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Enantioselective synthesis of tranylcypromine analogues as lysine demethylase (LSD1) inhibitors

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ABSTRACT

Asymmetric cyclopropanation of styrenes by *tert*-butyl diazoacetate followed by ester hydrolysis and Curtius rearrangement gave a series of tranylcypromine analogues as single enantiomers. The *o*,- *m*- and *p*-bromo analogues were all more active than tranylcypromine in a LSD1 enzyme assay. The *m*- and *p*-bromo analogues were micromolar growth inhibitors of the LNCaP prostate cancer cell line as were the corresponding biphenyl analogues prepared from the bromide by Suzuki crosscoupling.

1. Introduction

Eukaryotic DNA is packaged together with histone proteins to form chromatin in which genes are usually silenced. The DNA needs to unwind from the histone complex to be transcriptionally active and this interaction between DNA and histones is orchestrated by three sets of players. Firstly, there are the 'writers', enzymes that introduce post-translational modifications into DNA and histone proteins and thereby alter their affinity for one another. Next, there are corresponding 'erasers' that remove these modifications and return chromatin to its native state. Finally, 'readers' recognize and respond to the pattern of dynamic chromatin post-transcriptional modification, referred to as the 'epigenetic code'.[1]

Among the post-translational modifications, methylation is unique in that it occurs both in DNA (primarily at the C-5 position of cytosine) and in histone proteins (at lysine and arginine residues). It is also unique in that it was long considered to be an irreversible modification although there is no chemical reason why demethylation would be impossible. In 2004, the first lysine demethylase LSD1 (Lysine Specific Demethylase) was described.[2] Soon thereafter in 2006, a second family of lysine and arginine demethylases containing the JmjC domain was identified that are iron-dependent α-ketoglutarate dioxygenases.[3] In 2009, enzymes that convert 5-methylcytosine in DNA to 5-hydroxymethylcytosine were reported and there is evidence that DNA repair enzymes will replace the modified base by cytosine thus effecting an indirect demethylation.[4]

Currently, nearly 30 human lysine demethylases within the LSD and JmjC families are known.[5] LSDs comprising LSD1 and LSD2 are homologous to monoamine oxidases (MAOs) and demethylate mono- and dimethyllysine residues. The reaction involves electron transfer between the FAD cofactor and the nitrogen lone pair. This is why trimethyllysine is not a substrate, while mono- or dimethyllysine are oxidized to an iminium ion that is hydrolytically decomposed to formaldehyde (Fig. 1). The best characterized LSD substrate is histone H3 on K4 and K9 residues but there are additional non-histone client proteins of importance. LSD1 mediated demethylation of the tumor suppressor p53 inhibits its function by preventing interaction with the co-activator 53BP1,[6] while demethylation of the DNA methyltransferase Dnmt1 is necessary for maintaining DNA methylation activity.[7]

Overexpression of LSDs is observed in neuroblastoma, prostate, breast and bladder cancers where it is believed to repress transcription of repair pathways that would normally lead to apoptosis instead of proliferation.[8] Thus, LSD inhibitors are of interest as anticancer agents as well as potentially applicable to other human diseases that exhibit misregulated gene expression. The homology between LSDs and MAOs, a clinically validated target, suggests that LSDs are druggable. Indeed, screening known MAO inhibitors has uncovered micromolar LSD inhibitors among which the best known is the antidepressant drug tranylcypromine (*trans*-2-

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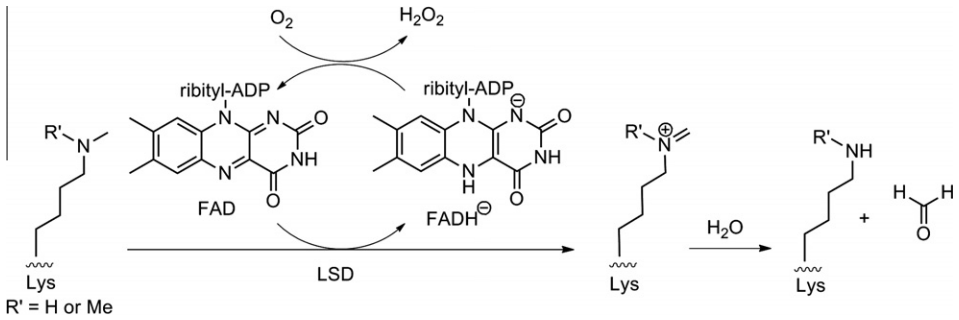

Figure 1. The catalytic mechanism of lysine specific demethylases (LSDs).

phenylcyclopropylamine, Parnate).[9] The compound acts as an irreversible inhibitor forming a covalent adduct with the FAD cofactor. X-ray structures of this complex bound within the LSD1 active site are not completely unambiguous, and it is likely that more than one adduct is formed (Fig. 2).[10]

Recently, several groups have reported the evaluation of tranylcypromine analogues as LSD inhibitors. Tranylcypromine itself is clinically used as a racemate and the same is true of the analogues described (Fig. 3).[10c,11] Here, we report our independent results in developing a high-throughput LSD1 assay and the synthesis of tranylcypromine analogues by a route that provides compounds as single enantiomers.

2. Results and discussion

2.1. LSD1 assays

We expressed His-tagged full length recombinant human LSD1 using a plasmid kindly provided by Shi.[2] The purified enzyme was assayed by Shi's immunoblot method. Briefly, the enzyme was incubated with purified histones or a methylated histone peptide substrate and activity detected with a commercial H3K4(me_2) antibody. In our hands, this did not give reproducible results. SELDI-TOF mass spectrometric analysis of the reaction mixture was more reliable (Fig. 4) and we observed both single and double demethylation of the dimethyllysine containing peptide to the corresponding methyllysine and lysine derivatives. Furthermore, substrate turnover was significantly reduced by the addition of tranylcypromine confirming inhibition of the enzyme.

