



ORIGINAL ARTICLE

# Cysteine-based 3-substituted 1,5-benzoxathiepin derivatives: Two new classes of anti-proliferative agents



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**Abstract** Two distinct series of the 3-amino-1,5-benzoxathiepin scaffold, derived from L-cysteine, were synthesized and evaluated for their anti-proliferative activity in the breast cancer MDA-MB-231 and MCF-7 cells, and in the ovarian carcinoma SKOV-3 cell line. (3*R*)-Amino-3,4-dihydro-2*H*-1,5-benzoxathiepin [(*R*)-**10**] was diversified into two forms: (a) by incorporating different amino acids at its position 3, through an amide bond; and (b) by construction of the purine ring to give 6-chloro-9-[2-(3,4-dihydro-2*H*-1,5-benzoxathiepin-(3*R*)-yl)]-9*H*-purine [(*R*)-**28**]. Nevertheless, when the introduction of iodine was tried at position 2 of the purine ring of (*R*)-**28**, 2-[[2-(6-chloro-2-iodo-9*H*-purin-9-yl)prop-2-en-1-yl]thio]phenol (**34**) was obtained. Compound **34** shows activity against cancer cells. Interestingly, **34** inhibits mammosphere formation at the micromolar range,

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demonstrating activity against cancer stem cells. Although further studies of its targets and mechanism of action are needed, these findings support the therapeutic potential of this compound in cancer.

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## 1. Introduction

It is well known that cancer continues to be a major health problem in developing as well as undeveloped countries. Although major advances have been made in the chemotherapeutic management of some patients, the continued commitment to the laborious task of discovering new anticancer agents remains critically important, in the course of identifying various chemical substances which may serve as a lead for designing novel anti-tumor agents.

Breast cancer still remains as the second most common cancer worldwide. Although chemotherapy and radiotherapy can effectively reduce tumor mass and provide temporary remission, relapse occurs in many cases. This is due to an intrinsically chemotherapy-resistant subpopulation of cells with a strong ability for self-renewal, known as cancer stem cells (CSCs) (Li et al., 2008). This subset of cells may also contribute to tumor initiation, progression and metastasis. Thus, drugs that target CSCs offer great promise for cancer treatment, particularly in combination with chemotherapy (García-Rubiño et al., 2016).

The design, synthesis and biological evaluation of a series of 2- and 6-disubstituted (*RS*)-9-(2,3-dihydro-1,4-benzoxathiin-3-ylmethyl)-9*H*-purine derivatives and the most active compounds as anti-proliferative agents were **1** and **2** (Fig. 1) (Díaz-Gavilán et al., 2008).

We have also reported the synthesis and anti-proliferative activity against the human breast cancer cell line MCF-7 of a series of substituted (*RS*)-9-(2,3-dihydro-1,4-benzoxathiin-2-ylmethyl)-9*H*-purines **12–14**. The most active compound (*RS*)-2,6-dichloro-9-(2,3-dihydro-1,4-benzoxathiin-2-ylmethyl)-9*H*-purine (**5**) shows an  $IC_{50} = 2.75 \pm 0.02 \mu\text{M}$  (Fig. 1) (Conejo-García et al., 2011).

The issue of chiral drug is now a major theme in the design, discovery and development of new drugs. It has been shown that for many pharmaceuticals only one enantiomer contains the desired activity, and the synthesis of such drug molecules in their optically pure form is becoming increasingly important. We have recently reported an efficient enantiospecific synthesis of the (*R*)- and (*S*)-9-(2,3-dihydro-1,4-benzoxathiin-2 and 3-ylmethyl)-9*H*-purines **3–5** under microwave (MW) irradiation, together with the mechanism and their corresponding anti-tumor activity against the human breast cancer cell lines MCF-7 and SKBR-3. All homochiral compounds included in this study show different apoptotic effects between both enantiomers (García-Rubiño et al., 2013).

Preparation has been developed for alkylated aminopurines with the (*R*)- and (*S*)-3,4-dihydro-2*H*-1,5-benzoxathiepin-3-ol moieties by the Mitsunobu reaction under microwave-assisted conditions. It reveals a complete inversion of the stereogenic center of the secondary alcohol giving an alkylated adenine linked to a homochiral six-membered ring (García-Rubiño et al., 2014). In both publications, when (*RS*)-3-hydroxy-3,4-dihydro-2*H*-1,5-benzoxathiepin reacts with several purines under the Mitsunobu conditions, a contraction of the seven-membered ring takes place into the six-membered one. The reactions deviate from the normal behavior because the sulfur atom competes as an alternative nucleophile, through an intermediate episulfonium ion (García-Rubiño et al., 2014, 2013).

The secondary hydroxyl group was previously transformed into the hydroxyethyleneoxy or hydroxypropyleneoxy fragments. This allowed the separation of a newly generated primary hydroxyl group of the seven-membered cycle in order to prevent the *S*-3 participation of neighboring group and concomitant contraction of the ring to a six-membered cycle. With this objective, we published the synthesis and

anti-tumor activity of a series of (*RS*)-9-[3-(3,4-dihydro-2*H*-1,5-benzoxathiepin-3-yloxy)alkyl]-9*H*-purines **6–8**. The most active compounds are **7** and **8** with  $IC_{50} = 6.67 \pm 0.06 \mu\text{M}$ , and  $5.14 \pm 0.06 \mu\text{M}$ , respectively (Fig. 1). Our results demonstrate that the anti-proliferative activities displayed by compounds **6–8** against the MCF-7 human breast cancer cells are due to the inhibition of protein synthesis by the eIF-2 $\alpha$  phosphorylation and inhibition of the PI3K pathway (Kimatrai et al., 2011).

Benzoxazepins are well-known pharmacophores in medicinal chemistry showing a promising activity against various diseases such as psychosis with a central nervous system activity along with an anti-cancer profile against breast cancer cells (Liao et al., 1999; James et al., 1993; Mulligan et al., 2006). One of these groups of benzoxazepins has been identified as a target for the microtubule assembly in order to induce anticancer activity (Mulligan et al., 2006). However, amino acid-based benzoxazepins were little explored to evaluate their pharmacological activity. Syntheses of amino acid-based polycycles were reported (Mishra et al., 2007a; Mishra and Panda, 2007b, 2005). Easy availability of amino acid-based benzoxazepins promoted Samanta et al. (2010) to evaluate them for anticancer breast activity, and they designed amino acid-derived benzoxazepin derivatives with alkyl amino ethyl chains and evaluated anti-tumor activity in human breast cancer cells and a xenograft model. The most active compound was (*S*)-**9** (Fig. 1) (Samanta et al., 2010).

In an attempt to further explore the biological properties of benzoxathiepins as active pharmacophores, we propose herein to prepare two different structural types: (a) a new kind of L-cysteine-based 1,5-benzoxathiepin derivatives with an amino group at position 3 of the heterocycle with an *S*-configuration, and (b) a purine linked to a 1,5-benzoxathiepin-derived structure.

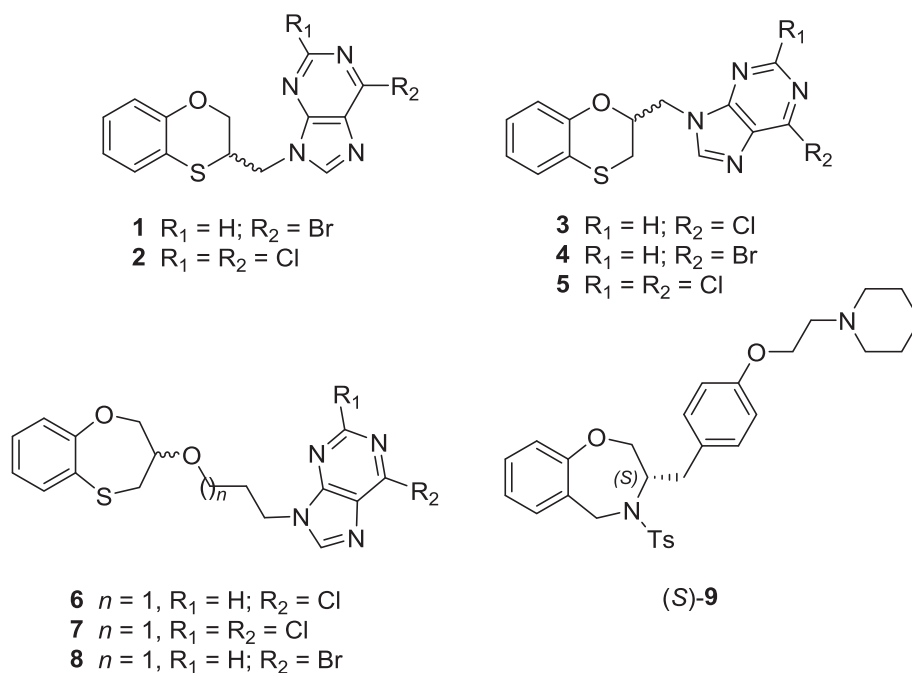
## 2. Results and discussion

### 2.1. Chemistry

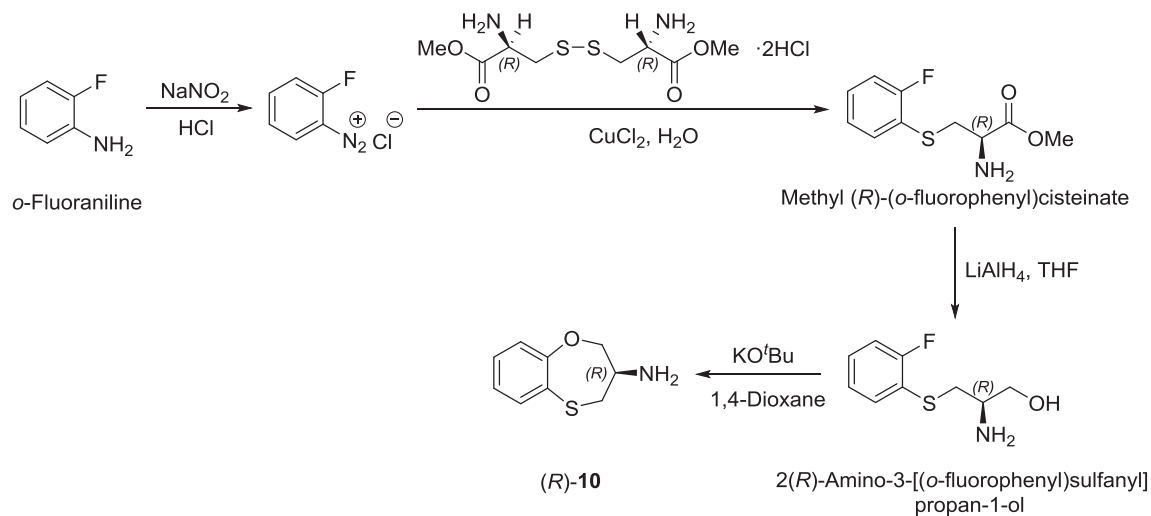
(3*R*)-Amino-3,4-dihydro-2*H*-1,5-benzoxathiepin [(*R*)-**10**] was diversified into two forms: (a) the incorporation of different amino acids through an amide bond, and (b) by the construction of the purine ring to give rise to a new prototype. The primary amine (*R*)-**10** has been prepared according to a patent (Vacher et al., 2005) and its synthesis is displayed in Scheme 1. Reaction of L-cystine dimethyl ester in its form of a dihydrochloride salt [absolute configuration (*R,R*)] with the appropriate diazonium salt yielded the methyl (*o*-fluorophenyl)-L-cysteinate [absolute configuration (*R*)], which was reduced with lithium aluminum hydride to the corresponding (2*R*)-amino-3-[*o*-fluorophenyl]sulfanyl]propan-1-ol. This amino alcohol was finally cyclized to (*R*)-**10**. The four-step synthesis resulted in a 51% overall yield of pure (*R*)-**10**. We have prepared two derivatives of (*R*)-**10** to confirm its structure: the acetamide (*R*)-**11**, whose structure has been corroborated by X-ray, and the *p*-nitrobenzenesulfonamide (*R*)-**12** (Scheme 2).

#### 2.1.1. X-ray crystallography of (*R*)-**11**

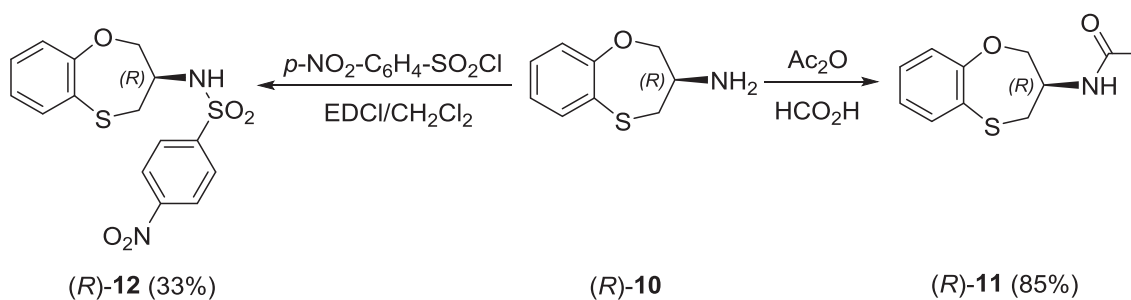
Derivative (*R*)-**11** has been crystallized from  $\text{CH}_2\text{Cl}_2$  and its 3D structure has been determined using X-ray diffraction. A



**Fig. 1** Benzofused six- and seven-membered heterocycles with interesting anti-proliferative (**1–8**) and anticancer [(*S*)-**9**] activities.



