_5$] at the HEA sub-unit by transforming [$^2H_5$]-bromobenzene into a common intermediate for these agents. The intermediate was made using established reactions that included an asymmetric reaction step. The intermediate was used to create the [phenyl ring-$^2H_5$] labeled HEA core structure of DPH 140662 and DPH 153893. Very high deuterium incorporation was achieved by the sequence we have described and the synthetic approach may be applicable, with some modifications, to other HIV-protease inhibitors.

Experimental

All reactions were carried out under an atmosphere of argon unless otherwise specified. Solvents were commercial grade and used without purification or drying. Column chromatography was carried out on Merck Kiesegel 60 (230 m) silica gel. Flash chromatographic separations were on a Biotage Flash System using a pre-packed silica gel cartridge. TLC visualization reagents included (10% iodine plus 10% AcOH) in 40% aqueous KI. ^1H NMR spectra were recorded at 300, 400 or 500 MHz. Chemical shifts are given in ppm relative to tetramethylsilane (TMS). Only the important IR absorptions (cm^{-1}) and the molecular ion and/or base peaks in MS are given. HPLC columns used include Zorbax Rx C_{18}, Discovery Amide C_{16}, and Waters Symmetry C_{18} columns.

4S-Benzyl-3-(3-[$^2H_5$]phenyl-propionyl)-oxazolidin-2-one, 5

To a stirred solution of [$^2H_5$]-phenylacrylic acid (10.1 g, 65.07 mmol, see Scheme 1 for preparation) and 4-methylmorpholine (7.15 ml, 65.07 mmol) in dry THF (120 ml) maintained at −78°C with a dry ice-acetone bath was added trimethylacetyl chloride (8.01 ml, 65.07 mmol). After 30 min the cold bath was replaced with an ice-water bath and the reaction was stirred for another 45 min. The precipitate was removed by filtration, and the filtrate was transferred to a solution of *N*-lithio-oxazolidinone that was pre-generated as follows. (S)-(-)-4-Benzyl-2-oxazolidinone (11.53 g, 65.07 mmol) in dry THF (120 ml) was stirred under argon atmosphere at −78°C while BuLi (1.6 M solution in hexanes, 40.6 ml, 65.07 mmol) was added dropwise. The solution was stirred at −78°C for 1.0 h and the above filtrate, a solution of mixed anhydride in THF, was added in one portion. The reaction was quenched after 1 h with saturated NH_4Cl solution (100 ml) and the organic phase was separated. The aqueous portion was further extracted with ethyl acetate (3 × 200 ml), the combined organic extract was dried and evaporated to dryness to give (S)-4-benzyl-3-(3-[$^2H_5$]phenylacryloyl)-oxazolidin-2-one 4. Compound 4 was dissolved in ethyl acetate (150 ml) and a suspension of 10% Pd/C (1.2 g) in ethyl acetate (20 ml) was added. After the reaction mixture was degassed, it was stirred under a hydrogen atmosphere for 3 h and filtered through a pad of Celite to remove the spent catalyst. The filtrate was evaporated to a product which crystallized from EtOAc-hexane to give 5 (17.10 g, 84%). ^1H NMR (400 MHz) δ 2.74 (dd, 1H), 3.03 (m, 2H), 3.28 (m, 3H), 4.16 (m, 2H), 4.65 (m, 1H), 7.17 (d, 2H) and 7.32 (m, 3H). ^{13}C NMR (75 MHz) δ 30.30, 37.12, 37.94, 55.17, 66.26, 127.4, 128.0, 128.2, 129.0, 129.5, 140.4, 153.4 and 172.5. IR $\nu_{\max}$ ($CHCl_3$) 2276, 1785, 1699, 1490, 1478, 1390, 1372, and 1202 cm^{-1}.