We next examined colorimetric LSD assays suitable for higher throughput. Shi has reported[2] a coupled assay in which formaldehyde, the byproduct of LSD demethylation, is converted by formaldehyde dehydrogenase to formic acid with the concomitant reduction of NAD^+ to NADH which is spectrophotometrically monitored at 340 nM. However, no enzyme activity was detected with this assay. We then turned to the quantitation of hydrogen peroxide, the other byproduct of LSD demethylation. Forneris has reported a horseradish peroxidase coupled LSD assay based on the formation of a chromogenic quinoneimine dye between 3,5-dichloro-2-hydroxybenzenesulfonic acid and 4-aminoantipyrine.[12] The results with this assay were inconclusive in our hands. An alternative method[13] based on the peroxidase catalyzed oxidation of 10-acetyl-3,7-dihydroxyphenoxazine (AmplexRed) to the fluorescent product resorufin however gave reliable and reproducible results. As a fluorescence-based method, it has intrinsically higher sensitivity and lower background than the colorimetric readouts. While this work was in progress, the Cole group has also noted[14] the improved sensitivity of the AmplexRed coupled assay and we recommend it as the preferred LSD assay for screening. As a secondary assay to eliminate false positives and artefacts, we find the mass spectrometric method described above to be a useful and rapid means of directly measuring substrate demethylation.

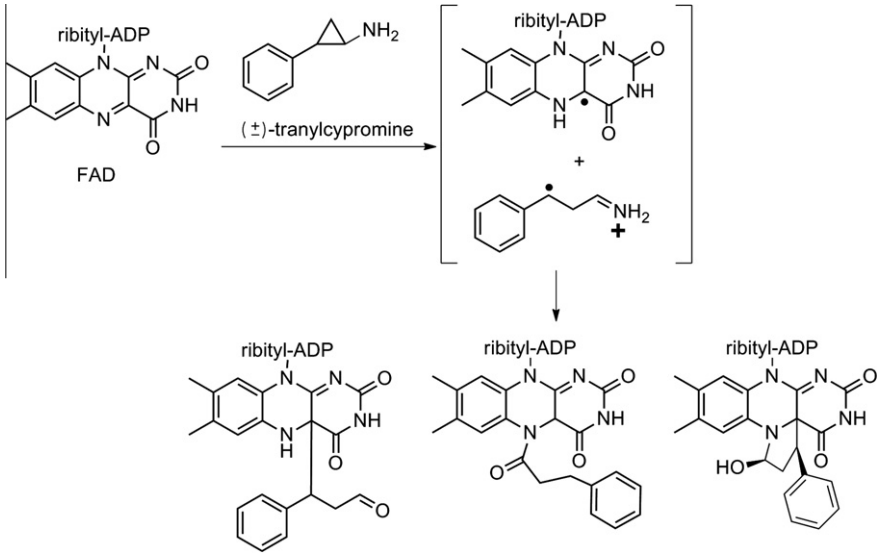

Figure 2. The mechanism of LSD inhibition by tranylcypromine.

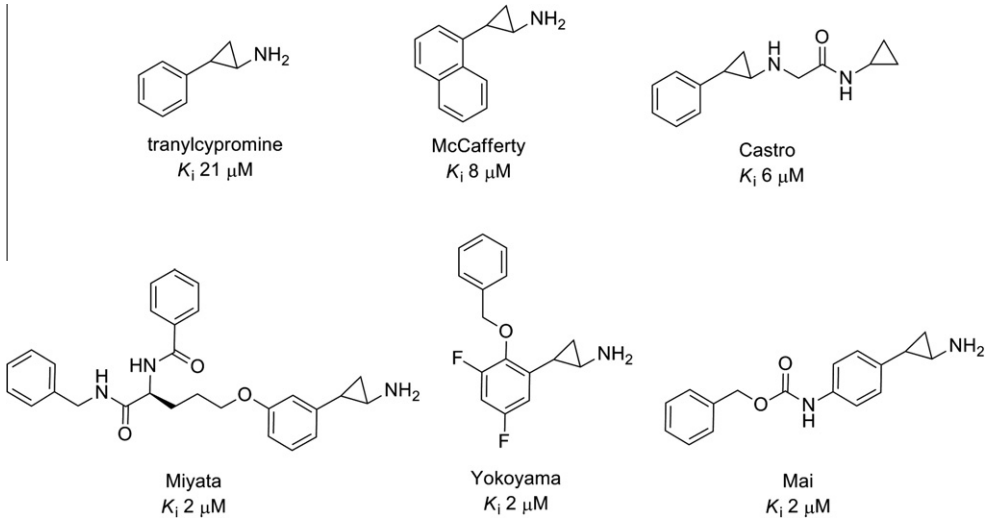

Figure 3. Examples of tranylcypromine analogues reported as LSD inhibitors. $K_{i(inact)}$ values are from the literature and not necessarily comparable due to differences in assay conditions.

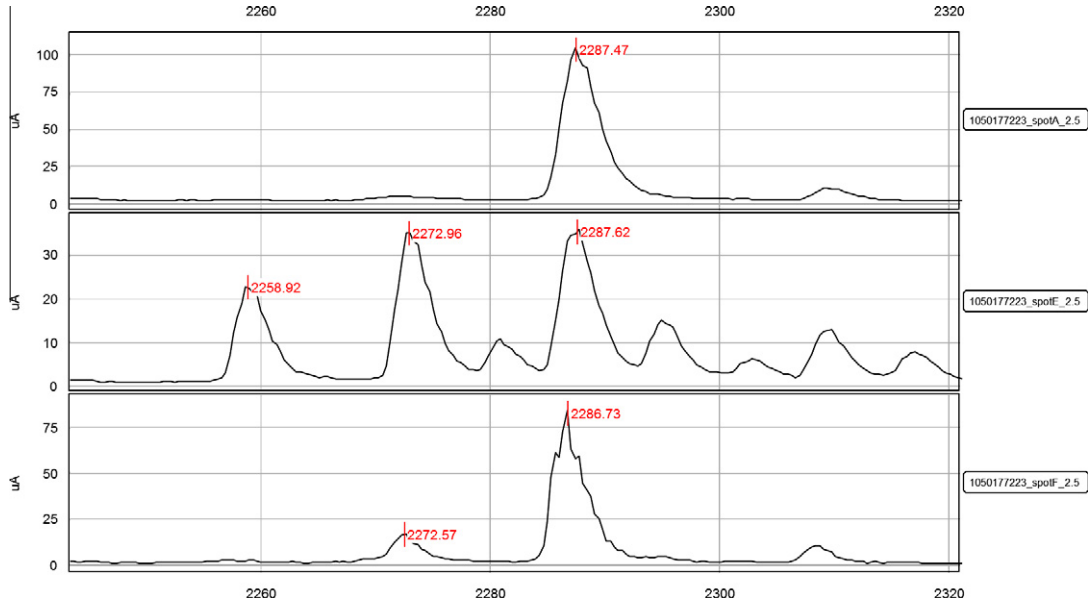

Figure 4. Mass spectrometric analysis of LSD1 activity with the synthetic peptide ARTK(me$_2$)QTARKSTGGKAPRKQLA. Due to a calibration error, all masses are M+5. *Top*: MS of peptide alone, MW 2283. *Middle*: MS of peptide after incubation with LSD1 showing monomethyl (MW 2269) and nonmethylated products (MW 2255). *Bottom*: MS of peptide incubated with LSD1 and inhibitor tranylcypromine.