**Scheme 1** Synthesis of (*R*)-**10** (Vacher et al., 2005).



**Scheme 2** (*3R*)-Amino-3,4-dihydro-2*H*-1,5-benzoxathiepin derivatives.

view of the molecule is represented in Fig. 2. Each molecule is built up from two fused six- and seven-membered rings. The six-membered ring is planar, whereas the seven-membered ring displays a chair conformation with the puckering parameters:  $Q_2 = 0.453(3) \text{ \AA}$  and  $Q_3 = 0.710(3) \text{ \AA}$ ,  $\phi_2 = 29.2(4)^\circ$  and  $\phi_3 = 313.2(2)^\circ$  (Cremer and Pople, 1975), with a total puckering amplitude of  $0.842(2) \text{ \AA}$  (Boessenkool and Boyens, 1980). The acetamide fragment occupies an axial position in the seven-membered ring. The absolute stereochemistry of this homochiral molecule was confirmed by a Flack parameter of  $-0.003(13)$  (Parsons et al., 2013).

In the crystal, the acetamide moieties connect adjacent molecules by hydrogen bonding interactions generating a chain running along the *a*-axis (Fig. 3). Non-classical C—H hydrogen bonds connect chains to build up the supramolecular structure.

### 2.1.2. The 1,5-benzoxathiepin scaffold linked to distinct $\alpha$ -amino acids

We have used several proteinogenic amino acids assorted on the basis of their structure and the general chemical characteristics of their R groups, located at position  $\alpha$  in relation to the carboxylic acid group: acid (Glu), aliphatic (Leu), aromatic (Phe, Tyr), basic (Arg, Lys) and cyclic (Pro).

The strategy followed is shown in Scheme 3. The reaction of amine (*R*)-10 with different Boc-amino acids and subsequent Boc-deprotection of the corresponding derivatives is summarized in Table 1. Amide bond formation is a fundamentally important reaction in organic synthesis, and is typically mediated by the so-called coupling reagents. Valeur and Bradley wrote a critical review on the most used coupling reagents with particular attention paid to the pros and cons of these reagents (Valeur and Bradley, 2009). We have used *N*-[3-(dimethylamino)propyl]-*N'*-ethylcarbodiimide hydrochloride (EDC·HCl), followed by 1-hydroxybenzotriazole hydrate (HOBt) as coupling agents for amide formation.

### 2.1.3. Novel purine derivatives: 1. Preservation of the 3,4-dihydro-2*H*-1,5-benzoxathiepin scaffold and construction of the 6-chloropurine moiety linked to the seven-membered frame. 2. Ring opening when iodine is introduced at position 2 of the purine moiety

At this point, we have followed a different strategy (Kimatrai et al., 2011) to preserve the seven-membered ring: We propose herein to build up the purine ring from the amino group of the 3,4-dihydro-2*H*-1,5-benzoxathiepin-(3*R*)-yl amine (*R*)-10. Starting from (*R*)-10 and according to Scheme 4, (*R*)-28 was obtained in a sequence of two steps: a) condensation of (*R*)-10 with the 5-amino-4,6-dichloropyrimidine to produce

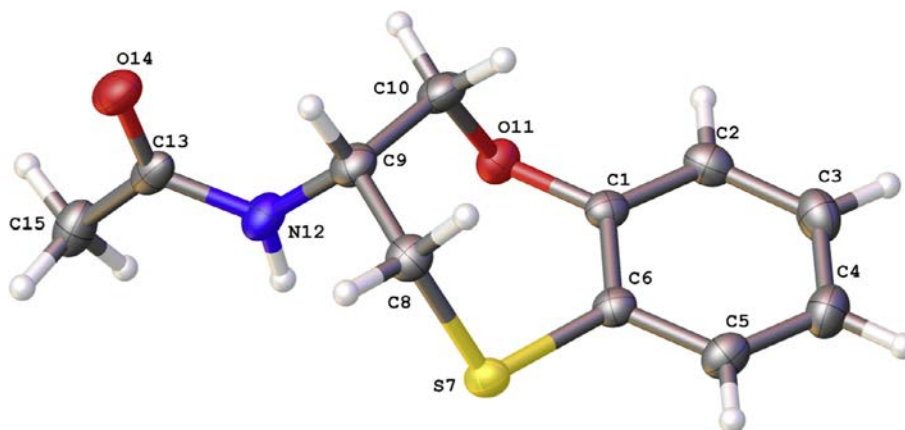


Fig. 2 The molecular structure of (*R*)-30 showing the atom labeling scheme.

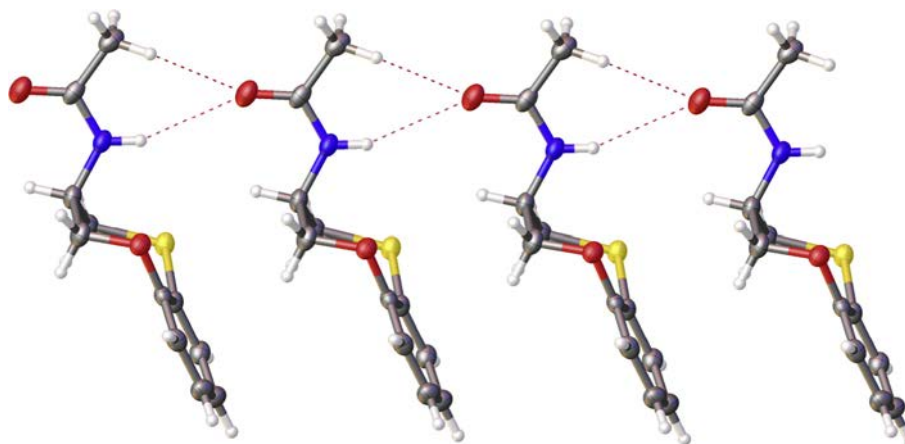
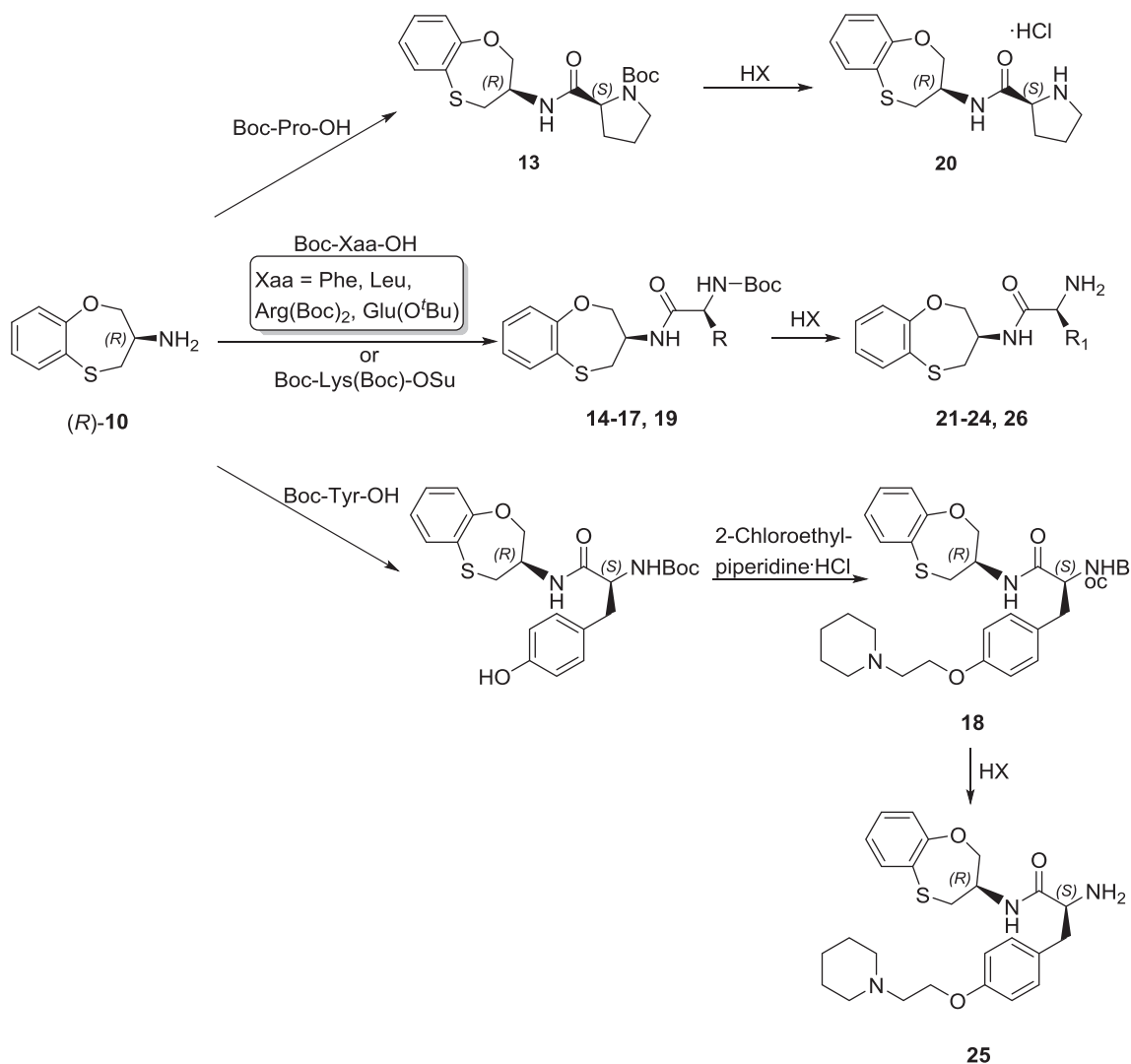


Fig. 3 A chain obtained from hydrogen bonding interaction between the acetamide moieties, running along the *a*-axis in (*R*)-30.



**Scheme 3** General synthetic procedure for dipeptides made up by condensation of the L-cysteine-derived heterocyclic amine **(R)-10** and Boc-protected α amino acids to give **13–19**, and the corresponding deprotected derivatives **20–26**.

the substituted diaminopyrimidine **(R)-27**; and b) reaction of this product with triethyl orthoformate in an acidic medium (Scheme 4) (Dejmek et al., 2012) to produce **(R)-28**.

As a part of a research program on the synthesis of some biologically active heterocyclic compounds containing nitrogen, we planned to synthesize some new 6-substituted purine derivatives carrying the 9-(3,4-dihydro-2H-1,5-benzoxathiepin-(3R)-yl)-9H-purine motif, aiming at an investigation of new heterocycles with enhanced biological activity. Scheme 5 shows several substitution products of the chlorine atom, and a modification of the oxidation state of the sulfur atom of **(R)-28**.

We have published substitution reactions on 6-chloro-7- or 9-(2,3-dihydro-5H-1,4-benzodioxepin-3-yl)-7H- or 9H-purines (Núñez et al., 2006). In the present paper we find that in the amination of **(R)-28** with NH<sub>4</sub>OH, together with ammonia, a second nucleophile arose (hydroxyl ion) and the two products **(R)-29** and **(R)-30** were obtained and easily purified by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH: 9.9/0.1), eluting **(R)-29** faster than **(R)-30**. In the case of **(R)-31**, the dimethylamine nucleophile

results as a consequence of the decomposition of DMF under microwave irradiation, high temperature (180 °C) and basic conditions in a sealed microwave vial. Finally, oxidation of the sulfur atom of **(R)-28** with potassium peroxymonosulfate (Oxone®, Scheme 5) produces the sulfone **(R)-32**. We have previously used this oxidation for benzo-fused sulfur-containing seven-membered *O,N*-acetals (Núñez et al., 2007).

In our experience, 2,6-dihalogenopurines in general show a better anti-proliferative activity than the 6-chloropurine derivatives (Conejo-García et al., 2011; García-Rubiño et al., 2013; Kimatrai et al., 2011; López-Cara et al., 2011; Morales et al., 2014; Morales et al., 2015) and therefore this prompted us to introduce an iodine atom at position 2 of the purine ring of **(R)-28**. According to the work of Taddei et al. (2004), a regioselective 2-lithiation of 6-chloropurine derivatives is achievable at a low temperature in THF with 5 equivalents of Harpoon's base (lithium 2,2,6,6-tetramethylpiperidine, LTMP). Quenching the lithiated species with 5 equivalents of tributyltin chloride affords the 2-stannylated product exclusively, and finally an iodination product under mild conditions.

**Table 1** 3-(*R*)-Amino-3,4-dihydro-2*H*-1,5-benzoxathiepin has been linked to different protected  $\alpha$ -amino acids through an amide bond to give the protected compounds **13–19**, which produce **20–26**, after deprotection.