*4S-Benzyl-3-[(2S-[$^2H_5$]benzyl-4-(benzyloxy)-3S-hydroxy-butyryl)]-oxazolidin-2-one, **6***

To a stirred solution 4S-benzyl-3-(3-[$^2H_5$]phenylpropionyl)-oxazolidin-2-one **5** (23.68 g, 75.3 mmol) in CH_2Cl_2 (140 ml) at 0°C was added dibutylboron triflate (1.0 M solution in dichloromethane, 98.0 ml, 98 mmol) followed by diisopropylethylamine (19.67 ml, 112.97 mmol). After stirring the mixture for 1 h at room temperature it was cooled to −78°C and a solution of (benzyloxy)acetaldehyde (20 g, 133.17 mmol) in CH_2Cl_2 (30 ml) was added. The reaction mixture was stirred for 30 min at −78°C and warmed to room temperature for 2 h. A phosphate buffer of pH 7.3 (40 ml) was added and while the reaction was cooled to 0°C, methanol (140 ml) was added followed by a mixture of (2:1) MeOH and 30% H_2O_2 (180 ml). The resulting mixture was stirred for 1 h, concentrated to a small volume and extracted with EtOAc (2 × 200 ml). The organic portions were combined and washed with saturated $NaHCO_3$ (200 ml), brine (200 ml) and dried over anhydrous magnesium sulfate. Evaporation of the solvent gave the crude product which was purified by chromatography on silica gel. The column was eluted with 10–25% ethyl in hexane to give **6** (25 g, 71.46%). ^{1}H NMR (500 MHz) δ 2.10 (dd, Hz, 1H), 2.64 (brs, 1H), 2.83 (dd, 1H), 3.08 (q, Hz, 1H), 3.17 (q, 1H), 3.59 (m, 2H), 3.78 (tr, 1H), 3.86 (q, 1H), 4.18 (q, 1H), 4.42 (m, 1H), 4.50 (s, 2H), 4.64 (m, 1H), 6.93 (dd, 2H), 7.20–7.343 (m, 10H). IR $\nu_{\max}$ ($CHCl_3$) 2274, 1778, 1694, 1496, 1386, 1210, and 1104 cm^{-1}.

*S-2-[$^2H_5$]Benzyl-4-(benzyloxy)-3S-hydroxy-butyric acid, **7***

To a stirred solution of 4S-benzyl-3-[(2S-[$^2H_5$]benzyl-4-(benzyloxy)-3S-hydroxy-butyryl)]-oxazolidin-2-one **6** (23.1 g, 49.72 mmol) in 50% aqueous THF (105 ml) at 0°C were added 30% H_2O_2 (25 ml, 220 mmol) and $LiOH.H_2O$ (4.25 g, 101.2 mmol) in succession. The reaction mixture was stirred in an ice bath and monitored by TLC until acyloxazolidinone was consumed in a calculated 6 h. Saturated sodium metabisulfite (100 ml) was added and the mixture was extracted with EtOAc (3 × 200 ml). After acidification of the aqueous portion to pH 2 with dilute HCl, it was extracted with EtOAC (2 × 120 ml). The organic portions were combined, dried over anhydrous Na_2SO_4, filtered and evaporated to an oil. TLC on silica gel (ether: pet. ether: acetic acid, 60/40/1 v/v/v) showed fast running desired product and slow running oxazolidinone. The mixture was applied to a column of silica gel and eluted with (ether: pet. ether: acetic acid 60/40/1,v/v/v/) to afford crystalline (CH_2Cl_2-hexane) 7 (12.98 g, 84%). ^{1}H NMR (500 MHz) δ 2.95 (m, 1H), 3.10 (2H), 3.49 (dd, 1H), 3.59 (dd, 1H), 4.03 (m, 1H), 4.54 (s, 2H), 7.31–7.36 (m, 5H). ^{13}C NMR (75 MHz) δ 33.67, 50.09, 70.38, 71.64, 73.57, 127.9, 128.0, 128.2, 128.3, 128.6, 137.6, 138.6, and 177.9. IR ν_{max} ($CHCl_3$) 3254, 2275, 1728, 1454 and 1169 cm^{-1}.