2.2. Tranylcypromine stereochemistry

Four stereoisomers of 2-phenylcylopropylamine are possible. Of these, it is the racemate of the *trans* diastereomer which is used clinically as a MAO inhibitor. MAO inhibition was found to be relatively insensitive to stereochemistry, with the *trans* diastereomer being three fold more potent than the *cis*. Within the *trans* series, the (+) antipode with (1*R*,2*S*) stereochemistry was fourfold more potent than its enantiomer.[15] At the time of our studies, the situation with regards to LSD inhibition was unknown. We took racemic tranylcypromine and resolved[16] it into its two enantiomers which were individually tested (Fig. 5). The $K_{i(inact)}$ values observed are similar to those reported (21 μM) by Schmidt and McCafferty.[9b] Our results indicate a slightly higher activity for the (1*R*,2*S*) enantiomer, which is also the more active in MAO inhibition. While this manuscript was in preparation, Mai has reported[10c] that (+)-tranylcypromine has a $K_{i(inact)}$ of 284 μM compared to 168 μM for (−)-tranylcypromine and the two enantiomers form different adducts with FAD in the LSD active site. It is possible their assay underestimates activity as it uses the less sensitive colorimetric detection method. Furthermore, their results are based on truncated LSD1 lacking the N-terminal 184 amino acids whereas our assay uses the full length protein.

2.3. Enantioselective analogue synthesis

The reported syntheses of tranylcypromine analogues as LSD inhibitors are in racemic form. Although we found only a small difference in activity between the two tranylcypromine enantiomers, it was unclear if this would be further magnified in the case of analogues. For this reason, we designed an enantioselective route which would exclusively provide the (1*R*,2*S*) stereochemistry that

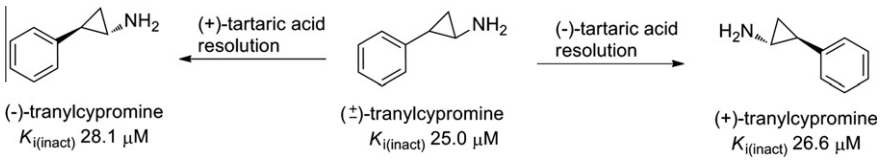

Figure 5. LSD1 inhibition by tranylcypromine as a racemate and single enantiomers.

was the more active of the two enantiomers. Our synthesis began with the copper-catalyzed cyclopropanation of styrenes by α-diazoesters. Pioneering work by Doyle, Pfaltz and Evans among others has led to chiral catalysts that provide the product with high enantioselectivity.[17] Evans, for example, reports the reaction between *tert*-butyl diazoacetate and styrene in the presence of chiral bisoxazoline ligands to give the cyclopropane in 81:19 *trans*:*cis* diastereoselectivity and 96% ee.[18] We repeated the procedure with substituted styrenes and isolated the pure *trans* cyclopropane esters by chromatography. Comparison of optical rotation with literature values indicated high enantioselectivity. These were then converted to the chiral tranylcypromine analogue in three steps by conversion of the ester to the acyl azide and Curtius rearrangement (Fig. 6).[19]

2.4. Biological evaluation

Our first three analogues **4a**–**c** probed the electronic effects of phenyl substitution. In the LSD enzyme assay (Table 1), both the *para*-methoxy and *para*-fluoro compounds were poorer inhibitors than tranylcypromine suggesting that strongly electron-donating or electron-withdrawing substituents are detrimental. On the other hand, the *para*-bromo analogue **4c** was a significantly better LSD inhibitor than tranylcypromine, a result that was independently reported by Mai.[10c] We then prepared the *meta*- and *ortho*- bromo isomer **4d**–**e** and these showed improved activity as well compared to tranylcypromine. The X-ray structure of LSD1 indicates a roomy active site compared to MAOs,[10] and our results demonstrate that substituents with the right electronic properties can be accommodated at *ortho*, *meta* and *para* positions.

We chose the bromo derivatives for a second reason, namely the ability to convert these compounds into additional analogues by palladium catalyzed crosscoupling reactions. This was demonstrated by Suzuki coupling to give biphenyl analogues **4f–h** (Fig. 7). While substitution of bromide by phenyl in the *para* position was well tolerated, the *meta* and *ortho* biphenyl analogues had low activity in the LSD enzyme assay.

Functional biology studies have indicated potential for inhibitors of LSD1 as anticancer therapeutics. For example, in prostate cancer cell lines, siRNA induced depletion of LSD1 attenuates its complex formation with the androgen receptor (AR) and subsequent AR mediated transcriptional activation and cell proliferation. Chemical inhibition of LSD1 in these models (using the MAO inhibitor pargyline) was also shown to block demethylation of repressive histone H3 lysine 9 (H3K9) marks and to inhibit androgen mediated AR dependent gene activation.[20] We therefore tested the in vivo effects of tranylcypromine and our analogues for their inhibitory effects in cell proliferation assays using the LNCaP prostate adenocarcinoma cell line (Table 1).