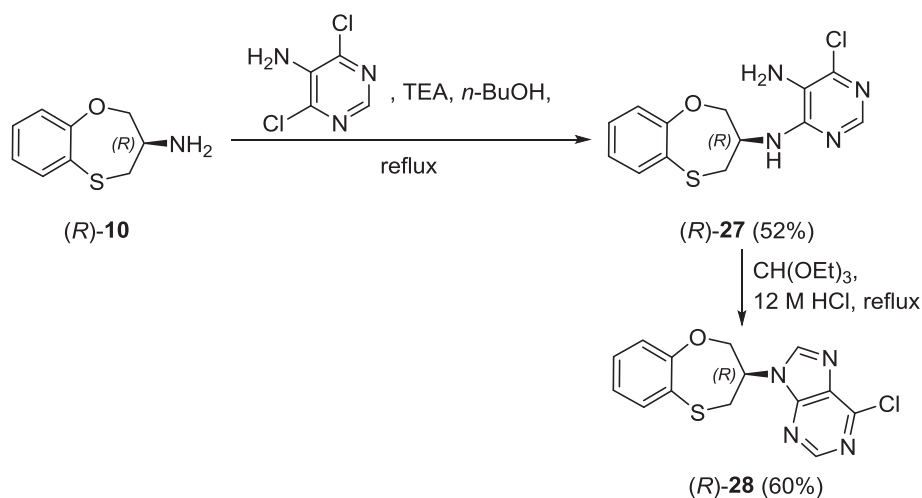
Starting amino acid	Protected product <sup>a</sup>	Product after deprotection
Boc-Pro-OH	<b>13</b> (93%)	<b>20</b> (92%)
To Boc-Phe-OH	<b>14</b> (98%)	<b>21</b> (75%)
Boc-Leu-OH	<b>15</b> (96%)	<b>22</b> (92%)
Boc-Arg(Boc) <sup>b</sup>	<b>16</b> (95%)	<b>23</b> (97%)
Boc-Glu(O <sup>t</sup> Bu)-OH	<b>17</b> (98%)	<b>24</b> (98%)
Boc-Tyr-OH	<b>18</b> <sup>2</sup> (90%)	<b>25</b> (97%)
Boc-Lys(Boc)-Osu <sup>c</sup>	<b>19</b> (98%)	<b>26</b> (90%)

<sup>a</sup> The  $\alpha$  amino group is protected with a Boc (*tert*-butyloxycarbonyl) group and the lateral chain is protected with Boc or <sup>t</sup>Bu, if necessary.

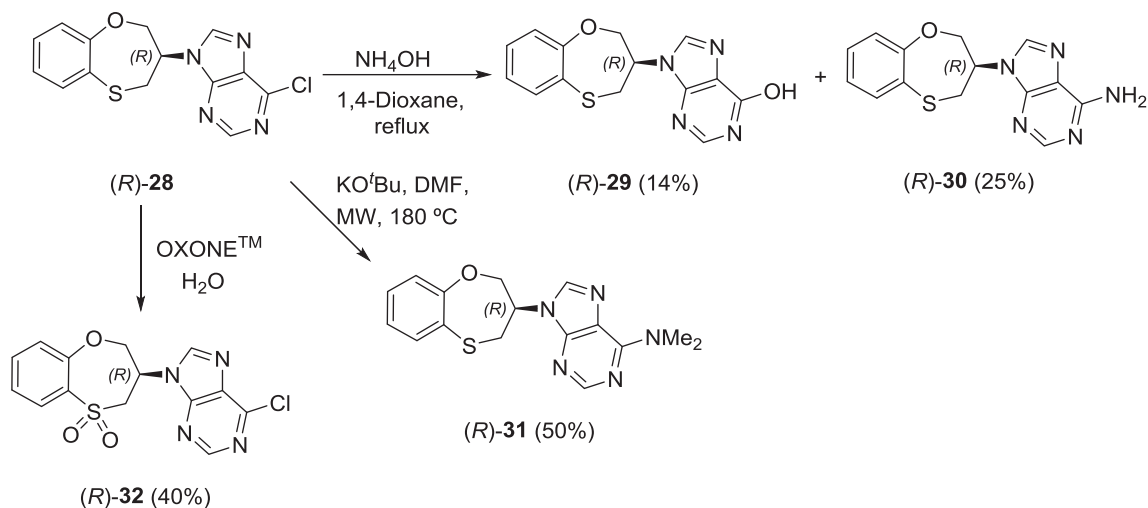
<sup>b</sup> Compound **18** was obtained by treatment of {(2*S*)-*tert*-butoxycarbonylamino-*N*-[3,4-dihydro-2*H*-1,5-benzoxathiepin-(3*R*)-yl]-3-[4-hydroxybenzene]}propionamide with 2-chloroethylpiperidine chlorhydrate.

<sup>c</sup> *N*<sub>2</sub>*N*<sub>ε</sub>-Di-Boc-*l*-lysine hydroxysuccinimide ester.





**Scheme 4** Construction of the 6-chloropurine ring to obtain (R)-10.



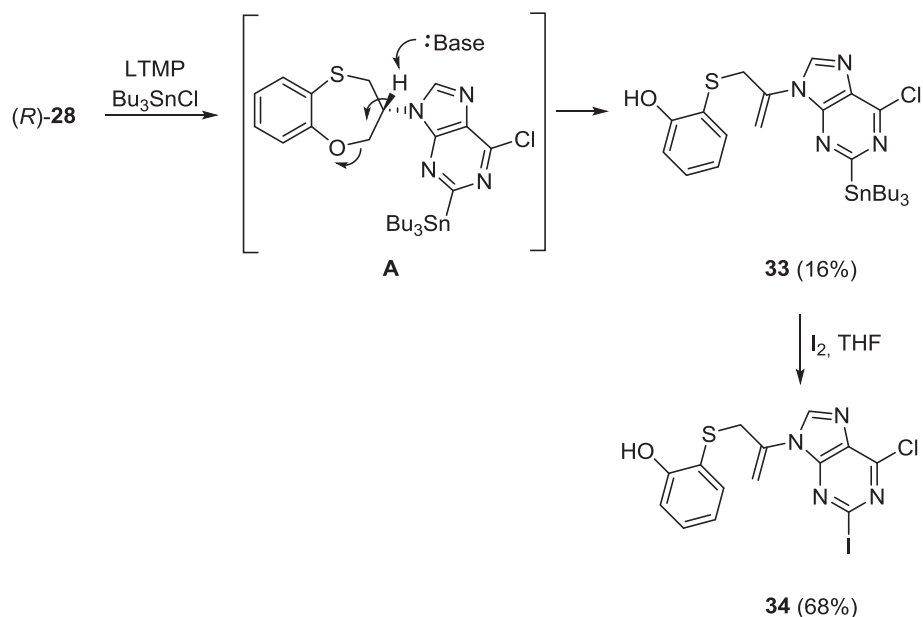
**Scheme 5** Several substitution products of the chlorine atom, and modification of the oxidation state of the sulfur atom of (R)-28.

The opening of the seven-membered ring of the non-isolated intermediate **A** took place, giving rise to **33** and **34** (Scheme 6). In fact, it is well known that ethers may be cleaved by organolithium compounds and, although the rates of these reactions depend on both the structure of the organolithium compound and the ether, such cleavages may occur even at low temperatures because of the negative temperature coefficients associated with these reactions (Wakefield, 1974).

Characterization of **33** and **34** by NMR and high resolution mass spectra (HRMS) was obtained after purification of the crude oils by flash chromatography on silica gel [(hexane/EtOAc: 3/1) and (hexane/EtOAc: 5/1), respectively]. In both cases,  $^1\text{H}$  NMR (in a  $\text{CDCl}_3$  solution) showed only one purine proton ( $\delta$  8.05 ppm) assignable to H-8, as a result of a 2-substitution. The  $^1J$ ,  $^2J$ , and  $^3J$  ( $^{13}\text{C}$ - $^{119}\text{Sn}$ ) couplings between the tin atom and the corresponding *n*-butyl aliphatic carbon atoms were observed in the  $^{13}\text{C}$  NMR of **33** (see Experimental Part). As spectra of **33** and **34** are similar (and therefore with the same scaffold) and in order to justify their structures, we will develop the reasoning on the 2,6-dihalopurine derivative **34**.

When 1,3-benzoxathioles were heated under reflux of benzene with a lithium amide (lithium diethylamide), cleavage of a single bond (ether and thioether) or both bonds takes place (Melis et al., 1983). However, by performing the reaction with LTMP at  $-78^\circ\text{C}$ , we achieved the selective cleavage of the ether bond of (R)-28 to produce **33**. This result may seem contradictory at first sight because the C—O bond dissociation energies are usually higher than the corresponding C—S energies (Oae and Doi, 1991). However, one must consider that hindered lithium amides such as LTMP are useful for the selective deprotonation of functionalized molecules precisely because of the unique ability of lithium to coordinate to heteroatoms (Woltermann et al., 2004).

We hypothesized that the first step for the cleavage of the ether/thioether bond of (R)-28 requires as the primary step coordination between lithium and the heteroatom to bring the chiral base and the substrate into close proximity, thus enforcing the removal of the proton. Such approach was previously used by Wiedemann et al. (2003) to explain the  $\beta$ -lithiation of epoxides that afford allylic alcohols, where the lithium atom of LTMP coordinates with the epoxide oxygen



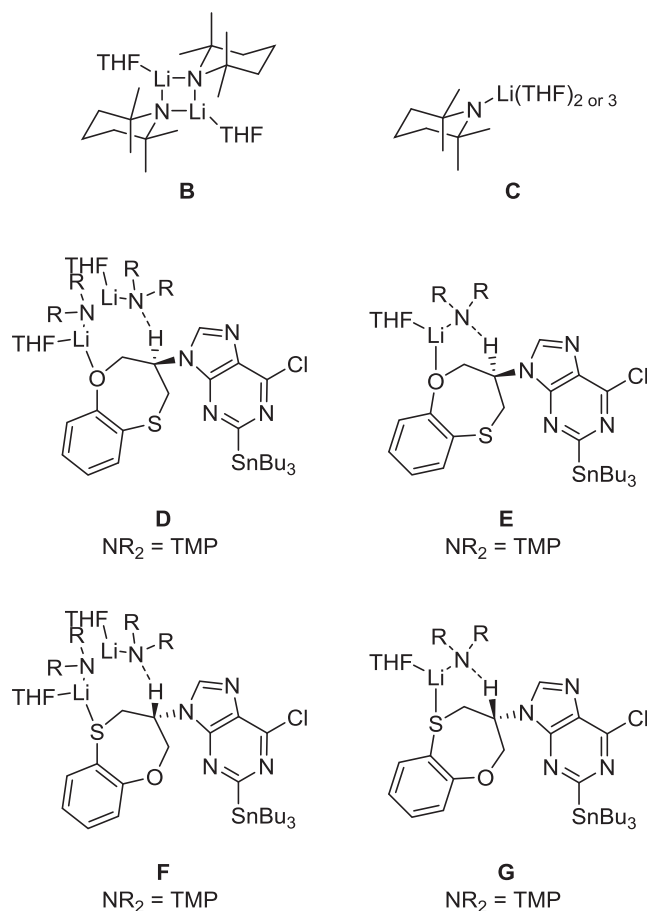
**Scheme 6** Opening of the seven-membered ring of **(R)-28** when introducing iodine at position 2 of the purine ring, by using an excess of the Harpoon's base and tributyltin chloride, and iodine.

to direct the abstraction of the proton by the nitrogen of the base. Coordination of the oxygen to the metal would also favor the cleavage of the C–O bond by polarizing the flanking oxygen–carbon bonds, producing a more stable leaving group (Houghton, 1979).

Therefore, the exclusive obtaining of **34** as the reaction product would reflect the preferential coordination of the lithium of LTMP with the oxygen atom (and not the sulfur) of **(R)-28**, which would generate a more stable transition structure. Alternatively, the preferential abstraction of the proton by the complex when the lithium binds to the oxygen atom could also reflect a transition state structure where the proton and the nitrogen are closer to the collinearity (*i.e.* N–H–C optimum abstraction bond angle  $\sim 180^\circ$ ).

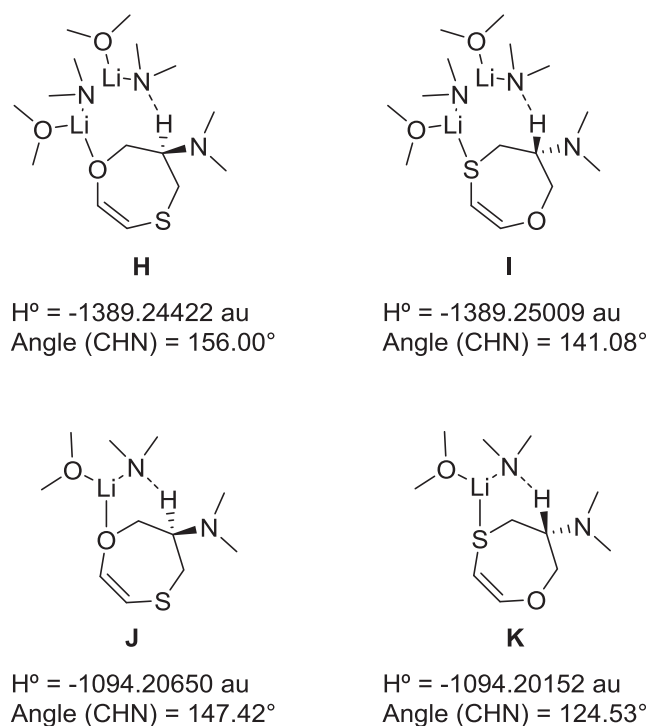
Wiedemann et al. (2003) also demonstrated that LTMP exists in mixtures of THF and pentane as the C<sub>2h</sub> dimer **B**, whereas low concentrations of the monomer **C** ( $\approx 20\%$ ) appear in neat THF (Fig. 4). Consequently, four possible transition structures (**D–G**, Fig. 4) could be formed through the coordination of lithium atoms in the aggregation/solvation states of LTMP with the oxygen or sulfur atoms of **(R)-28**. Based on the selective cleavage of the ether bond observed for **(R)-28**, one may expect that the transition structures **D** and **F** are more stable than the corresponding **E** and **G**.