S-4-[$^2H_5$]Benzyl-5S-((benzyloxymethyl)-oxazolidin-2-one, **8**

A mixture of S-(2-[$^2H_5$]benzyl-4-(benzyloxy)-3S-hydroxy-butyric acid 7 (11.0 g, 36.0 mmol), diphenylphosphoryl azide (9.84 ml, 45.66 mmol) and triethylamine (6.68 ml, 47.92 mmol) in toluene (78 ml) was refluxed for 4 h and cooled to room temperature. Ethyl acetate (250 ml) was added and the solution was washed with saturated $NaHCO_3$ (100 ml), dried and evaporated to give a residue that was chromatographed on silica gel. The fast running impurities were first eluted with 25% EtOAc in hexane, and 35% EtOAc in hexane was used to elute the desired product **8** (11.7g, 98%). ^{1}H NMR (300 MHz) δ 2.75 (dd, 1H), 2.92 (dd, 1H), 3.797 (d, 2H), 4.12 (m, 1H), 5.62 (s, 2H), 5.8 (m, 2H) and 7.40 (m, 5H).

(S)-1-[N-tert-Butyloxycarbonyl)amino]-1-[$^2H_5$]benzyl-3-(benzyloxy)propan-2S-ol, **9**

S-4-[$^2H_5$]Benzyl-5S-(benzyloxymethyl)-oxazolidin-2-one **8** (11.7 g, 38.74 mmol) in 50% aqueous ethanol (40 ml) containing KOH (9.0 g, 160.4 mmol) was heated at 70°C for 3 h and then cooled to room temperature. The solution was adjusted to pH 7 with 1.0 M HCl and concentrated under reduced pressure. Di-*tert*-butyl dicarbonate (16.99 g, 77.84 mmol) in CH_2Cl_2 (60 ml) was added at 0°C and the reaction was stirred for 2 h at room temperature. Saturated NH_4Cl solution (40 ml) was added. The mixture was extracted with CH_2Cl_2 (2 × 50 ml), the combined organic portions were dried and evaporated to yield a solid residue. The crude product was applied to a column of silica gel and the excess di-*tert*-dibutyl dicarbonate was eluted with 10% EtOAc in hexane. Further elution with 35% EtOAc in hexane gave a solid which crystallized from hexane to afford **9** (15.2g, 94%). ^{1}H NMR (300 MHz) δ 1.35 (s, 9H), 2.62 (brs), 2.88 (d, 2H), 3.56 (m, 2H), 3.73 (brs, 1H), 3.93 (brs, 1H), 4.51 (s, 2H), 4.72 (d, 1H), 7.33 (m, C_6H_5). ^{13}C NMR (75 MHz) δ 28.34, 36.36, 54.20, 71.72, 71.82, 73.68, 79.53, 125.9(tr) 127.8, 127.9, 128.1, 128.5, 129.1(tr), 137.8, 137.9 and 155.9. IR ν_{max} ($CHCl_3$) 1685, 2273, 1526, and 1172 cm^{-1}.

S-1-[N-tert-Butyloxycarbonyl)amino]-1-[$^2H_5$]benzyl-propan-2S,3-diol, **10**

To a solution of S-1-[*N-tert*-butyloxycarbonyl)amino]-1-[$^2H_5$]benzyl-3-(benzyloxy)-propan-2S-ol **9** (14.0 g, 37.18 mmol) in EtOAc (140 ml) was added a suspension of 5% Pd/C (1.40 g) in ethyl acetate (10 ml). The stirred reaction mixture was purged to remove air and stirred under hydrogen atmosphere for 18 h. The spent catalyst was filtered off and the filtrate was concentrated to a white solid that crystallized from hexane-ethyl acetate to give **<u>10</u>** (10.48 g, ≈ 100%). ^{1}H NMR (500 MHz) δ 1.37 (s, 9H), 1.59 (brs, 1H), 2.75 (d, 1H), 2.91 (dd, 1H), 3.09 (dd, 1H), 3.33 (brs, 2H), 3.65 (m, 2H), 3.82 (m, 1H), 4.53 (d, 1H). ^{13}C NMR (75 MHz) δ 28.30, 36.55, 52.59, 63.06, 73.27, 80.41, 126.2(tr), 128.2 (tr), 129.0 (tr), 137.3, and 157.1. IR ν_{max} (CHCl$_3$) 3356, 2273, 1685, 1525, 1445, 1250, and 1171 cm^{-1}.