Tranylcypromine was a very poor inhibitor of the cell line in a six-day proliferation assay with an IC_{50} of 174 mM. The *meta*- and *para*-bromo analogues were significantly more potent micromolar inhibitors with a 1000 fold increase in activity compared to tranylcypromine. Surprisingly, all three biphenyl analogues were micromolar inhibitors of cell growth inhibition including the *meta*- and *ortho*- analogues with weak activity in the enzyme assay. The trends observed together with the low

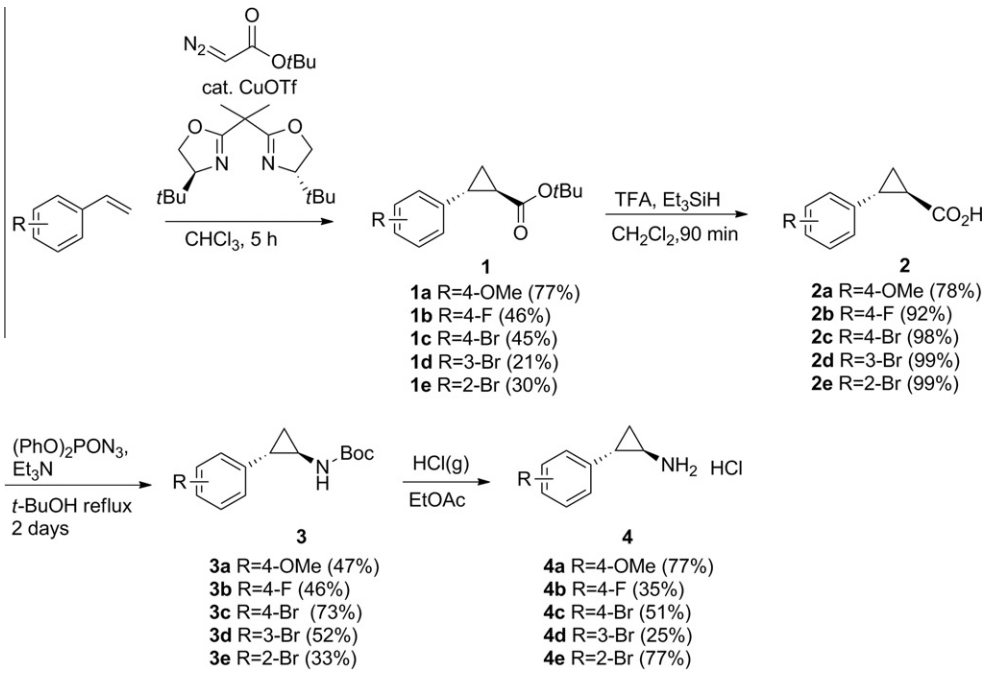

Figure 6. Enantioselective synthesis of tranylcypromine analogues.

Table 1
Activity of tranylcypromine analogues in LSD enzyme and LNCaP growth inhibition assays

Compound	Substitution R	LSD inhibition $K_{i(inact)}$ (μM)	LNCaP inhibition IC_{50} (μM)
(±)-Tranylcypromine	H	25.0 ± 9.5	>100,000
(+)-Tranylcypromine	H	26.6 ± 12.2	>100,000
(−)-Tranylcypromine	H	28.1 ± 12.9	>100,000
4a	4-OMe	41.8 ± 1.0	1706 ± 120
4b	4-F	79.6 ± 1.0	>100,000
4c	4-Br	3.7 ± 0.5	111 ± 103
4d	3-Br	8.9 ± 3.2	129 ± 3
4e	2-Br	11.7 ± 0.9	5869 ± 470
4f	4-Ph	6.8 ± 0.3	110 ± 31
4g	3-Ph	>100	226 ± 72
4h	2-Ph	>100	181 ± 6

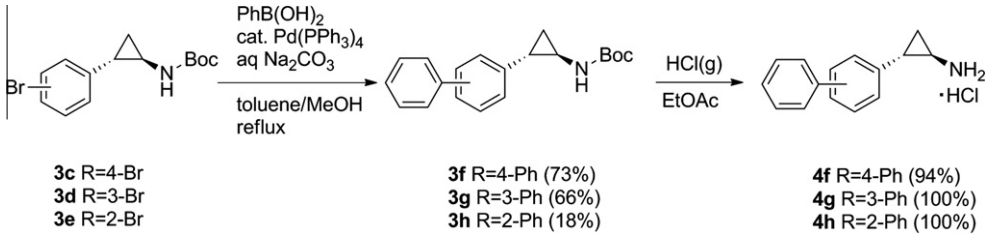

Figure 7. Synthesis of phenyltranylcypromine by Suzuki crosscoupling.

growth inhibition of tranylcypromine itself suggest that activity in the cell assay is driven by permeability and lipophilicity. Thus, the more lipophilic biphenyl analogues retain activity despite being poor in the enzyme assay. *ortho*-Bromide **4e** is an outlier as its cell growth inhibition is much lower compared to the isomeric bromides.

3. Conclusions

We report the first enantioselective synthesis of tranylcypromine analogues designed as LSD inhibitors. Our results indicate very little difference between the two enantiomers of tranylcypromine in LSD1 inhibition. The bromo substituted tranylcypromine analogues **4c–e** were superior to tranylcypromine in the enzyme assay regardless of the position of bromination, with the *para*-bromo derivative showing the highest activity. We have prepared the biphenyl derivatives **4f–h** and show that the *meta* or *ortho* analogues are relatively inactive while the *para* analogue is similar in activity to the bromide. We report the first observations of tranylcypromine analogues as single agents in cancer cell growth inhibition. In the LNCaP prostate cancer cell line, **4c** and **4f** are over 1000 fold more potent than tranylcypromine. Work is in progress to prepare further tranylcypromine analogues with a view to improving cellular potency and selectivity.

4. Experimental

4.1. Expression and purification of full length human LSD1

The plasmid pet15b-His-tagged full length human LSD1 was kindly provided by Fei Lan in Dr. Yang Shi's laboratory.[2] The plasmid was transformed into BL21 RIPL Codon Plus (DE3) bacteria (Stratagene) and protein expression was induced with 0.1 mM IPTG for 3 h at room temperature. The bacterial pellet was lysed (40 mM Tris–HCl pH 8.0, 300 mM NaCl, 0.2 % Triton X100, 5% glycerol, 10 μg/ml DNase I and 10 mM $MgCl_2$ in the presence of protease inhibitors), sonicated on ice and centrifuged. The supernatant was used to purify LSD1 recombinant protein using an AKTA Prime FPLC system. Three chromatography steps (Nickel, gel filtration and Q-sepharose columns from GE-Healthcare) were successively performed and the purified protein was stored in 20 mM Tris–HCl pH 8.0, 300 mM NaCl and 5% glycerol at −70 °C.

4.2. Fluorescence based LSD1 enzyme assay

LSD1 enzymatic activity was measured with a synthetic peptide corresponding to the first 21 amino acids of human histone H3 dimethylated on lysine 4: ARTK(me_2)QTARKSTGGKAPRKQLA (BPS Bioscience). Assays were carried out in a final volume of 100 μL in white 96 microtitre plates (Greiner).