Computations using density functional theory (DFT) were then employed to detail these transition states. As the computational cost for these simulations grows exponentially with the number of electrons of the system (Whitfield et al., 2013), we used structures **H** to **K** (Fig. 5) as simplified models for the transition structures **D** to **G**. According to the simulations, the reaction is more likely to proceed via dimer-based than monomer-based lithiation, although nearly no difference in the enthalpy values was observed when the lithium atom of LTMP coordinated with the oxygen or the sulfur atom. However, when we look at the N–H–C bond angles, the transition structures **I** and **K** display considerable deviation from the



**Fig. 4** Dimeric (**B**) and monomeric (**C**) aggregation/solvation states of lithium 2,2,6,6-tetramethylpiperidine (LTMP) in tetrahydrofuran (THF) and the corresponding transition structures predicted to be formed, during the  $\beta$ -lithiation of **(R)-28**.





**Fig. 5** Enthalpy ( $H^\circ$ ) and bond angle (CHN) values calculated for simplified models of the dimeric and monomeric transition structures predicted to be formed during the  $\beta$ -lithiation of (*R*)-**28**.

optimum  $180^\circ$  collinear proton transfer angle (N–H–C angles =  $141.08^\circ$  and  $124.53^\circ$ , respectively) when compared to the transition structures **H** and **J** (N–H–C angles =  $156.00^\circ$  and  $147.42^\circ$ , respectively), a result that is consistent with the formation of **34**.

## 2.2. Biological activities

MCF-7 and MDA-MB-231 human breast cancer cell lines were selected for the *in vitro* assays as they have proved to be excellent experimental models for the study of both tumorigenesis and metastasis as well as the efficacy of different therapies before its use in patients (Holliday and Speirs, 2011; Wilhelm et al., 2004). Due to the similar genetic susceptibility existing among breast and ovarian cancers (Miki et al., 1994), we decided to also test the compounds in the ovarian carcinoma SKOV-3 cell line.

Compounds evaluated for the anti-proliferative activity have been the following: (*R*)-**29**, (*R*)-**30**, (*R*)-**31**, **37**, **39–45**, (*R*)-**46**, (*R*)-**47** and **56**. To evaluate the anti-proliferative activity of the synthesized compounds, we consider active only those that showed an  $IC_{50}$  value below or equal to  $100 \mu\text{M}$ . In the first chemical library only compound **37** showed an  $IC_{50} = 100 \mu\text{M}$  in the three cancerous cell lines previously cited.

### 2.2.1. Compound **34** has a selective anti-proliferative effect on breast cancer cell lines

In relation to the purine-related compounds, **34** showed a selective anti-proliferative activity against breast cancer cell lines MCF-7 ( $IC_{50} = 41.8 \mu\text{M}$ ) and MDA-MB-231 ( $IC_{50} = 20.4 \mu\text{M}$ ), being inactive against SKOV-3. Interest-

ingly, **34** did not induce significant cytotoxic effects at concentrations up to  $100 \mu\text{M}$  in the non-tumor cell line of 142BR fibroblasts: 85% of the cells were viable at a concentration of  $100 \mu\text{M}$ , and 91% of them at  $50 \mu\text{M}$ , suggesting that the mechanism of action of the compound is selective for cancer cell lines.

### 2.2.2. Compound **34** decreases mammosphere formation in MDA-MB-231 and MCF-7 breast cancer cells

CSCs can be identified due to the ability to grow as 3D non-adherent structures when clonally seeded in serum-free media (tumor-spheres), among several characteristics (Cioce et al., 2010). We determined the effect of **34** on MDA-MB-231 and MCF-7 breast CSCs enriched populations by growing them into low attachment plates with sphere-forming medium for 2 weeks.

As previously described (Iglesias et al., 2013), MDA-MB-231 cells formed weak mammospheres that could be disaggregated mechanically. Nevertheless, the difference between MDA-MB-231 cells treated with vehicle or with **34** was unquestionable, as compound-treated cells were unable to grow as spheres while in DMSO-treated cultures a significant number of small-sized mammospheres were formed. In contrast to MCF-7 spherical solid masses of cells were formed upon incubation with DMSO, but this sphere formation ability was almost completely abolished by treatment with  $20 \mu\text{M}$  of **34**.

Importantly, the **34**-induced reduction in mammosphere formation was not due to non-specific cytotoxicity. Previous experiments in monolayer cultures showed that cell viability remained as high as 60% for MDA-MB-231 cells and as 97% for MCF-7 cells in the presence of  $20 \mu\text{M}$  of the compound.

## 3. Conclusions

The importance of sulfur in biological systems is known and also its increasing interest as a regulatory agent. Accordingly, the rationality that leads to the employment of sulfur-based heterocycle drugs comes to light. On the other hand, the incorporation of alpha-amino acids into heterocyclic structures is an effective strategy for generating numerous peptidomimetics and combinatorial library scaffolds. We have conducted these studies hoping to shed some light on the synthesis and reactivity of seven-membered benzo-fused diheterocycles. A diverse group of novel seven-membered heterocycles derived from naturally abundant proteinogenic amino acids have been synthesized. When (*R*)-**28** was treated with an excess of the Harpoon's base (lithium 2,2,6,6-tetramethylpiperidine) and tributyltin/iodination, 2-[[2-(6-chloro-2-iodo-9*H*-purin-9-yl)prop-2-en-1-yl]thio]phenol (**34**) was obtained as a result of breaking the O-CH<sub>2</sub> bond of the seven-membered ring. On the other hand, within the *N*-9 alkylated purines, **34** showed important activity against CSC. We are currently carrying out its detailed mechanistic biological studies, including the identifications of possible targets for future rational drug design.

## 4. Experimental section

### 4.1. General methods

Melting points were taken in open capillaries. Flash chromatography was performed on silica gel 60 with a particle size of 0.040–0.063 mm (230–400 mesh ASTM). Small scale microwave-assisted synthesis was carried out in an Initiator

2.0 single-mode microwave instrument producing controlled irradiation at 2.450 GHz (Biotage AB, Uppsala). IR spectra were registered on a Nicolet Avatar 360 FTIR spectrophotometer;  $\nu_{\max}$  is given for the main absorption bands. Nuclear magnetic resonance spectra have been carried out at the Centro de Instrumentación Científica/Universidad de Granada, and recorded on a 300 MHz  $^1\text{H}$  and 75 MHz  $^{13}\text{C}$ NMR Varian Inova-TM spectrometers at ambient temperature. On the other hand,  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra recorded on a Bruker AV-400 instrument (400 MHz  $^1\text{H}$  and 100 MHz  $^{13}\text{C}$  NMR spectrometer) at room temperature have been carried out at the University of Zaragoza. Chemical shifts ( $\delta$ ) are quoted in parts per million (ppm) and are referenced to the residual solvent peak. Signals are designated as follows: s, singlet; bs, broad singlet; d, doublet; dd, doublet of doublets; ddd, doublet of doublet of doublets; dt, double triplet; t, triplet; m, multiplet. High-resolution Nano-Assisted Laser Desorption/Ionization (NALDI-TOF), Electrospray Ionization (ESITOF) mass spectra were carried out on a Bruker Autoflex, on a Waters LCT Premier Mass Spectrometer, and on Bruker Microtof-Q spectrometer. All the compounds gave accurate mass spectra, having the correct isotope abundance and no extraneous peaks. Small-scale microwave-assisted syntheses were carried out in a single-mode microwave instrument producing controlled irradiation at 2.450 GHz and sealed reaction vessels were used. Reaction time refers to hold time from 130 °C to 160 °C, not to total irradiation time. The temperature was measured with an IR sensor outside the reaction vessel. Optical rotations were measured in a cell with a 1 dm path length at the temperature indicated in each case using a JASCO P-1020 polarimeter. All evaporations were carried out *in vacuum* with a Büchi rotary evaporator and the pressure controlled by a Vacuubrand CVCII apparatus. Anhydrous DMF was used as received.

#### 4.1.1. Crystal structure determination of (R)-11

Measured crystal was prepared under inert conditions immersed in perfluoropolyether as protecting oil for manipulation. A suitable crystal was mounted on MiTeGen Micro-mounts, and this sample was used for data collection. Data were collected on a Bruker D8 Venture diffractometer (Cu K $\alpha$ , 150 K). Data collection and processing were performed using the programs APEX3 (Bruker, 2016) and SAINT (Bruker, 2013), and a multi-scan absorption correction was applied using SADABS (Sheldrick, 2014). The structures were solved by direct methods (Sheldrick, 2008), which revealed the position of all non-hydrogen atoms. These atoms were refined on F<sup>2</sup> by a full-matrix least-square procedure using anisotropic displacement parameters (Sheldrick, 2015). All hydrogen atoms were located in the difference Fourier map and included as fixed contributions riding on attached atoms with isotropic thermal displacement parameters 1.2 (—CH, —CH<sub>2</sub>, —N—H) or 1.5 (—CH<sub>3</sub>) times than those of the respective atoms. Crystallographic data of (R)-30 for structural analysis have been deposited in the Cambridge Crystallographic Data Centre, CCDC 1477918.

#### 4.1.2. Incorporation of $\alpha$ -amino acids into the amino group of (R)-10

4.1.2.1. Procedure A: General procedure for amide bond formation from amine (R)-10 and Boc-Xaa-OH. To a solution of Boc-L-Xaa-OH (1.50 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (30 mL), cooled to

0 °C in an ice bath, was added *N*-[3-(dimethylamino)propyl]-*N'*-ethylcarbodiimide hydrochloride (EDC·HCl) (316 mg, 1.65 mmol) followed by 1-hydroxybenzotriazole hydrate (HOBt) (253 mg, 1.65 mmol) and the reaction was stirred for 15 min. Then, a solution of (R)-10 (Vacher et al., 2005) (1.24 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) and *N*-methylmorpholine (NMM) (0.18 mL, 1.65 mmol) was added and the mixture was stirred at rt for 24 h. The reaction mixture was washed with a 5% aqueous solution of NaHCO<sub>3</sub> (3 × 30 mL), followed by a 5% aqueous solution of KHSO<sub>4</sub> (3 × 30 mL). The organic phase was dried (MgSO<sub>4</sub>), filtered and evaporated to dryness. The crude products were purified by column chromatography to provide the corresponding compounds 13–19.

4.1.2.1.1. {1-*tert*-Butoxycarbonyl-(2*S*)-[3,4-dihydro-2*H*-1,5-benzoxathiepin-(3*R*)-carbamoyl]}pyrrolidine (13). A column chromatography (hexane/EtOAc: 3/7) afforded 13 as a white solid (435 mg, 1.15 mmol, 93% yield). Mp: 135–136 °C.  $[\alpha]_{\text{D}}^{27}$ : -15.9 ( $c = 0.60$ , acetone). IR (KBr)  $\nu$ : 3274, 1684, 1668 cm<sup>-1</sup>.  $^1\text{H}$  NMR (MeOH-*d*<sub>4</sub>, 400 MHz):  $\delta$  1.45 (s, 9H), 1.82–2.03 (m, 3H), 2.14–2.34 (m, 1H), 2.96 (dd, 1H,  $J = 14.3$  Hz,  $J = 6.7$  Hz), 3.10 (dd, 1H,  $J = 14.3$  Hz,  $J = 3.5$  Hz), 3.40–3.46 (m, 1H), 3.49–3.55 (m, 1H), 4.10–4.22 (m, 2H), 4.26 (dd, 1H,  $J = 8.6$  Hz,  $J = 3.7$  Hz), 4.42–4.48 (m, 1H), 6.97–7.02 (m, 2H), 7.16–7.21 (m, 1H), 7.37 (d, 1H,  $J = 7.8$  Hz).  $^{13}\text{C}$  NMR (MeOH-*d*<sub>4</sub>, 100 MHz):  $\delta$  24.61, 25.38, 28.71, 31.35, 32.60, 36.54, 47.98, 48.25, 51.38, 61.47, 61.74, 75.07, 75.18, 81.42, 81.60, 123.11, 124.95, 129.09, 129.38, 129.80, 133.03, 156.03, 156.42, 161.73, 161.86, 174.57, 175.03. HRMS (ESI) C<sub>19</sub>H<sub>26</sub>N<sub>2</sub>NaO<sub>4</sub>S [M + Na]<sup>+</sup>: calcd. 401.1505, found 401.1523. Anal. Calcd. for C<sub>19</sub>H<sub>26</sub>N<sub>2</sub>O<sub>4</sub>S: C, 60.29; H, 6.92; N, 7.40; Found: C, 60.32; H, 7.05; N, 7.32.