[(S)-1′-((N-tert-Butyloxycarbonyl)amino)-2S-[$^{2}H_5$]phenyl-ethyl]oxirane, **<u>11</u>**

1-Bromocarbonyl-1-methylethyl acetate (2.0 ml, 13.63 mmol) was added to a stirred suspension of (S)-1-[*N-tert*-butyloxycarbonyl)amino]-1-[^{2}H$_5$]benzyl-propan-2S,3-diol **<u>10</u>** (3.0 g, 10.48 mmol) in dry, ethanol free, chloroform (30 ml) maintained at 0°C in an ice water bath. After 3 h at room temperature the reaction was quenched by the addition of saturated NaHCO$_3$ solution (20 ml) and extracted with EtOAc (3 × 30 ml). The organic extract was washed with brine (30 ml), dried over anhydrous Na$_2$SO$_4$ and evaporated to give a solid. The solid was stirred as a solution in dry THF (40 ml), cooled to 0°C in an ice bath and NaOCH$_3$ (0.92 g, 17.03 mmol) was added. After stirring the reaction mixture at room temperature for 4 h, saturated ammonium chloride solution (30 ml) was added and the mixture extracted with EtOAc (3 × 30 ml). The organic extract was dried over anhydrous Na$_2$SO$_4$, filtered, and the filtrate was evaporated to a small volume. The material was applied to a column of silica gel and the compound was eluted with 22% EtOAc in hexane to give a white solid **<u>11</u>** (2.08 g, 73%). ^{1}H NMR (300 MHz) δ 1.75 (d, J = 7.0 Hz, 3H), 2.45 (s, 3H), 4.97 (q, J = 7.0 Hz, 1H), 7.10–7.27 (m, 3H), 7.32–7.43 (m, 2H), 7.51 (d, J = 8.2 Hz, 2H) and 7.75 (d, J = 7.3 Hz, 1H). ^{13}C NMR (75 MHz) δ 28.34, 37.65, 41.61, 46.81, 53.3, 79.71, 126.2 (tr), 128.1(tr), 129.1(tr), 136.7 and 155.3. IR ν_{max} (CHCl$_3$) 2275, 1679, 1523 and 1168 cm^{-1}.

3S-[N-(tert-Butyloxycarbonyl)amino-1-(2-methylpropyl)amino-4-[$^{2}H_5$]phenylbutan-2R-ol, **<u>12</u>**

To a solution of 2S-[1′-(S)-((*N-tert*-butyloxycarbonyl)amino)-2-[^{2}H$_5$]phenyl-ethyl]-oxirane **<u>11</u>** (2.06 g, 7.20 mmol) in isopropanol (6 ml) was added isobutylamine (6 ml, 111.5 mmol) and heated at 86°C for 4 h under argon atmosphere. The solvent was evaporated after cooling to room temperature. The white solid obtained was crystallized from ether-petroleum ether to give **<u>12</u>** (2.32 g, ≈ 100%). ^{1}H NMR (500 MHz) δ 0.89 (dd, 6H), 1.35 (s, 9H), 1.70 (m, 1H), 2.39 (d, 3H), 2.68 (d, 2H), 2.86 (dd, 1H), 2.98 (dd, 1H), 3.44 (m, 1H), 3.79 (brs, 1H), 4.67 (d, 1H). IR ν_{max} (CHCl$_3$) 3365, 1648, 1521, and 1173 cm^{-1}.