An aliquot of 1.25 μg of recombinant LSD1 enzyme was added to a 50 μL reaction containing 35 μM of peptide substrate in 50 mM potassium phosphate pH 7.2 and the reaction mixture was incubated at room temperature for 20 min. Fifty microliter of a mix containing 0.5 μL of 10 mM AmplexRed (InVitrogen) and 1 μL of 10 U/ml HRP (Sigma) in 50 mM potassium phosphate buffer were added and the reaction mixture kept at room temperature for another 30 min before quenching by adding 20 μL of AmplexRed Stop solution (InVitrogen). Fluorescence was measured at 530/590 nm on a Varioskan Flash microplate reader (Thermo Fisher). Under these conditions, a K_m of 21 μM was observed for the peptide substrate. For assays with inhibitors, the compound was incubated with LSD1 for 10 min prior to the addition of the peptide substrate. $K_{i(inact)}$ values are means determined from a minimum of three dose–response curves for each compound.

4.3. Mass spectrometric LSD1 enzyme assay

In a total volume of 50 μL, 50 μM of tranylcypromine was incubated alone or in the presence of 2.4 μg of LSD1 at room temperature for 10 min.

The peptide ARTK(me_2)QTARKSTGGKAPRKQLA was added at a concentration of 35 μM and the reaction was carried out for an extra 20 min at room temperature. The samples were kept on ice before mass spectrometric analysis, carried out with NP20 ProteinChip (BioRad) arrays.

An NP20 array was pre-rinsed with 5 μL of ultrapure H_2O and 3 μL sample was subsequently applied on each spot. The

sample-loaded arrays were then incubated for 15 min in a humidity chamber at room temperature. Then, 1 μL of energy absorbing matrix [EAM; α-cyano-4-hydroxycinnamic acid (CHCA) in 200 μL acetonitrile and 200 μL of 1% TFA] was added to each spot, air dried and reapplied. ProteinChips were read using a PBS II SELDI mass spectrometer (Bio-Rad). Data acquisition was performed using the following parameters: Mass range between 0.5 and 20 kDa with a focus mass of 3 kDa and detector blinding at 0.5 kDa. The laser settings used were: intensity of 3,000nj and 18 shots, 2 warming shots at an intensity of 3,300nj, acquisition of 1 shot every four pixels in a randomized fashion.

4.4. LNCaP cell growth inhibition assay

Cell proliferation assays were undertaken using the LNCaP prostate adenocarcinoma cell line to produce IC_{50} values for inhibition as previously described.[21] Experiments are mean values +/− SEM determined from a minimum of two experiments for each compound.

4.5. Compound synthesis

4.5.1. Styrene asymmetric cyclopropanation to give 1a–1e

(*S*,*S*)-2,2′-Isopropylidene-bis(4-*tert*-butyl-2-oxazoline) (0.01 equiv) and copper(II) trifluoromethanesulfonate (0.01 equiv) were dissolved in chloroform and stirred for 45 min under argon. Styrene derivatives (5 equiv) and *tert*-butyl diazoacetate (1 equiv) were then added and the reaction mixture was stirred for 5 h, followed by concentration in vacuo and purification of the crude material by flash chromatography using CH_2Cl_2/hexane (2/8) to provide the pure *trans*-diastereoisomers.

4.5.1.1. (*R*,*R*)-*tert*-Butyl 2-(4-methoxyphenyl)cyclopropanecarboxylate (1a)[22]. $[\alpha]_D^{23}$ −192.2 (*c* 0.24, $CHCl_3$), [lit.[23] $[\alpha]_D^{24}$ −241.4 (*c* 1.0, $CHCl_3$)]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (m, 1H), 1.48 (s, 9H), 1.50–1.52 (m, 1H), 1.76 (dt, *J* = 8.7, 4.6 Hz, 1H), 2.38–2.44 (m, 1H), 3.79 (s, 3H), 6.83 (dd, *J* = 9.0, 0.4 Hz, 2H), 7.03 (dd, *J* = 8.6, 0.3 Hz, 2H); ES$^+$ MS *m*/*z* 303 ([M+Na+MeOH]$^+$). Yield 77%.

4.5.1.2. (*R*,*R*)-*tert*-Butyl 2-(4-fluorophenyl)cyclopropanecarboxylate (1b)[22]. $[\alpha]_D^{25}$ −213.9 (*c* 0.44, $CHCl_3$), [lit.[22] $[\alpha]_D^{20}$ −182 (*c* 0.64, $CHCl_3$)]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.19 (ddd, *J* = 8.4, 6.4, 4.5 Hz, 1H), 1.48 (s, 9H), 1.52 (dt, *J* = 4.6, 0.8 Hz, 1H), 1.78 (ddd, *J* = 8.5, 5.3, 4.2 Hz, 1H), 2.43 (ddd, *J* = 9.2, 6.5, 4.1 Hz, 1H), 6.93–7.01 (m, 2H), 7.02–7.10 (m, 2H). Yield 46%.

4.5.1.3. (*R*,*R*)-*tert*-Butyl 2-(4-bromophenyl)cyclopropanecarboxylate (1c)[23]. $[\alpha]_D^{25}$ −196.1 (*c* 0.41, $CHCl_3$), [lit.[23] $[\alpha]_D^{24}$ −216.7 (*c* 1.00, $CHCl_3$)]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20 (ddd, *J* = 8.5, 6.4, 4.5 Hz, 1H), 1.47 (s, 9H), 1.54 (td, *J* = 4.6, 0.8 Hz, 1H), 1.80 (ddd, *J* = 8.4, 5.4, 4.2 Hz, 1H), 2.40 (ddd, *J* = 9.2, 6.3, 4.1 Hz, 1H), 6.70 (d, *J* = 8.2 Hz, 2H), 7.02–7.10 (d, *J* = 8.5 Hz, 2H). Yield 45%.