4.1.2.1.2. *tert*-Butyl {1(*S*)-[3,4-dihydro-2*H*-1,5-benzoxathiepin-(3*R*)-carbamoyl]-2-phenylethyl}carbamate (14). A column chromatography (hexane/EtOAc: 7/3) provided 14 as a white solid (522 mg, 1.22 mmol, 98% yield). Mp: 125–127 °C.  $[\alpha]_{\text{D}}^{28}$ : +30.4 ( $c = 0.55$ , acetone). IR (KBr)  $\nu$ : 3337, 3314, 1690, 1648 cm<sup>-1</sup>.  $^1\text{H}$  NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  1.41 (s, 9H), 2.85 (dd, 1H,  $J = 14.4$ ,  $J = 5.3$  Hz), 3.01 (dd, 1H,  $J = 14.4$ ,  $J = 2.9$  Hz), 3.05–3.16 (m, 2H), 3.87 (dd, 1H,  $J = 12.6$ ,  $J = 2.2$  Hz), 4.12 (dd, 1H,  $J = 12.4$ ,  $J = 3.2$  Hz), 4.36–4.46 (m, 1H), 4.47–4.54 (m, 1H), 5.05 (bs, 1H), 6.82 (d, 1H,  $J = 8.9$  Hz), 6.96–7.01 (m, 2H), 7.15–7.19 (m, 1H), 7.22–7.27 (m, 3H), 7.29–7.33 (m, 2H), 7.38 (dd, 1H,  $J = 7.6$  Hz,  $J = 1.7$  Hz).  $^{13}\text{C}$  NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  28.39, 36.33, 38.71, 48.91, 56.04, 74.35, 80.39, 122.35, 124.14, 127.17, 128.24, 128.86, 129.01, 129.42, 132.43, 136.64, 155.40, 160.88, 170.74. HRMS (ESI) C<sub>23</sub>H<sub>28</sub>N<sub>2</sub>NaO<sub>4</sub>S [M + Na]<sup>+</sup>: calcd. 451.1662, found 451.1673. Anal. Calcd. for C<sub>23</sub>H<sub>28</sub>N<sub>2</sub>O<sub>4</sub>S: C, 64.46; H, 6.59; N, 6.54; Found: C, 64.67; H, 6.74; N, 6.65.

4.1.2.1.3. *tert*-Butyl {1(*S*)-[3,4-dihydro-2*H*-1,5-benzoxathiepin-(3*R*)-carbamoyl]-3-methylbutyl}carbamate (15). A column chromatography (hexane/EtOAc: 7/3) provided 15 as a white solid (470 mg, 1.19 mmol, 96% yield). Mp: 111–112 °C.  $[\alpha]_{\text{D}}^{29}$ : +24.6 ( $c = 0.70$ , acetone). IR (KBr)  $\nu$ : 3327, 3267, 3062, 1686, 1643 cm<sup>-1</sup>.  $^1\text{H}$  NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  0.95 (d, 3H,  $J = 2.5$  Hz), 0.97 (d, 3H,  $J = 2.6$  Hz), 1.44 (s, 9H), 1.50–1.59 (m, 1H), 1.63–1.75 (m, 2H), 2.92 (ddd, 1H,  $J = 14.4$  Hz,  $J = 5.2$  Hz,  $J = 1.4$  Hz), 3.05 (dd, 1H,  $J = 14.4$  Hz,  $J = 2.9$  Hz), 3.94 (dd, 1H,  $J = 12.5$  Hz,  $J = 2.1$  Hz), 4.09–4.24 (m, 1H), 4.32 (ddd, 1H,  $J = 12.5$  Hz,  $J = 3.6$  Hz,  $J = 1.3$  Hz), 4.53–4.58 (m, 1H), 4.85–4.97

(m, 1H), 6.97–7.05 (m, 2H), 7.13 (d, 1H,  $J = 8.9$  Hz), 7.17–7.21 (m, 1H), 7.42 (dd, 1H,  $J = 7.7$  Hz,  $J = 1.7$  Hz).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz):  $\delta$  22.10, 23.14, 24.94, 28.42, 36.40, 41.46, 48.84, 53.41, 74.65, 80.26, 122.44, 124.21, 128.45, 129.10, 132.54, 155.72, 161.09, 172.27. HRMS (ESI)  $\text{C}_{20}\text{H}_{30}\text{N}_2\text{NaO}_4\text{S}$   $[\text{M} + \text{Na}]^+$ : calcd. 417.1818, found 417.1837. Anal. Calcd. for  $\text{C}_{20}\text{H}_{30}\text{N}_2\text{O}_4\text{S}$ : C, 60.89; H, 7.66; N, 7.10; Found: C, 60.58; H, 7.26; N, 7.07.

4.1.2.1.4. (2*S*)-*tert*-Butoxycarbonylamino-5-(*N*-*tert*-butoxycarbonylamino-*N'*-*tert*-butoxycarbonyliminoguanidino)-*N*-[3,4-dihydro-2*H*-1,5-benzoxathiepin-(3*R*)-yl]pentanoamide (**16**). A column chromatography (hexane/EtOAc: 7/3) yielded **16** as a white solid (759 mg, 1.19 mmol, 96% yield). Mp: 75–77 °C.  $[\alpha]_{\text{D}}^{25}$ : +21.5 ( $c = 0.67$ , acetone). IR (KBr) v: 3409, 3197, 3056, 1699, 1686, 1677, 1616  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  1.44 (s, 9H), 1.46 (s, 9H), 1.51 (s, 9H), 1.64–1.73 (m, 2H), 1.78–1.98 (m, 2H), 2.95 (dd, 1H,  $J = 14.4$  Hz,  $J = 5.6$  Hz), 3.04 (dd, 1H,  $J = 14.3$  Hz,  $J = 3.4$  Hz), 3.84 (t, 2H,  $J = 7.3$  Hz), 4.08–4.14 (m, 1H), 4.18–4.29 (m, 2H), 4.53–4.60 (m, 1H), 5.78 (d, 1H,  $J = 8.2$  Hz), 6.94–7.00 (m, 2H), 7.15 (ddd, 1H,  $J = 8.1$  Hz,  $J = 7.2$  Hz,  $J = 1.7$  Hz), 7.29 (d, 1H,  $J = 8.9$  Hz), 7.36 (dd, 1H,  $J = 7.7$  Hz,  $J = 1.7$  Hz), 9.22 (bs, 1H), 9.35 (bs, 1H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz):  $\delta$  25.03, 28.17, 28.33, 28.52, 35.96, 44.19, 49.35, 54.50, 74.44, 79.22, 80.12, 84.09, 122.28, 123.98, 127.87, 128.79, 132.23, 155.02, 155.84, 160.55, 160.80, 163.59, 171.85. HRMS (ESI)  $\text{C}_{30}\text{H}_{48}\text{N}_5\text{O}_8\text{S}$   $[\text{M} + \text{H}]^+$ : calcd. 638.3218, found 638.3227. Anal. Calcd. for  $\text{C}_{30}\text{H}_{47}\text{N}_5\text{O}_8\text{S}$ : C, 56.50; H, 7.43; N, 10.98; Found: C, 56.34; H, 7.52; N, 11.09.

4.1.2.1.5. *tert*-Butyl {4-*tert*-butoxycarbonylamino-(4*S*)-[3,4-dihydro-2*H*-1,5-benzoxathiepin-(3*R*)-yl]carbamoyl}butyrate (**17**). A column chromatography (hexane/EtOAc: 6/4) provided **17** as a white solid (568 mg, 1.22 mmol, 98% yield). Mp: 57–58 °C.  $[\alpha]_{\text{D}}^{25}$ : +27.7 ( $c = 0.50$ , acetone). IR (KBr) v: 3375, 3277, 1717, 1702, 1672  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  1.43 (s, 9H), 1.44 (s, 9H), 1.90–2.00 (m, 1H), 2.08–2.16 (m, 1H), 2.30–2.46 (m, 2H), 2.92 (ddd, 1H,  $J = 14.4$  Hz,  $J = 5.3$  Hz,  $J = 1.3$  Hz), 3.05 (dd, 1H,  $J = 14.4$  Hz,  $J = 2.9$  Hz), 3.94 (dd, 1H,  $J = 12.6$  Hz,  $J = 2.1$  Hz), 4.14–4.25 (m, 1H), 4.32 (ddd, 1H,  $J = 12.5$  Hz,  $J = 3.7$  Hz,  $J = 1.3$  Hz), 4.52–4.58 (m, 1H), 5.31 (d, 1H,  $J = 7.8$  Hz), 6.97–7.04 (m, 2H), 7.18 (ddd, 1H,  $J = 8.1$  Hz,  $J = 7.3$  Hz,  $J = 1.7$  Hz), 7.23 (d, 1H,  $J = 8.9$  Hz), 7.41 (dd, 1H,  $J = 7.6$  Hz,  $J = 1.7$  Hz).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz):  $\delta$  27.69, 28.17, 28.38, 31.91, 36.28, 48.97, 54.32, 74.46, 80.16, 80.97, 122.37, 124.12, 128.28, 129.00, 132.44, 155.72, 160.96, 171.33, 172.66. HRMS (ESI)  $\text{C}_{23}\text{H}_{34}\text{N}_2\text{NaO}_6\text{S}$   $[\text{M} + \text{Na}]^+$ : calcd. 489.2030, found 489.2046. Anal. Calcd. for  $\text{C}_{23}\text{H}_{34}\text{N}_2\text{O}_6\text{S}$ : C, 59.21; H, 7.34; N, 6.00; Found: C, 59.54; H, 7.19; N, 6.00.

4.1.2.1.6. {(2*S*)-*tert*-Butoxycarbonylamino-*N*-[3,4-dihydro-2*H*-1,5-benzoxathiepin-(3*R*)-yl]-3-[4-hydroxybenzene]}propionamide. Procedure A for amide bond formation from amine (*R*)-**10** and Boc-Tyr-OH, followed by column chromatography purification (hexane/EtOAc: 4/6) provided {(2*S*)-*tert*-butoxycarbonylamino-*N*-[3,4-dihydro-2*H*-1,5-benzoxathiepin-(3*R*)-yl]-3-[4-hydroxybenzene]}propionamide as a white solid (516 mg, 1.16 mmol, 94% yield). Mp: 94–96 °C.  $[\alpha]_{\text{D}}^{25}$ : +23.3 ( $c = 0.50$ , acetone). IR (KBr) v: 3311, 3063, 3010, 1699, 1658  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  1.41 (s, 9H), 2.83 (dd, 1H,  $J = 14.2$  Hz,  $J = 5.3$  Hz), 2.93–3.03 (m, 3H), 3.87 (dd, 1H,  $J = 12.6$  Hz,  $J = 2.2$  Hz), 4.04–4.17 (m, 1H), 4.30–

4.43 (m, 1H), 4.43–4.53 (m, 1H), 5.22 (bs, 1H), 6.71–6.76 (m, 2H), 6.93–7.04 (m, 5H), 7.15 (ddd, 1H,  $J = 8.2$  Hz,  $J = 8.2$  Hz,  $J = 1.7$  Hz), 7.36 (dd, 1H,  $J = 7.6$  Hz,  $J = 1.6$  Hz).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz):  $\delta$  28.37, 36.12, 37.82, 49.07, 56.20, 74.27, 80.65, 115.82, 122.34, 124.14, 127.60, 128.11, 129.01, 130.44, 132.41, 155.58, 160.77, 171.32. HRMS (ESI)  $\text{C}_{23}\text{H}_{28}\text{N}_2\text{NaO}_5\text{S}$   $[\text{M} + \text{Na}]^+$ : calcd. 467.1611, found. 467.1613. Anal. Calcd. for  $\text{C}_{23}\text{H}_{28}\text{N}_2\text{O}_5\text{S}$ : C, 62.14; H, 6.35; N, 6.30; Found: C, 62.32; H, 6.21; N, 6.12.

4.1.2.1.7. *tert*-Butoxycarbonylamino-[(1*S*)-[3,4-dihydro-2*H*-1,5-benzoxathiepin-(3*R*)-yl]carbamoyl]-2-[4-(2-piperidin-1-ylethoxy)phenyl]ethyl carbamate (**18**). To a solution of {(2*S*)-*tert*-butoxycarbonylamino-*N*-[3,4-dihydro-2*H*-1,5-benzoxathiepin-(3*R*)-yl]-3-[4-hydroxybenzene]}propionamide (444 mg, 1.0 mmol) in acetone (25 mL) was added  $\text{K}_2\text{CO}_3$  (414 mg, 3.0 mmol) and NaI (19 mg, 0.13 mmol) followed by 2-chloroethylpiperidine chlorhydrate (276 mg, 1.5 mmol). The reaction was refluxed for 24 h. Then, the mixture was filtered and the filtrate was evaporated at reduced pressure. The crude product was purified by column chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  9/1) to provide **18** as a white solid (500 mg, 0.9 mmol, 90% yield). Mp: 108–110 °C.  $[\alpha]_{\text{D}}^{19}$ : +19.1 ( $c = 0.50$ , acetone). IR (KBr) v: 3416, 3312, 1706, 1663  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  1.41 (s, 9H), 1.45–1.52 (m, 2H), 1.63–1.71 (m, 4H), 2.55–2.67 (m, 4H), 2.82–2.83 (m, 1H), 2.85 (t, 2H,  $J = 5.7$  Hz), 2.97–3.12 (m, 3H), 3.86 (dd, 1H,  $J = 12.5$  Hz,  $J = 2.1$  Hz), 4.13 (t, 2H,  $J = 5.8$  Hz), 4.09–4.18 (m, 1H), 4.31–4.41 (m, 1H), 4.45–4.54 (m, 1H), 5.05 (bs, 1H), 6.76–6.87 (m, 3H), 6.96–7.01 (m, 2H), 7.12–7.19 (m, 3H), 7.38 (dd, 1H,  $J = 7.7$  Hz,  $J = 1.7$  Hz).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz):  $\delta$  23.27, 24.72, 28.30, 36.19, 37.70, 48.84, 54.63, 55.96, 57.30, 64.67, 74.28, 80.21, 114.84, 122.28, 124.05, 128.15, 128.95, 129.08, 130.42, 132.34, 155.31, 157.28, 160.78, 170.72. HRMS (ESI)  $\text{C}_{30}\text{H}_{42}\text{N}_3\text{O}_5\text{S}$   $[\text{M} + \text{H}]^+$ : calcd. 556.2840, found 556.2847. Anal. Calcd. for  $\text{C}_{30}\text{H}_{41}\text{N}_3\text{O}_5\text{S}$ : C, 64.84; H, 7.44; N, 7.56; Found: C, 65.10; H, 7.25; N, 7.62.