N-(3S-Amino-2R-hydroxy-4-[$^2H_5$]phenyl-butyl)-N-isobutyl-4-nitro-benzenesulfonamide, **13**

A solution of K_2CO_3 (430 mg, 3.11 mmol) in water (3 ml) was added to a solution of 3S-[*N*-(*tert*-butyloxycarbonyl)amino-1-(2-methylpropyl)amino-4-[$^2H_5$]phenylbutan-2R-ol **12** (575.19 mg, 1.68 mmol) in isopropyl acetate (3 ml). The mixture was stirred at 56°C to attain solution. 4-Nitro-benzenesulfonyl chloride (360 mg, 1.62 mmol) in isopropyl acetate (3 ml) was added and stirring continued for 30 min after the addition was completed. A solid product separated upon cooling the reaction to room temperature, and it was collected by filtration. This material (Boc protected amine) (885.8 mg, 1.681 mmol) was stirred in isopropyl acetate (18 ml) and the temperature was brought to 85°C under argon atmosphere. Methanesulfonic acid (130.9 µl, 2.0 mmol) in isopropyl acetate (5 ml) was added slowly and the reaction mixture was stirred for another 30 min. Upon cooling to room temperature a crystalline product separated and it was collected by filtration to afford **13** (722.6 mg, 80%). ^{1}H NMR (300 MHz) (DMSO-d_6) δ 0.89 (dd, 6H), 1.80 (m, 1H), 2.36 (s, 3H), 2.85 (m, 2H), 3.10 (m, 3H), 3.49 (dd, 1H), 3.95 (m, 1H), 5.58 (d, 1H), 7.80 (brs, 2H), 8.15 (d, 1H), 8.40 (d, 1H).

2-Amino-N-{1-[$^2H_5$]benzyl-2-hydroxy-3-[isobutyl-(4-nitro-benzenesulfonyl)-amino]-propyl-3,3-dimethylbutyramide, **14**

N-*tert*-Butyloxycarbonyl *tert*-butylglycine (Boc-L-*tert*-leucine) (360.80 mg, 1.56 mmol), 1-hydroxybenzotriazole hydrate (210.8 mg, 1.56 mmol) in anhydrous DMF (10 ml) was treated with 4-methylmorpholine (343 µl, 3.12 mmol) followed by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (298.8 mg, 1.56 mmol). Compound **13** (680 mg, 1.302 mmol) was added and the reaction mixture was stirred under argon atmosphere overnight. The reaction mixture was concentrated to a small volume, partitioned between ethyl acetate (20 ml) and water (15 ml), and the aqueous portion was separated. The aqueous portion was further extracted with ethyl acetate (3 × 20 ml) and the combined organic portions were washed sequentially with saturated NH_4Cl solution (20 ml), saturated K_2CO_3 solution (20 ml), brine (20 ml) and dried. The filtrate was evaporated to a residue (843 mg), and then dissolved in isopropyl acetate (10 ml). While the stirred solution was maintained at 85°C, methanesulfonic acid (102 µl, 1.85 mmol) in isopropyl acetate (3 ml) was added dropwise. The reaction mixture was cooled to room temperature after 30 min, and concentrated to a small volume. Upon the addition of ether, a crystalline solid precipitated, and the material was collected by filtration to afford **14** (708 mg, 84%). HPLC analysis on a Zorbax Rx C_{18} column 4.6 × 250 mm at a flow rate of 1.0 ml/min under isocratic conditions using 40% water in acetonitrile containing 0.05% TFA gave a retention time of 3.3 min. ^{1}H NMR (300 MHz) (DMSO-d_6) δ 0.75 (dd, 6H), 0.96 (s, 9H), 1.89 (m, 1H), 2.28 (s, 3H), 2.65 (dd, 1H), 2.82 (m, 1H), 2.96 (dd, 1H), 3.08 (dd, 1H), 3.40 (m, 2H), 3.65 (m, 1H), 3.85 (brs), 4.10 (m, 1H), 7.85 (brs, 2H), 8.10 (d, 1H), 8.38 (d, 1H). IR ν_{max} ($CHCl_3$) 2276, 1691, 1530, 1352, 1350, and 1150 cm^{-1}.