4.5.1.4. (*R*,*R*)-*tert*-Butyl 2-(3-bromophenyl)cyclopropanecarboxylate (1d)[24]. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22 (ddd, *J* = 8.5, 6.3, 4.6 Hz, 1H), 1.48 (s, 9H), 1.53 (dd, *J* = 9.5, 5.1 Hz, 1H), 1.83 (ddd, *J* = 8.4, 5.3, 4.2 Hz, 1H), 2.41 (ddd, *J* = 9.2, 6.2, 4.0 Hz, 1H), 7.03 (dt, *J* = 7.7, 1.3 Hz, 1H), 7.14 (t, *J* = 7.9 Hz, 1H), 7.23 (t, *J* = 1.8 Hz, 1H), 7.33 (dt, *J* = 7.7, 1.5 Hz, 1H). Yield 21%.

4.5.1.5. (*R*,*R*)-*tert*-Butyl 2-(2-bromophenyl)cyclopropanecarboxylate (1e). Colorless oil; $[\alpha]_D^{25}$ −66.3 (*c* 0.27, CH_3OH); IR 3060, 1716, 1442 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (ddd, *J* = 8.4, 6.7, 4.6 Hz, 1H), 1.50 (s, 9H), 1.54–1.59 (m, 1H), 1.69 (ddd, *J* = 8.4, 5.2, 4.6 Hz, 1H), 2.64 (ddd, *J* = 9.0, 6.7, 4.5 Hz, 1H), 7.01 (dd, *J* = 7.7, 1.4 Hz, 1H), 7.09 (dt, *J* = 7.7, 1.6 Hz, 1H), 7.20–7.26 (m, 1H), 7.57 (dd, *J* = 7.9, 1.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.1, 24.3, 26.8, 28.2, 80.6, 126.4, 127.3, 127.4, 128.0, 132.6, 139.4, 172.4; EI MS *m*/*z* 240, 242 ([M+-*t*Bu]). Yield 30%.

4.5.2. Ester hydrolysis of 1 to 2

The carboxylate esters **1** (1 equiv), TFA (13 equiv) and triethylsilane (2.5 equiv) were taken up in CH_2Cl_2. The reaction mixture was stirred for 90 min followed by concentration in vacuo. The crude material was used with no further purification or purified by flash chromatography using CH_2Cl_2 as eluent if required to provide **2**.

4.5.2.1. (*R*,*R*)-2-(4-Methoxyphenyl)cyclopropane carboxylic acid (2a)[19a]. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37 (ddd, *J* = 8.3, 6.7, 4.5 Hz, 1H), 1.63 (dt, *J* = 9.4, 4.8 Hz, 1H), 1.84 (ddd, *J* = 8.3, 5.1, 4.2 Hz, 1H), 2.58 (ddd, *J* = 9.2, 6.6, 4.2 Hz, 1H), 3.80 (s, 3H), 6.81–6.87 (m, 2H), 7.03–7.09 (m, 2H). Yield 78%.

4.5.2.2. (*R*,*R*)-2-(4-Fluorophenyl)cyclopropane carboxylic acid (2b)[19a]. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.53 (ddd, *J* = 8.4, 6.7, 4.7 Hz, 1H), 1.82 (dt, *J* = 9.4, 4.9 Hz, 1H), 1.96–2.08 (m, 1H), 2.71–2.82 (m, 1H), 7.08–7.20 (m, 2H), 7.20–7.32 (m, 2H), 10.83 (br s, 1H). Yield 92%.

4.5.2.3. (*R*,*R*)-2-(4-Bromophenyl)cyclopropanecarboxylic acid (2c)[25]. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (ddd, *J* = 8.4, 6.6, 4.8 Hz, 1H), 1.68 (dt, *J* = 9.4, 5.0 Hz, 1H), 1.88 (ddd, *J* = 8.5, 5.2, 4.2 Hz, 1H), 2.57 (ddd, *J* = 9.2, 6.6, 4.0 Hz, 1H), 6.99 (d, *J* = 8.4 Hz, 2H), 7.42 (d, *J* = 8.4 Hz, 2H). Yield 98%.

4.5.2.4. (*R*,*R*)-2-(3-Bromophenyl)cyclopropanecarboxylic acid (2d)[26]. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (ddd, *J* = 8.4, 6.8, 4.6 Hz, 1H), 1.59 (m, 1H), 1.91 (ddd, *J* = 8.5, 5.2, 4.2 Hz, 1H), 2.58 (ddd, *J* = 9.2, 6.6, 4.0 Hz, 1H), 7.05 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.17 (t, *J* = 7.7 Hz, 1H), 7.25–7.28 (m, 1H), 7.33–7.40 (m, 1H). Yield 99%.

4.5.2.5. (*R*,*R*)-2-(2-Bromophenyl)cyclopropanecarboxylic acid (2e)[27]. $[\alpha]_D^{25}$ −76.4 (*c* 0.29, $CHCl_3$), [lit.[26] (*S*,*S*)-enantiomer $[\alpha]_D$ +109.9 (*c* 0.77, $CHCl_3$)]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 (ddd, *J* = 8.4, 7.0, 4.8 Hz, 1H), 1.71 (dt, *J* = 9.2, 4.7 Hz, 1H), 1.77–1.90 (m, 1H), 2.80 (ddd, *J* = 9.2, 7.0, 4.4 Hz, 1H), 7.05 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.12 (dt, *J* = 7.6, 1.7 Hz, 1H), 7.20–7.26 (m, 1H), 7.59 (dd, *J* = 7.9, 1.3 Hz, 1H). Yield 99%.

4.5.3. Curtius rearrangement of 2 to 3

The acids **2** (1 equiv), diphenylphosphoryl azide (1.1 equiv) and triethylamine (1.5 equiv) were combined in *tert*-butanol under argon and heated at reflux for 48 h. The mixture was diluted with EtOAc and saturated Na_2CO_3 solution. The organic layer was separated and the aqueous layer was extracted with EtOAc. The organics layers were combined, dried over $MgSO_4$ and concentrated in vacuo. The crude material was purified by flash chromatography (EtOAc/hexane 2/8) to provide the desired carbamates **3**.

4.5.3.1. (1*R*,2*S*)-*tert*-Butyl 2-(4-methoxyphenyl)cyclopropylcarbamate (3a)[19a]. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.03–1.16 (m, 2H), 1.48 (s, 9H), 1.95–2.05 (m, 1H), 2.64–2.70 (m, 1H), 3.78 (s, 3H), 4.91 (br s, 1H), 6.76–6.90 (m, 2H), 7.05–7.15 (m, 2H). Yield 47%.