4.1.2.2. Procedure B: Procedure for amide bond formation from amine (*R*)-**10** and Boc-Lys(Boc)-OSu: Synthesis of 2(*S*), 6-di(*tert*-butoxycarbonylamino)-*N*-[3,4-dihydro-2*H*-1,5-benzoxathiepin-(3*R*)-yl]hexanoamide (**19**). To a solution of Boc-Lys(Boc)-OSu (665 mg, 1.50 mmol) in  $\text{CH}_2\text{Cl}_2$  (40 mL), cooled to 0 °C in an ice bath, was added a solution of (*R*)-**10** (1.24 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 mL). After stirring at rt for 24 h, the reaction mixture was washed with a 5% aqueous solution of  $\text{NaHCO}_3$  (3 × 30 mL) followed by a 5% aqueous solution of  $\text{KHSO}_4$  (3 × 30 mL). The organic phase was dried ( $\text{MgSO}_4$ ), filtered and evaporated to dryness. The residue was purified by column chromatography (eluent: hexane/EtOAc: 1/1) to provide **19** as a white solid (621 mg, 1.21 mmol, 98% yield). Mp: 61–63 °C.  $[\alpha]_{\text{D}}^{25}$ : +19.6 ( $c = 0.90$ , acetone). IR (KBr) v: 3333, 1703, 1694  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  1.32–1.53 (m, 4H), 1.37 (s, 18H), 1.57–1.69 (m, 1H), 1.76–1.84 (m, 1H), 2.85 (ddd, 1H,  $J = 14.4$  Hz,  $J = 5.3$  Hz,  $J = 1.4$  Hz), 2.98 (dd, 1H,  $J = 14.4$  Hz,  $J = 2.8$  Hz), 3.02–3.12 (m, 2H), 3.86 (dd, 1H,  $J = 12.6$  Hz,  $J = 2.0$  Hz), 4.02–4.13 (m, 1H), 4.26 (ddd, 1H,  $J = 12.5$  Hz,  $J = 3.6$  Hz,  $J = 1.3$  Hz), 4.46–4.52 (m, 1H), 4.60 (bs, 1H), 5.16 (bs, 1H), 6.91–6.99 (m, 1H), 7.06 (d, 1H,  $J = 8.9$  Hz), 7.10–7.15 (m, 1H), 7.36 (dd, 1H,  $J = 7.7$  Hz,  $J = 1.7$  Hz).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz):  $\delta$  22.71, 28.42, 28.56, 29.78, 32.18, 36.34,

39.98, 48.85, 54.70, 74.58, 79.22, 80.17, 122.42, 124.22, 128.35, 129.11, 132.53, 155.80, 156.27, 161.04, 171.74. HRMS (ESI)  $C_{25}H_{39}N_3NaO_6S$   $[M+Na]^+$ : calcd. 532.2452, found 532.2476. Anal. Calcd. for  $C_{25}H_{39}N_3O_6S$ : C, 58.92; H, 7.71; N, 8.24; Found: C, 58.55; H, 7.59; N, 8.49.

**4.1.2.3. Procedure C for Boc deprotection.** Compounds **13–16** and **19** (0.65 mmol) were treated with a 3 M HCl solution in cyclopentyl methyl ether (5 mL) and the reaction mixture was stirred at rt for 3 to 5 h. After completion, the solvent was evaporated to dryness and the resulting product was purified as indicated in each case, to yield **20–22**, **25** and **26**.

**4.1.2.3.1. (2S)-[3,4-Dihydro-2H-1,5-benzoxathiepin-(3R)-yl]pyrrolidine-2-carboxamide hydrochloride (20).** The resulting residue was taken up in water and lyophilized. After lyophilization, the solid was triturated with diethyl ether and filtered at reduced pressure to yield **20** as a white solid (188 mg, 0.60 mmol, 92% yield). Mp: 170–172 °C.  $[\alpha]_D^{27}$ : +1.9 ( $c = 0.60$ , methanol). IR (KBr)  $\nu$ : 3500–2500, 1665  $cm^{-1}$ .  $^1H$  NMR (MeOH- $d_4$ , 400 MHz):  $\delta$  2.02–2.11 (m, 3H), 2.46–2.54 (m, 1H), 3.02 (dd, 1H,  $J = 14.3$  Hz,  $J = 6.8$  Hz), 3.14 (dd, 1H,  $J = 14.3$  Hz,  $J = 3.6$  Hz), 3.34–3.39 (m, 1H), 3.41–3.46 (m, 1H), 4.17 (dd, 1H,  $J = 12.6$  Hz,  $J = 5.0$  Hz), 4.25 (dd, 1H,  $J = 12.5$  Hz,  $J = 3.0$  Hz), 4.35–4.38 (m, 1H), 4.46–4.49 (m, 1H), 6.98–7.03 (m, 2H), 7.17–7.21 (m, 1H), 7.37 (dd, 1H,  $J = 7.7$  Hz,  $J = 1.7$  Hz).  $^{13}C$  NMR (MeOH- $d_4$ , 100 MHz):  $\delta$  25.07, 31.34, 36.13, 47.49, 52.25, 61.14, 74.81, 123.05, 124.96, 129.01, 129.76, 132.94, 161.63, 169.37. HRMS (ESI)  $C_{14}H_{19}N_2O_2S$   $[M+H]^+$ : calcd. 279.1162, found 279.1160.

**4.1.2.3.2. (2S)-Amino-N-[3,4-dihydro-2H-1,5-benzoxathiepin-(3R)-yl]-3-phenylpropionamide (21).** The resulting residue was dissolved ( $H_2O$ ) and washed ( $CH_2Cl_2$ ). Lyophilization of the aqueous phase yielded **21** as a white solid (179 mg, 0.49 mmol, 75% yield). Mp: 109–111 °C.  $[\alpha]_D^{26}$ : +78.4 ( $c = 0.60$ , methanol). IR (KBr)  $\nu$ : 3500–2500, 1672  $cm^{-1}$ .  $^1H$  NMR (MeOH- $d_4$ , 400 MHz):  $\delta$  2.94 (dd, 1H,  $J = 14.3$ ,  $J = 6.9$  Hz), 3.10 (dd, 1H,  $J = 14.3$ ,  $J = 3.7$  Hz), 3.11–3.21 (m, 2H), 3.84 (dd, 1H,  $J = 12.5$ ,  $J = 5.3$  Hz), 4.07 (dd, 1H,  $J = 12.5$ ,  $J = 3.1$  Hz), 4.17 (t, 1H,  $J = 7.5$  Hz), 4.34–4.39 (m, 1H), 6.96–7.00 (m, 2H), 7.15–7.19 (m, 1H), 7.30–7.40 (m, 6H).  $^{13}C$  NMR (MeOH- $d_4$ , 100 MHz):  $\delta$  36.14, 38.81, 51.94, 55.67, 74.57, 123.01, 124.91, 128.84, 128.92, 129.71, 130.08, 130.59, 132.89, 135.66, 161.50, 169.10. HRMS (ESI)  $C_{18}H_{21}N_2O_2S$   $[M+H]^+$ : calcd. 329.1318, found 329.1311.

**4.1.2.3.3. (2S)-Amino-[3,4-dihydro-2H-1,5-benzoxathiepin-(3R)-yl]-4-methylpentanoamide hydrochloride (22).** The resulting residue was taken up ( $H_2O$ ). After lyophilization, the solid was dissolved ( $H_2O$ ) and washed ( $Et_2O$ ). Evaporation of the aqueous phase yielded **22** as a white solid (199 mg, 0.60 mmol, 92% yield). Mp: 114–116 °C.  $[\alpha]_D^{28}$ : +40.5 ( $c = 0.70$ , methanol). IR (KBr)  $\nu$ : 3500–2700, 1667  $cm^{-1}$ .  $^1H$  NMR ( $D_2O$ , 400 MHz):  $\delta$  0.97 (d, 3H,  $J = 6.4$  Hz), 0.99 (d, 3H,  $J = 6.4$  Hz), 1.66–1.85 (m, 3H), 3.00 (dd, 1H,  $J = 14.4$  Hz,  $J = 6.3$  Hz), 3.14 (dd, 1H,  $J = 14.5$  Hz,  $J = 3.1$  Hz), 4.09–4.16 (m, 2H), 4.22 (dd, 1H,  $J = 12.8$  Hz,  $J = 4.8$  Hz), 4.47–4.51 (m, 1H), 7.10–7.14 (m, 2H), 7.29–7.33 (m, 1H), 7.50 (dd, 1H,  $J = 7.6$  Hz,  $J = 1.7$  Hz).  $^{13}C$  NMR (MeOH- $d_4$ , 100 MHz):  $\delta$  21.16, 23.11, 25.54, 36.10, 41.86, 52.12, 53.03, 74.79, 123.06, 124.95, 128.95, 129.75, 132.93,

161.61, 170.38. HRMS (ESI)  $C_{15}H_{23}N_2O_2S$   $[M+H]^+$ : calcd. 295.1475, found 295.1484.

**4.1.2.3.4. (2S)-Amino-5-guanidino-N-[3,4-dihydro-2H-1,5-benzoxathiepin-(3R)-yl]pentanoamide dihydrochloride (23).** The resulting residue was taken up ( $H_2O$ ). After lyophilization, the solid was dissolved in water and washed with diethyl ether. Evaporation of the aqueous phase yielded **23** as a white solid (259 mg, 0.63 mmol, 97% yield). Mp: 109–111 °C.  $[\alpha]_D^{28}$ : +30.2 ( $c = 0.60$ , methanol). IR (KBr)  $\nu$ : 3500–2500, 1664  $cm^{-1}$ .  $^1H$  NMR ( $D_2O$ , 400 MHz): 1.71–1.78 (m, 2H), 1.99–2.05 (m, 2H), 3.02 (dd, 1H,  $J = 14.5$  Hz,  $J = 5.9$  Hz), 3.15 (dd, 1H,  $J = 14.5$  Hz,  $J = 3.1$  Hz), 3.28 (t, 2H,  $J = 6.8$  Hz), 4.13 (dd, 1H,  $J = 12.8$  Hz,  $J = 2.5$  Hz), 4.19 (t, 1H,  $J = 6.6$  Hz), 4.28 (dd, 1H,  $J = 12.7$  Hz,  $J = 4.8$  Hz), 4.47–4.57 (m, 1H), 7.11–7.16 (m, 2H), 7.33 (ddd, 1H,  $J = 8.1$  Hz,  $J = 7.3$  Hz,  $J = 1.7$  Hz), 7.51 (dd, 1H,  $J = 7.7$  Hz,  $J = 1.7$  Hz).  $^{13}C$  NMR (MeOH- $d_4$ , 100 MHz):  $\delta$  25.51, 29.84, 36.09, 41.78, 52.19, 53.97, 74.77, 123.04, 124.95, 129.00, 129.75, 132.94, 158.54, 161.55, 169.50. HRMS (ESI)  $C_{15}H_{24}N_5O_2S$   $[M+H]^+$ : calcd. 338.1645, found 338.1643.

**4.1.2.3.5. {2-Amino-N-[3,4-dihydro-2H-1,5-benzoxathiepin-(3R)-yl]-3-[4-piperidin-1-ylethoxy]phenyl}propionamide (25).** To the resulting residue, a saturated solution of  $NaHCO_3$  was added and extracted ( $EtOAc$ : 3  $\times$  15 mL). The combined organic extracts were dried ( $MgSO_4$ ), filtered and evaporated to dryness. The crude compound was purified by column chromatography ( $CH_2Cl_2/MeOH$ : 8:2) to afford **25** as a white solid (287 mg, 0.63 mmol, 97% yield). Mp: 96–98 °C.  $[\alpha]_D^{18}$ : +30.9 ( $c = 0.50$ , methanol). IR (KBr)  $\nu$ : 3406, 3351, 1645  $cm^{-1}$ .  $^1H$  NMR ( $CDCl_3$ , 400 MHz):  $\delta$  1.42–1.48 (m, 2H), 1.63 (tt, 4H,  $J = 5.6$  Hz), 1.81 (bs, 2H), 2.49–2.62 (m, 4H), 2.74 (dd, 1H,  $J = 13.8$  Hz,  $J = 8.9$  Hz), 2.81 (t, 2H,  $J = 6.0$  Hz), 2.86 (ddd, 1H,  $J = 14.4$  Hz,  $J = 5.4$  Hz,  $J = 1.3$  Hz), 3.04 (dd, 1H,  $J = 14.3$  Hz,  $J = 2.9$  Hz), 3.16 (dd, 1H,  $J = 13.8$  Hz,  $J = 4.1$  Hz), 3.64 (dd, 1H,  $J = 8.9$  Hz,  $J = 4.1$  Hz), 3.96 (dd, 1H,  $J = 12.5$  Hz,  $J = 2.2$  Hz), 4.12 (t, 2H,  $J = 6.0$  Hz), 4.30 (ddd, 1H,  $J = 12.5$  Hz,  $J = 3.8$  Hz,  $J = 1.2$  Hz), 4.52–4.57 (m, 1H), 6.82–6.87 (m, 2H), 6.96–7.03 (m, 2H), 7.11–7.19 (m, 3H), 7.40 (dd, 1H,  $J = 7.7$  Hz,  $J = 1.7$  Hz), 8.22 (d, 1H,  $J = 9.1$  Hz).  $^{13}C$  NMR ( $CDCl_3$ , 100 MHz):  $\delta$  24.14, 25.82, 36.57, 40.26, 48.47, 55.07, 56.67, 57.93, 65.85, 74.74, 114.92, 122.38, 124.14, 128.45, 128.99, 129.81, 130.42, 132.47, 157.82, 160.98, 173.95. HRMS (ESI)  $C_{25}H_{34}N_3O_3S$   $[M+H]^+$ : calcd. 456.2315, found 456.2309.