*[{1-{1-[$^2H_5$]Benzyl-2-hydroxy-3-[isobutyl-{4-nitro-benzenesulfonyl)-amino]-propylcarbamoyl}-2,2-dimethyl-propylcarbamoyl}-methyl](3-fluoro-benzyl)-carbamic acid tert-butyl ester, **15***

To a solution of *N*-[(*tert*-butyloxycarbonyl)-(3-fluorobenzyl)]glycine (IS066) (373.95 mg, 1.32 mmol), 1-hydroxybenzotriazole hydrate (HOBT), 178.38 mg, 1.32 mmol) in anhydrous DMF (5 ml) was added 4-methylmorpholine (290 μl, 2.64 mmol) followed by EDC.HCl (252.56 mg, 1.32 mmol). While stirring under an argon atmosphere, **14** (700 mg, 1.1 mmol) was added. After stirring overnight, the mixture was concentrated under high vacuum to a small volume and partitioned between EtOAc (20 ml) and water (15 ml). The organic phase was separated and the aqueous portion was further extracted with ethyl acetate (2 × 20 ml). The combined organic portions were washed with water (20 ml), saturated NH_4Cl solution (20 ml), saturated K_2CO_3 (20 ml), brine (20 ml) and dried over magnesium sulfate. The solvent was evaporated to give a foamy solid **15** (880 mg, 91%). HPLC analysis on a Zorbax Rx C_{18} column 4.6 × 250 mm at a flow rate of 1.0 ml/min under isocratic conditions using 40% water in acetonitrile containing 0.05% TFA gave a retention time of 11.12 min.

N-{3-[(4-Amino-benezenesulfonyl)-isobutyl-amino]-1-[$^2H_5$]benzyl-2-hydroxy-propyl}-2-[2-(3-fluorobenzylamino)-acetylamino]-3,3-dimethyl-butyramide, [$^2H_5$]-DPH 140662

To a solution of **15** (700 mg, 0.869 mmol) in methanol (25 ml) was added a suspension of 10% Pd/C (224 mg) in methanol (8 ml). The reaction mixture was stirred at room temperature in an atmosphere of hydrogen for 3 h and then filtered. The solvent was evaporated to yield a foamy solid. Trifluoroacetic acid (2.616 ml) and triethylsilane (Et_3SiH) (0.4 ml) were added successively to a solution of the solid in methylene chloride (18 ml). The mixture was stirred at room temperature and the reaction was monitored by TLC (3% MeOH in dichloromethane). After 2 h the reaction mixture was evaporated to a residue and, EtOAc (20 ml), water (15 ml), and solid $NaHCO_3$ (1.3 g) were added. The mixture was stirred for 20 min and the organic portion was separated. The aqueous portion was further extracted with ethyl acetate (2 × 20 ml) and the combined organic extract was dried over $MgSO_4$. The product was purified by chromatography on silica gel column eluted with 8% EtOH in toluene to give an oil. Trituration of the oil with ether gave