4.5.3.2. (1*R*,2*S*)-*tert*-Butyl 2-(4-fluorophenyl)cyclopropylcarbamate (3b)[19a]. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.04–1.19 (m, 2H), 1.46 (s, 9H), 1.98–2.12 (m, 1H), 2.60–2.76 (m, 1H), 4.83 (br s, 1H), 6.88–7.01 (m, 2H), 7.11–7.15 (m, 2H). Yield 46%.

4.5.3.3. (1*R*,2*S*)-*tert*-Butyl 2-(4-bromophenyl)cyclopropylcarbamate (3c)[28]. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.08–1.19 (m, 2H),

1.46 (s, 9H), 1.94–2.09 (m, 1H), 2.64–2.73 (m, 1H), 4.82 (br s, 1H), 7.03 (d, J = 8.3 Hz, 2H), 7.38 (d, J = 8.3 Hz, 2H); ES^+ MS m/z 334, 336 ($[M+Na]^+$). Yield 73%.

4.5.3.4. (1*R*,2*S*)-*tert*-Butyl 2-(3-bromophenyl)cyclopropylcarbamate (3d)[29]. ^1H NMR (300 MHz, $CDCl_3$) δ 1.13–1.20 (m, 2H), 1.46 (s, 9H), 2.02 (td, J = 7.8, 3.0 Hz, 1H), 2.69–2.77 (m, 1H), 4.82 (br s, 1H), 7.04–7.10 (m, 1H), 7.11–7.16 (m, 1H), 7.31 (m, 1H), 7.37–7.43 (m, 1H); ES^+ MS m/z 334, 336 ($[M+Na]^+$). Yield 52%.

4.5.3.5. (1*R*,2*S*)-*tert*-Butyl 2-(2-bromophenyl)cyclopropylcarbamate (3e)[30]. ^1H NMR (300 MHz, $CDCl_3$) δ 1.14–1.40 (m, 2H), 1.52 (s, 9H), 2.09–2.34 (m, 1H), 2.65–2.90 (m, 1H), 5.05 (br s, 1H), 7.04–7.18 (m, 2H), 7.22–7.36 (m, 1H), 7.53–7.64 (m, 1H); ES^+ MS m/z 334, 336 ($[M+Na]^+$). Yield 33%.

4.5.4. Suzuki coupling of 3c–e to 3f–h

To a solution of the aryl bromide in a mixture of toluene/methanol/water (80/18/2) were added tetrakis(triphenylphosphine)palladium (0.2 equiv), phenylboronic acid (4 equiv), and Na_2CO_3 (2 equiv). The reaction mixture was refluxed overnight. The mixture was then diluted with EtOAc, washed with water and brine and purified by flash chromatography (EtOAc/hexane 5/95) to provide the biphenyl product.

4.5.4.1. (1*R*,2*S*)-*tert*-Butyl-2-(biphenyl-4-yl)cyclopropylcarbamate (3f). Mp 98–100 °C; $[\alpha]_D^{23}$ −99.4 (c 0.25, $CHCl_3$); IR 3338, 1687, 1523, 1483 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.08–1.33 (m, 2H), 1.48 (s, 9H), 2.09 (ddd, J = 9.3, 6.4, 3.1 Hz, 1H), 2.77 (m, J = 15.6 Hz, 1H), 4.88 (br s, 1H), 7.21 (d, J = 8.1 Hz, 2H), 7.35 (d, J = 7.4 Hz, 1H), 7.44 (t, J = 7.5 Hz, 2H), 7.48–7.61 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.4, 24.8, 28.4, 32.6, 79.7, 126.8, 126.9, 127.0, 128.7, 139.0, 139.9, 141.0, 156.3; ES^+ MS m/z 332 ($[M+Na]^+$); HRMS (ESI) m/z calcd for $C_{20}H_{23}NNaO_2$ $(M+Na)^+$ 332.1621, found 332.1616. Yield 73%.

4.5.4.2. (1*R*,2*S*)-*tert*-Butyl-2-(biphenyl-3-yl)cyclopropylcarbamate (3g). Mp 68–70 °C; $[\alpha]_D^{25}$ −50.8 (c 0.52, CH_3OH); IR 3330, 1687, 1511, 1483 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.13–1.32 (m, 2H), 1.48 (s, 9H), 2.12 (ddd, J = 9.5, 6.4, 3.3 Hz, 1H), 2.73–2.89 (m, 1H), 4.87 (br s, 1H), 7.13 (d, J = 7.6 Hz, 1H), 7.31–7.38 (m, 3H), 7.39–7.50 (m, 3H), 7.52–7.67 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.6, 25.1, 28.4, 32.6, 79.7, 125.0, 125.3, 125.4, 127.19, 127.23, 128.69, 128.73, 141.2, 141.3, 156.2; ES^+ MS m/z 373 ($[M+Na+CH_3CN]^+$); HRMS (ESI) m/z calcd for $C_{20}H_{23}NNaO_2$ $(M+Na)^+$ 332.1621, found 332.1628. Yield 66%.

4.5.4.3. (1*R*,2*S*)-*tert*-Butyl-2-(biphenyl-2-yl)cyclopropylcarbamate (3h). Mp 64–66 °C; IR 3338, 1696, 1480 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.98–1.08 (m, 1H), 1.15 (q, J = 6.1 Hz, 1H), 1.46 (s, 9H), 2.00 (ddd, J = 9.9, 6.3, 3.0 Hz, 1H), 2.62–2.87 (m, 1H), 4.56 (br s, 1H), 7.04 (d, J = 7.1 Hz, 1H), 7.20–7.33 (m, 3H), 7.38 (dq, J = 8.8, 4.3 Hz, 1H), 7.45 (d, J = 5.1 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.0, 23.3, 28.3, 33.2, 79.5, 125.0, 125.9, 126.9, 127.5, 128.0, 129.6, 129.7, 137.8, 141.6, 142.5, 156.2; ES^+ MS m/z 373 ($[M+Na+CH_3CN]^+$); HRMS (ESI) m/z calcd for $C_{20}H_{23}NNaO_2$ $(M+Na)^+$ 332.1621, found 332.1626. Yield 18%.

4.5.5. Boc deprotection of 3 to 4

Carbamate derivative **3** was taken up in anhydrous EtOAc cooled at 0 °C and $HCl_{(g)}$ was bubbled into the solution for 40–90 min. The reaction was monitored by TLC. The resulting HCl salt was filtered and washed with diethyl ether.