**4.1.2.3.6. 2(S)-6-Diamino-[3,4-dihydro-2H-1,5-benzoxathiepin-(3R)-yl]hexanoamide dihydrochloride (26).** The resulting residue was taken up ( $H_2O$ ). After lyophilization, the solid was dissolved ( $H_2O$ ) and washed ( $Et_2O$ ). Evaporation of the aqueous phase yielded **26** as a white solid (245 mg, 0.59 mmol, 90% yield). Mp: 71–73 °C.  $[\alpha]_D^{27}$ : +32.8 ( $c = 0.50$ , methanol). IR (KBr)  $\nu$ : 3500–2500, 1677  $cm^{-1}$ .  $^1H$  NMR (MeOH- $d_4$ , 400 MHz):  $\delta$  1.05–1.58 (m, 2H), 1.71–1.78 (m, 2H), 1.86–2.01 (m, 2H), 2.92–3.01 (m, 2H), 3.02 (dd, 1H,  $J = 14.4$  Hz,  $J = 6.7$  Hz), 3.16 (dd, 1H,  $J = 14.4$  Hz,  $J = 3.6$  Hz), 4.01 (t, 1H,  $J = 6.6$  Hz), 4.22–4.23 (m, 2H), 4.45–4.50 (m, 1H), 6.99–7.03 (m, 2H), 7.18–7.22 (m, 1H), 7.39 (dd, 1H,  $J = 7.6$  Hz,  $J = 1.7$  Hz).  $^{13}C$  NMR (MeOH- $d_4$ , 100 MHz):  $\delta$  22.96, 28.08, 32.18, 36.08, 40.29, 52.17, 54.12, 74.76, 123.02, 124.96, 129.03, 129.76, 132.96, 161.59, 169.66. HRMS (ESI)  $C_{15}H_{24}N_3O_2S$   $[M+H]^+$ : calcd. 310.1584, found 310.1585.

**4.1.2.4. Procedure D for Boc deprotection: Synthesis of (4*S*)-amino-4-[3,4-dihydro-2*H*-1,5-benzoxathiepin-(3*R*)-yl]-carbamoyl]butyric acid trifluoroacetate (**24**).** Compound **17** (0.65 mmol) was treated with trifluoroacetic acid (TFA) in CH<sub>2</sub>Cl<sub>2</sub> (3/4.5 mL) and the reaction mixture was stirred at rt for 4 h. After evaporation of the solvent, the residue was taken up (H<sub>2</sub>O) and washed (Et<sub>2</sub>O, 3 × 15 mL). Lyophilization of the aqueous phase yielded **24** as a white solid (272 mg, 0.64 mmol, 98% yield). Mp: 73–75 °C. [ $\alpha$ ]<sub>D</sub><sup>28</sup>: +42.4 (*c* = 0.50, methanol). IR (KBr)  $\nu$ : 3500–2500, 1690, 1665 cm<sup>-1</sup>. <sup>1</sup>H NMR (MeOH-*d*<sub>4</sub>, 400 MHz):  $\delta$  2.11–2.21 (m, 2H), 2.52 (t, 2H, *J* = 7.4 Hz), 3.00 (dd, 1H, *J* = 14.4 Hz, *J* = 6.7 Hz), 3.14 (dd, 1H, *J* = 14.3 Hz, *J* = 3.6 Hz), 4.03 (t, 1H, *J* = 6.4 Hz), 4.18–4.26 (m, 2H), 4.44–4.49 (m, 1H), 6.97–7.03 (m, 2H), 7.16–7.21 (m, 1H), 7.37 (dd, 1H, *J* = 7.7 Hz, *J* = 1.7 Hz). <sup>13</sup>C NMR (MeOH-*d*<sub>4</sub>, 100 MHz):  $\delta$  27.98, 30.72, 36.20, 52.03, 53.86, 74.78, 123.09, 124.96, 129.05, 129.77, 132.96, 161.71, 169.51, 176.20. HRMS (ESI) C<sub>14</sub>H<sub>18</sub>N<sub>2</sub>NaO<sub>4</sub>S [M + Na]<sup>+</sup>: calcd. 333.0879, found 333.0876.

#### 4.1.3. Synthesis of an amide and a *p*-nitrobenzenesulfonamide of (*R*)-**10**

**4.1.3.1. *N*-(3,4-Dihydro-2*H*-1,5-benzoxathiepin-(3*R*)-yl)acetamide [(*R*)-**11**].** Acetic anhydride (5 mL) was added to a solution of (*R*)-**10** (260 mg, 1.44 mmol) in formic acid (16 mL) at 0 °C. After 15 min the reaction mixture was warmed to rt and was stirred for further 16 h. Then, the solvent was removed and the reaction mixture was extracted with EtOAc. The organic layer was washed with H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated *in vacuo*. The crude was purified by column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH: 9.5/0.5) to afford (*R*)-**11** (222 mg, 85%), as a microcrystalline white solid. Mp 220–222 °C; [ $\alpha$ ]<sub>D</sub><sup>26</sup>: +18.3 (*c* = 1, in MeOH); <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  8.14 (1H, d, *J* = 7.5 Hz, NH), 7.32 (dt, *J* = 7.9, 1.5 Hz, 1H), 7.22–7.14 (m, 1H), 7.03–6.94 (m, 2H), 4.34–4.24 (2H, m, 2H), 3.94 (dd, *J* = 12.9, 6.6 Hz, 1H), 3.09 (dd, *J* = 14.1, 3.9 Hz, 1H), 2.86 (dd, *J* = 14.1, 7.5 Hz, 1H), 1.85 (d, *J* = 1.2 Hz, 3H). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  168.82, 159.25, 131.37, 128.29, 126.98, 123.54, 121.74, 73.64, 49.79, 34.60, 22.54. HRMS *m/z* [M + Na]<sup>+</sup> calcd for C<sub>11</sub>H<sub>13</sub>NNaO<sub>2</sub>S: 246.2798, found: 246.2796. Anal. Calc. for C<sub>11</sub>H<sub>13</sub>NO<sub>2</sub>S: C, 59.17; H, 5.87; N, 6.27. Found: C, 59.21; H, 5.99; N, 6.15.

**4.1.3.2. *N*-[3,4-Dihydro-2*H*-1,5-benzoxathiepin-(3*R*)-yl]-*p*-nitrobenzenesulfonamide [(*R*)-**12**].** A solution of (*R*)-**10** in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) at rt was added to a round-bottom flask (250 mL) that contained a solution of *p*-nitrobenzenesulfonyl chloride (344 mg, 1.55 mmol), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI, 642 mg, 3.3 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (15 mL). The resulting mixture was stirred at rt for 12 h, then cooled to 5 °C, and acidified to pH 1 with addition of an aqueous HCl solution (10%), which was followed by extraction with CH<sub>2</sub>Cl<sub>2</sub>/MeOH (9:1, 3 × 100 mL). The combined organic layers were washed with H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated *in vacuo*. The mixture was purified by flash chromatography (hexane/EtOAc: 1/0.5) to obtain (*R*)-**12** (183 mg, 33%) as a white solid; mp 160–162 °C; [ $\alpha$ ]<sub>D</sub><sup>26</sup>: +11.5 (*c* = 1, in MeOH); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  8.41–8.37 (m, 2H), 8.16–8.12 (m, 2H), 7.41 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.23–7.17 (m, 1H), 7.04–6.99 (m, 2H), 6.07 (d, *J* = 9.9 Hz, 1H, NH), 4.19 (ddd, *J* = 12.6,

3.7, 1.6 Hz, 1H), 4.09–4.03 (m, 1H), 3.76 (dd, *J* = 12.5, 1.9 Hz, 1H), 2.96 (dd, *J* = 14.4, 2.5 Hz, 1H), 2.78 (ddd, *J* = 14.5, 5.0, 1.6 Hz, 1H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>):  $\delta$  160.97, 150.27, 147.43, 132.81, 129.62, 128.28, 128.00, 124.70, 122.51, 74.90, 53.28, 37.62. HRMS *m/z* [M + H]<sup>+</sup> calcd for C<sub>15</sub>H<sub>15</sub>N<sub>2</sub>O<sub>5</sub>S<sub>2</sub>: 367.0422, found: 367.0423. Anal. Calc. for C<sub>15</sub>H<sub>14</sub>N<sub>2</sub>O<sub>5</sub>S<sub>2</sub>: C, 49.17; H, 3.85; N, 7.65. Found: C, 49.32; H, 3.95; N, 7.48.

**4.1.3.3. 6-Chloro-4-*N*-[3,4-dihydro-2*H*-1,5-benzoxathiepin-(3*R*)-yl]pyrimidine-4,5-diamine [(*R*)-**27**].** 4,6-Dichloropyrimidin-5-amine (133 mg, 0.81 mmol) and Et<sub>3</sub>N (0.44 mL, 0.0032 mmol) were added to a solution of (*R*)-**10** (100 mg, 0.55 mmol) in *n*-BuOH, and the reaction mixture was heated under reflux and stirred for 24 h under an atmosphere of dry argon. The solvent was evaporated *in vacuo*, and the reaction mixture was extracted (EtOAc). The organic layer was washed with H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated *in vacuo*. The crude was purified by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH: 9.9/0.1) to isolate (*R*)-**27** as a yellowish syrup (340 mg, 52%), [ $\alpha$ ]<sub>D</sub><sup>26</sup>: +38.4 (*c* = 1, in MeOH). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  8.06 (s, 1H), 7.47 (dd, *J* = 7.7, 1.7 Hz, 1H), 7.22 (ddd, *J* = 9.1, 7.4, 1.7 Hz, 1H), 7.07 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.03 (dt, *J* = 7.5, 1.4 Hz, 1H), 6.06 (d, *J* = 9.1 Hz, 1H, NH), 4.86 (ddd, *J* = 8.0, 4.8, 2.8 Hz, 1H), 4.50 (ddd, *J* = 12.5, 3.5, 1.4 Hz, 1H), 3.99 (dd, *J* = 12.5, 1.8 Hz, 1H), 3.13 (dd, *J* = 14.4, 2.8 Hz, 1H), 3.08 (ddd, *J* = 14.3, 4.9, 1.5 Hz, 1H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>):  $\delta$  161.35, 153.60, 149.18, 143.06, 132.81, 129.38, 128.73, 124.47, 122.72, 122.54, 74.88, 50.17, 36.51. HRMS *m/z* [M + H]<sup>+</sup> calcd for C<sub>13</sub>H<sub>14</sub>N<sub>4</sub>O<sub>2</sub>SCl: 309.0577, found: 309.0569.

Anal. Calc. for C<sub>13</sub>H<sub>13</sub>N<sub>4</sub>O<sub>2</sub>SCl: C, 50.57; H, 4.24; N, 18.14. Found: C, 50.40; H, 4.35; N, 17.99.

**4.1.3.4. 6-Chloro-9-[3,4-dihydro-2*H*-1,5-benzoxathiepin-(3*R*)-yl]-9*H*-purine [(*R*)-**28**].** A solution of (*R*)-**27** (654 mg, 2.05 mmol) in triethyl orthoformate (30 mL) was stirred while concentrated HCl (37%, 1.3 mL) was added in one portion; then the reaction mixture was stirred at rt for 24 h under an atmosphere of dry argon. The solvent was removed and the residue partitioned between CH<sub>2</sub>Cl<sub>2</sub> (50 mL) and H<sub>2</sub>O (50 mL), the phases were separated and the organic layer was washed with H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated *in vacuo*. The crude was purified by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH: 9/0.1) to afford (*R*)-**28** as a yellowish solid (392 mg, 60%); mp 152–154 °C; [ $\alpha$ ]<sub>D</sub><sup>26</sup>: +24.2 (*c* = 1, in MeOH). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  8.93 (s, 1H), 8.75 (s, 1H), 7.44 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.29–7.24 (m, 1H), 7.13 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.07 (td, *J* = 7.6, 1.4 Hz, 1H), 5.49–5.40 (m, 1H), 4.77–4.64 (m, 2H), 3.4–3.31 (m, 2H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>):  $\delta$  159.86, 152.00, 151.58, 151.42, 145.12, 132.33, 131.41, 129.34, 126.97, 124.73, 122.41, 73.54, 55.15, 35.95. HRMS *m/z* [M + H]<sup>+</sup> calcd for C<sub>14</sub>H<sub>12</sub>N<sub>4</sub>O<sub>2</sub>SCl: 319.0415, found: 319.0412. Anal. Calc. for C<sub>14</sub>H<sub>11</sub>N<sub>4</sub>O<sub>2</sub>SCl: C, 52.75; H, 3.48; N, 17.58. Found: C, 52.91; H, 3.52; N, 17.48.