[$^2H_5$]-DPH 140662 (385 mg, 61%; D_5 98.9%, D_4, 1.1%). $[\alpha]_D^{20}$ – 15.64° ($c = 0.41\%$ in MeOH). The product was analyzed by HPLC on a Discovery Amide C_{16} Supelco 250 × 4.6 mm, 5 μm column. The solvent combination of A (10 mmol ammonium formate) and B (acetonitrile) was used under gradient conditions of 20–30% B in 0–5 min, 30–50% B in 5–18 min and 20–30% B in 18–20 min at a flow rate of ml/min. The product peak was detected by UV at a wavelength of 254 nm and gave >98% chemical purity at a retention time of 14.13 min. IR ν_{max} ($CHCl_3$) 1314, 1285, and 1143 cm^{-1}. ^1H NMR (300 MHz, d_6-DMSO) δ 0.85 (m, 15H), 1.82 (m, 1H), 2.52 (dd, 1H), 2.68 (m, 1H), 2.85 (m, 2H), 2.95 (dd, 1H), 3.05 (s), 3.22 (dd, 1H), 3.30 (s, 3H), 3.50 (s), 3.60 (m, 1H), 3.89 (m, 1H), 4.18 (d, 1H), 4.89 (d, 1H), 5.98 (brs, 2H), 6.60 (d, 2H), 7.12 (m, 3H), 7.42 (m, 3H), 7.78 (d, 1H), 7.98 (d, 1H). HRMS (TOF MS ES+; M+1); calcd. 675.3762, found 675.3766. ESI full ms gave *z* at 675 (M+1, 100%) and 397 (5%), 372 (15%) and 354 (2%).

N-{1-[$^2H_5$]Benzyl-2-hydroxy-3-[isobutyl-(3-nitro-benzenesulfonyl)-amino]-propyl}-2-[2-(3-fluorobenzylamino)-acetylamino]-3,3-dimethyl-butyramide, [$^2H_5$]-DPH 153893

N-[(*tert*-butyloxycarbonyl)-(3-fluorobenzyl)]glycine (356.0 mg, 1.256 mmol) was reacted with 2-amino-*N*-{1-[$^2H_5$]benzyl-2-hydroxy-3-[isobutyl-(3-nitro-benzenesulfonyl–amino]-propyl-3,3-dimethyl-butyramide methanesulfonic acid salt **17** (799 mg, 1.256 mmol) to make [(1{1-[$^2H_5$]benzyl-2-hydroxy-3-[isobutyl-(3-nitro-benzenesulfonyl)-amino]-propyl-carbamoyl}-2,2-dimethyl-propylcarbamoyl)-methyl]–(3-fluorobenzylcarbamic acid tert-butyl ester (902 mg, 89%) as described under experiment **15**. Sequential hydrogenolysis and boc deprotection with methanesulfonic acid in isopropyl acetate gave *N*-{1-[$^2H_5$]benzyl-2-hydroxy-3-[isobutyl-(3-nitro-benzenesulfonyl)-amino]propyl}-2-[2-(3-fluorobenzylamino)-acetylamino]-3,3-dimethyl-butyramide **[$^2H_5$]-DPH 153893** (947 mg, 1.09 mmol, 98%; D_5, 98.9%, D_4, 1.1%) as the methanesulfonic acid salt. $[\alpha]_D^{20}$ – 17.84° ($c = 0.43\%$ in MeOH). HPLC analysis on Discovery Amide C_{16} Supelco 250 × 4.6 mm, 5 μm column with elution solvent combination of A (10 mmol ammonium formate) and B (acetonitrile), under the gradient conditions of 20–30% B in 0–5 min, 30–50% B in 5–18 min and 20–30% B in 18–20 min and a flow rate of 1.0 ml/min. The product peak was detected by UV at a wavelength of 254 nm, and gave >99.6% chemical purity at a retention time of 10.7 min. HRMS (TOF MS ES+; M+1); calcd. 675.3762, found 675.3765. ^1H NMR (300 MHz, d_6-DMSO) δ 0.85 (dd, and s, overlapped, 15 H), 1.92 (m, 1 H), 2.50 (dd, 1 H overlapped with DMSO), 2.80 (m, 2H), 2.90 (m, 2H), 3.03 (s), 3.50 (s), 3.60 (m, 1H), 3.95 (m, 1H), 4.20 (d, 1H), 4.90 (d, 1H), 5.51 (brs), 6.70 (d, 1H), 6.80 (d, 1H), 6.90 (s, 1H), 7.10 (m, 3H), 7.30 (m, 1H), 6.65 (d, 1H), 7.90 (d, 1H). ESI full MS gave *z* 675 (M+1, 100%), 397 (2%), 372 (18%), and 354 (5%).

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