4.5.5.1. (1*R*,2*S*)-2-(4-Methoxyphenyl)cyclopropanamine hydrochloride (4a)[19a]. ^1H NMR (300 MHz, CD_3OD) δ 1.18–1.31 (m, 1H), 1.32–1.43 (m, 1H), 2.28–2.41 (m, 1H), 2.76 (dt, J = 7.7, 3.7 Hz, 1H), 3.75 (s, 3H), 6.82–6.91 (m, 2H), 7.04–7.16 (m, 2H); HRMS (ESI) m/z calcd for $C_{10}H_{14}NO$ $(M+H)^+$ 164.1070, found 164.1074. Yield 77%.

4.5.5.2. (1*R*,2*S*)-2-(4-Fluorophenyl)cyclopropanamine hydrochloride (4b)[19a]. ^1H NMR (300 MHz, CD_3OD) δ 1.30 (dt, J = 7.8, 6.7 Hz, 1H), 1.42 (ddd, J = 10.2, 6.7, 4.4 Hz, 1H), 2.39 (ddd, J = 10.1, 6.5, 3.6 Hz, 1H), 2.82 (ddd, J = 7.9, 4.4, 3.6 Hz, 1H), 6.96–7.10 (m, 2H), 7.14–7.25 (m, 2H); ES^+ MS m/z 193 ($[M+H+CH_3CN]^+$); HRMS (ESI) m/z calcd for $C_9H_{11}FN$ $(M+H)^+$ 152.0870, found 152.0870. Yield 35%.

4.5.5.3. (1*R*,2*S*)-2-(4-Bromophenyl)cyclopropanamine hydrochloride (4c)[29]. ^1H NMR (300 MHz, CD_3OD) δ 1.39 (m, 1H), 1.49 (ddd, J = 10.3, 6.8, 4.5 Hz, 1H), 2.42 (ddd, J = 10.3, 6.5, 3.8 Hz, 1H), 2.90 (dt, J = 7.9, 3.8 Hz, 1H), 7.16 (d, J = 8.5 Hz, 2H), 7.51 (d, J = 8.5 Hz, 2H); ES^+ MS m/z 253, 255 ($[M+H+CH_3CN]^+$); HRMS (ESI) m/z calcd for $C_9H_{11}BrN$ $(M+H)^+$ 212.0069, found 212.0073. Yield 51%.

4.5.5.4. (1*R*,2*S*)-2-(3-Bromophenyl)cyclopropanamine hydrochloride (4d)[29]. ^1H NMR (300 MHz, CD_3OD) δ 1.41 (q, J = 7.0 Hz, 1H), 1.49 (ddd, J = 10.3, 6.3, 4.5 Hz, 1H), 2.42 (ddd, J = 10.0, 6.5, 3.5 Hz, 1H), 2.93 (dt, J = 8.0, 4.0 Hz, 1H), 7.17–7.23 (m, 1H), 7.29 (t, J = 7.8 Hz, 1H), 7.40–7.48 (m, 2H); ES^+ MS m/z 253, 255 ($[M+H+CH_3CN]^+$); HRMS (ESI) m/z calcd for $C_9H_{11}BrN$ $(M+H)^+$ 212.0069, found 212.0072. Yield 25%.

4.5.5.5. (1*R*,2*S*)-2-(2-Bromophenyl)cyclopropanamine hydrochloride (4e)[30]. ^1H NMR (300 MHz, CD_3OD) δ ppm 1.35–1.62 (m, 2H), 2.53–2.73 (m, 1H), 2.79–2.96 (m, 1H), 7.09–7.26 (m, 2H), 7.27–7.42 (m, 1H), 7.63 (d, J = 7.9 Hz, 1H). Yield 77%.

4.5.5.6. (1*R*,2*S*)-2-(Biphenyl-4-yl)cyclopropanamine hydrochloride (4f)[31]. ^1H NMR (300 MHz, CD_3OD) δ 1.33–1.54 (m, 2H), 2.44 (ddd, J = 10.1, 6.7, 3.6 Hz, 1H), 2.91 (dt, J = 7.7, 4.1 Hz, 1H), 7.21–7.39 (m, 3H), 7.44 (t, J = 7.5 Hz, 2H), 7.53–7.70 (m, 4H); ES^+ MS m/z 251 ($[M+H+CH_3CN]^+$); HRMS (ESI) m/z calcd for $C_{15}H_{16}N$ $(M+H)^+$ 210.1277, found 210.1282. Yield 94%.

4.5.5.7. (1*R*,2*S*)-2-(Biphenyl-3-yl)cyclopropanamine hydrochloride (4g)[31]. ^1H NMR (300 MHz, CD_3OD) δ 1.38–1.58 (m, 2H), 2.50 (ddd, J = 10.1, 6.7, 3.6 Hz, 1H), 2.95 (dt, J = 7.7, 4.1 Hz, 1H), 7.18 (d, J = 7.5 Hz, 1H), 7.32–7.56 (m, 6H), 7.56–7.73 (m, 2H); ES^+ MS m/z 210 ($[M+H]^+$), 251 ($[M+H+CH_3CN]^+$); HRMS (ESI) m/z calcd for $C_{15}H_{16}N$ $(M+H)^+$ 210.1277, found 210.1281. Yield 100%.

4.5.5.8. (1*R*,2*S*)-2-(Biphenyl-2-yl)cyclopropanamine hydrochloride (4h). Mp 52–54 °C; $[\alpha]_D^{25}$ −15.3 (c 0.07, CH_3OH); IR 1597, 1487, 1454 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 1.19–1.29 (m, 2H), 2.39 (td, J = 8.6, 3.5 Hz, 1H), 2.87–3.03 (m, 1H), 6.98–7.12 (m, 1H), 7.26–7.56 (m, 8H); ^{13}C NMR (100 MHz) δ 14.8, 21.0, 32.3, 125.4, 127.7, 128.3, 128.9, 129.5, 130.5, 130.9, 136.7, 142.5, 143.9; ES^+ MS m/z 210 ($[M+H]^+$), 251 ($[M+H+CH_3CN]^+$); HRMS (ESI) m/z calcd for $C_{15}H_{16}N$ $(M+H)^+$ 210.1277, found 210.1279. Yield 100%.

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Supplementary data

Supplementary data (^{1}H and ^{13}C NMR spectra for novel compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.02.017.

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