**4.1.3.5. 9-[3,4-Dihydro-2*H*-1,5-benzoxathiepin-(3*R*)-yl]-9*H*-purin-6-ol [(*R*)-**29**] and 9-[3,4-dihydro-2*H*-1,5-benzoxathiepin-(3*R*)-yl]-9*H*-purin-6-amine [(*R*)-**30**].** A solution of (*R*)-**28** (100 mg, 0.3 mmol) in 1,4-dioxane (5 mL) was



heated, and  $\text{NH}_4\text{OH}$  (10 mL) was added. The reaction mixture was stirred under reflux for 6 h, then the solvent was removed *in vacuo* and the residue was purified by column chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$ : 9.9/0.1) to give (*R*)-**29** and (*R*)-**30**.

(*R*)-**29**: White solid (13 mg, 14%); mp 140–142 °C;  $[\alpha]_{\text{D}}^{26}$ : +61.0 ( $c = 1$ , in MeOH).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  12.89 (s, 1H, OH), 8.50 (s, 1H), 8.20 (s, 1H), 7.39 (dd,  $J = 7.7$ , 1.6 Hz, 1H), 7.22 (ddd,  $J = 8.1$ , 7.3, 1.7 Hz, 1H), 7.09 (dd,  $J = 8.1$ , 1.3 Hz, 1H), 7.04 (dt,  $J = 7.5$ , 1.4 Hz, 1H), 5.28–5.26 (m, 1H), 4.74 (d,  $J = 13.3$  Hz, 1H), 4.64 (dd,  $J = 13.1$ , 3.4 Hz, 1H), 3.44 (dd,  $J = 14.6$ , 7.5 Hz, 1H), 3.33 (dd,  $J = 14.5$ , 4.4 Hz, 1H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  159.42, 159.21, 148.75, 145.09, 139.72, 131.96, 128.95, 126.62, 124.38, 122.24, 110.15, 73.45, 55.27, 35.88. HRMS  $m/z$   $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{14}\text{H}_{13}\text{N}_4\text{O}_2\text{S}$ : 301.0754, found: 301.0750. Anal. Calc. for  $\text{C}_{14}\text{H}_{12}\text{N}_4\text{O}_2\text{S}$ : C, 55.99; H, 4.03; N, 18.66. Found: C, 56.11; H, 4.12; N, 18.49.

(*R*)-**30**: Yellowish solid (24 mg, 25%); mp 231–233 °C;  $[\alpha]_{\text{D}}^{26}$ : +67.8 ( $c = 1$ , in MeOH).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.61 (s, 1H), 8.35 (s, 1H), 7.42 (dd,  $J = 7.8$ , 1.7 Hz, 1H), 7.25–7.22 (m, 1H), 7.11 (dd,  $J = 8.1$ , 1.4 Hz, 1H), 7.05 (dt,  $J = 7.5$ , 1.4 Hz, 1H), 6.18 (s, 2H,  $\text{NH}_2$ ), 5.33 (dd,  $J = 7.1$ , 3.3 Hz, 1H), 4.67 (d,  $J = 3.1$  Hz, 2H), 3.44–3.31 (m, 2H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  152.41, 151.15, 146.77, 140.90, 132.18, 129.11, 127.05, 125.95, 124.53, 122.32, 121.97, 73.65, 54.80, 35.98. HRMS  $m/z$   $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{14}\text{H}_{14}\text{N}_5\text{OS}$   $[\text{M} + \text{H}]^+$ : 300.0914, found 300.920. Anal. Calc. for  $\text{C}_{14}\text{H}_{13}\text{N}_5\text{OS}$ : C, 56.17; H, 4.38; N, 23.40. Found: C, 56.01; H, 4.23; N, 23.10.

4.1.3.6. 9-[3,4-Dihydro-2H-1,5-benzoxathiepin-(3*R*)-yl]-*N,N*-dimethyl-9*H*-purin-6-amine [(*R*)-**31**]. A mixture of (*R*)-**28** (100 mg, 0.3 mmol) and  $\text{KO}^t\text{Bu}$  (56 mg, 0.5 mmol) in DMF (5 mL) was heated under microwave irradiation at 180 °C for 25 min. The reaction mixture was evaporated *in vacuo*, and the residue was purified by flash chromatography (hexane/EtOAc: 1/1) to afford (*R*)-**31** as a colorless syrup (50 mg, 50%),  $[\alpha]_{\text{D}}^{26}$ : +91.6 ( $c = 1$ , in MeOH).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.40 (s, 1H, 1H), 8.34 (s, 1H), 7.38 (dd,  $J = 7.7$ , 1.6 Hz, 1H), 7.20 (ddd,  $J = 8.1$ , 7.3, 1.7 Hz, 1H), 7.07 (dd,  $J = 8.1$ , 1.4 Hz, 1H), 7.02 (dt,  $J = 7.5$ , 1.4 Hz, 1H), 5.32 (s, 1H), 4.72–4.59 (m, 2H), 3.55 (s, 6H,  $\text{N}(\text{CH}_3)_2$ ), 3.41 (dd,  $J = 14.6$ , 7.0 Hz, 1H), 3.31 (dd,  $J = 14.5$ , 3.9 Hz, 1H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  159.58, 154.84, 152.08, 150.07, 137.75, 131.96, 128.80, 126.94, 124.24, 122.21, 119.79, 73.66, 54.50, 38.84 ( $\text{CH}_3$ )<sub>2</sub>, 35.78. HRMS  $m/z$   $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{16}\text{H}_{18}\text{N}_5\text{OS}$ : 328.1227, found: 328.1228. Anal. Calc. for  $\text{C}_{16}\text{H}_{17}\text{N}_5\text{OS}$ : C, 58.70; H, 5.23; N, 21.39. Found: C, 58.75; H, 5.27; N, 21.23.

4.1.3.7. 6-Chloro-9-[5,5-dioxide-3,4-dihydro-2H-1,5-benzoxathiepin-(3*R*)-yl]-9*H*-purine [(*R*)-**32**]. Potassium peroxy-monosulfate (Oxone™, 470 mg, 3.1 mmol) in  $\text{H}_2\text{O}$  (2 mL) was added to a solution of (*R*)-**28** (100 mg, 0.31 mmol) in MeOH (6 mL) and the resulting suspension was stirred at rt for 2 h. After filtration and washing with  $\text{H}_2\text{O}$  and  $\text{CH}_2\text{Cl}_2$ , the residue was recrystallized from  $\text{Et}_2\text{O}$  to yield (*R*)-**32** (44 mg, 40%) as a white solid; mp: 185–187 °C;  $[\alpha]_{\text{D}}^{26}$ : +35.0 ( $c = 1$ , in MeOH).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.71 (d,  $J = 1.8$  Hz, 1H), 8.54 (s, 1H), 8.02 (dd,  $J = 7.9$ , 1.7 Hz, 1H), 7.67 (ddd,  $J = 8.1$ , 7.4, 1.7 Hz, 1H), 7.41 (dt,  $J = 7.7$ , 1.1 Hz, 1H), 7.32 (dd,  $J = 8.1$ , 1.1 Hz, 1H), 5.52 (d,  $J = 8.1$  Hz, 1H), 4.93–4.83 (m, 1H), 4.70 (d,  $J = 13.0$  Hz,

1H), 4.25 (dd,  $J = 14.9$ , 8.7 Hz, 1H), 3.82 (dd,  $J = 15.0$ , 4.1 Hz, 1H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  156.36, 152.21, 151.93, 151.46, 144.69, 136.22, 134.16, 131.81, 128.44, 125.79, 123.51, 74.48, 56.27, 52.38. HRMS  $m/z$   $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{14}\text{H}_{12}\text{N}_4\text{O}_3\text{SCl}$ : 351.7845, found: 351.7849. Anal. Calc. for  $\text{C}_{14}\text{H}_{11}\text{N}_4\text{O}_3\text{SCl}$ : C, 47.94; H, 3.16; N, 15.97. Found: C, 48.01; H, 3.20; N, 15.85.

4.1.3.8. 2-({2-[6-Chloro-2-(tributylstannyl)-9*H*-purin-9-yl]prop-2-en-1-yl}thio)phenol (**33**). To a stirred solution of lithium 2,2,6,6-tetramethylpiperidine (LTMP, 2 g, 14 mmol) in dry THF (30 mL) was added (*R*)-**28** (884 mg, 2.8 mmol) at –78 °C under a positive pressure of dry argon. Following stirring at the same temperature for 10 min,  $\text{Bu}_3\text{SnCl}$  (3.8 mL, 14 mmol) was added. After 30 min of stirring at –70 °C, the reaction was quenched by adding an aqueous  $\text{NH}_4\text{Cl}$  solution, and the mixture was partitioned between a saturated aqueous  $\text{NaHCO}_3$  solution and EtOAc. The organic layer was purified by flash chromatography (hexane/EtOAc: 3/1) to obtain **33** (260 mg, 16%) as a yellowish syrup,  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.05 (s, 1H,  $\text{H}^8$ ), 7.21 (ddd,  $J = 7.7$ , 6.0, 1.7 Hz, 2H), 6.93 (dd,  $J = 8.2$ , 1.3 Hz, 1H), 6.77 (td,  $J = 7.5$ , 1.3 Hz, 1H), 6.56 (s, 1H, OH), 5.29 (d,  $J = 1.5$  Hz, 1H,  $\text{H}^{3''}$ ), 4.88 (d,  $J = 1.3$  Hz, 1H,  $\text{H}^{3''}$ ), 4.25–4.23 (m, 2H), 1.64–1.57 (m, 6H), 1.36 (dt,  $J = 14.8$ , 7.4 Hz, 6H), 1.26–1.14 (m, 6H), 0.89 (t,  $J = 7.3$  Hz, 9H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  182.78 ( $\text{C}^{2''}$ ), 157.37, 150.43, 149.92, 142.89, 138.17, 136.25, 132.00, 130.90, 120.94, 116.67, 115.37, 111.35, 38.73, 29.10 (t,  $^3J(^{13}\text{C}-^{119}\text{Sn})$  10.5 Hz,  $[\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2]_3\text{Sn}$ ), 27.44 (t,  $^2J(^{13}\text{C}-^{119}\text{Sn})$  27.1 Hz  $[\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2]_3\text{Sn}$ ), 13.88 ( $\text{CH}_3(\text{CH}_2)_3\text{Sn}$ ), 10.91 (t,  $^1J(^{13}\text{C}-^{119}\text{Sn})$  165.3 Hz,  $[\text{CH}_3(\text{CH}_2)_2\text{CH}_2]_3\text{Sn}$ ), 12.7  $[\text{CH}_3(\text{CH}_2)_3\text{Sn}$ ). HRMS  $m/z$   $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{26}\text{H}_{38}\text{N}_4\text{OSSnCl}$ : 609.1471, found: 609.1468. Anal. Calc. for  $\text{C}_{26}\text{H}_{37}\text{N}_4\text{OSSnCl}$ : C, 51.38; H, 6.14; N, 9.22. Found: C, 51.23; H, 5.99; N, 9.23.

4.1.3.9. 2-({2-[6-Chloro-2-iodo-9*H*-purin-9-yl]prop-2-en-1-yl}thio)phenol (**34**). A solution of **33** (216 mg, 0.35 mmol) and iodine (90 mg, 0.35 mmol) in dry THF (5 mL) was stirred at rt for 24 h under a nitrogen atmosphere. The reaction mixture was diluted with a 5% aqueous  $\text{Na}_2\text{S}_2\text{O}_3$  and extracted ( $\text{CH}_2\text{Cl}_2$ , 3 × 100 mL). The extract was washed with brine, dried ( $\text{Na}_2\text{SO}_4$ ), filtered and concentrated *in vacuo*. The mixture was purified by flash chromatography (hexane/EtOAc: 5/1) to give **34** (71 mg, 68%) as a yellowish solid; mp: 136–138 °C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.05 (s, 1H,  $\text{H}^8$ ), 7.21–7.14 (m, 2H), 6.90 (dd,  $J = 8.1$ , 1.3 Hz, 1H), 6.74 (dt,  $J = 7.5$ , 1.3 Hz, 1H), 6.36 (s, 1H, OH), 5.30 (d,  $J = 1.6$  Hz, 1H,  $\text{H}^{3''}$ ), 5.04 (d,  $J = 1.9$  Hz, 1H,  $\text{H}^{3''}$ ), 4.14 (s, 2H,  $\text{H}^{1''}$ ).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  156.95, 151.63, 150.94, 144.46, 138.00, 135.83, 132.15, 131.99, 121.03, 117.00, 116.35, 115.39, 112.95, 38.15. HRMS  $m/z$   $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{14}\text{H}_{12}\text{N}_4\text{OSClI}$ : 444.9381, found: 444.9391. Anal. Calc. for  $\text{C}_{14}\text{H}_{10}\text{N}_4\text{OSClI}$ : C, 37.81; H, 2.27; N, 12.60. Found: C, 37.68; H, 2.28; N, 12.45.

#### 4.2. Density functional theory (DFT) computations

Three-dimensional structures of the compounds were initially built using the online version of Corina ([https://www.mn-am.com/online\\_demos/corina\\_demo](https://www.mn-am.com/online_demos/corina_demo)), which ascribes to 3D



structures pre-defined bond lengths and angles depending on the type of bond, type of atom and hybridization state. Corina also defines the most probable torsional angles according to the nature of the structure (acyclic, small/medium rings, macro/polycyclic, etc.), being able to correctly reproduce a varied number of X-ray structures (Gasteiger et al., 1990). Transition ground state geometries were then calculated in Spartan 14 using the DFT B3LYP functional combined with the 6-31G\* basis set (Shao et al., 2006). Although Spartan allows some calculations to be performed in THF, we performed all the simulations in vacuum because our transition state structures displayed some elements not parameterized for solvation in THF.

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### Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.arabjc.2017.01.011>.

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