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Stereoselective access to allenic sphingosine analogues as SphK inhibitors

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Axially chiral allenes are distinctive structural motifs whose potential in sphingolipid-based inhibitor design remains largely unexplored. Herein, we describe a concise and stereodivergent synthetic strategy to access allenic sphingosine analogues, in which an allene unit is incorporated into the sphingoid backbone as a rigid surrogate of the native *trans*-olefin. The approach relies on a copper-promoted allenation of terminal alkynes (ATA reaction), enabling efficient access to 1,3-disubstituted allenes from propargylic alcohols and aliphatic aldehydes. Fine tuning of the catalytic system with achiral secondary amines afforded high-yielding allenation reactions that produced nearly equimolar mixtures of allenic diastereomers, while the use of either enantiomer of a chiral prolinol-derived secondary amines under double stereodifferentiating conditions enabled stereodivergent access to each allenic diastereoisomer with high selectivity. These chiral allene intermediates constitute versatile platforms for late-stage diversification of the polar head group, affording structurally well-defined sphingosine analogues. Preliminary inhibitory studies revealed that the allene scaffold is well tolerated and leads to low micromolar sphingosine kinase inhibitors, with a preference for SphK2.

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Introduction

Sphingosine kinases, SphK1 and SphK2, are enzymes that catalyse the conversion of sphingosine into sphingosine 1-phosphate, a promyogenic bioactive lipid involved in cellular signalling. Both isoforms are widely overexpressed in most cancers,¹ promoting tumour progression and being associated with bad clinical prognosis. Consequently, their inhibition has emerged as a promising strategy for cancer treatment.^{1c,2} In particular, inhibition of SphK1 may help overcome chemotherapeutic resistance.³ While the role of SphK2 in cancer⁴ is less understood than that of SphK1, increasing evidence suggests that targeting SphK2 may offer a more favorable anti-tumour pharmacological profile. Accordingly, significant efforts have been devoted to the identification of effective and selective sphingosine kinase inhibitors.⁵

Although SphK1 and SphK2 share the same catalytic function, they differ in subcellular localization, trafficking, and regulatory mechanisms,⁶ having distinct preferences for both natural and synthetic ligands. To date, only the structure of SphK1 has been resolved,⁷ whereas the structural determinants underlying the differential substrate selectivity of SphK2 remains poorly understood.

Homology studies revealed structural differences between SphK1 and SphK2,⁸ particularly within the J-shaped sphingosine-binding cavity, which may influence ligand recognition and selectivity. A key difference between the two isoforms is the substitution of Phe288 in SphK1 by Cys533 in SphK2 at the toe of the J-channel, resulting in a larger and more flexible hydrophobic pocket in SphK2. In contrast, variations in residues adjacent to the conserved leucine (Leu299 in SphK1 and Leu517 in SphK2) at the heel induce a localized contraction. Overall, SphK2 exhibits a bipartite remodelling of the binding pocket, with expansion at the toe and contraction at the heel relative to SphK1.⁹

In light of these structural differences, strategies to improve SphK2 selectivity might focus on designing inhibitors that preferentially engage the extended hydrophobic pocket of SphK2, while minimizing productive interactions with the more restricted toe region of SphK1. Furthermore, reinforcing polar interactions at the head of the binding pocket could lead to the development of more effective inhibitors.

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In this context, our research has focused on the development of synthetic methodologies for sphingoid bases¹⁰ aimed at generating sphingolipid-derived SphKs inhibitors.¹¹ Building on our previous work, this study explores the synthesis of allenic sphingosine analogues and their evaluation as SphK inhibitors.

Allenes are distinctive structural motifs present in over 150 natural products and pharmaceuticals,¹² highlighting their relevance in medicinal chemistry. Their characteristic cumulated π -system with orthogonal orbital arrangement, can generate axial chirality when the terminal substituents differ. This unique structural and electronic configuration imparts properties¹³ that clearly distinguish allenes from both conjugated and isolated alkenes, supporting their growing relevance in chemical biology and drug development.

The initial rationale for introducing an allene moiety was based on the hypothesis that replacing the native alkene linker of sphingosine with an allene, featuring a rigid central sp-hybridized carbon, could influence the spatial disposition of the ligand and favor its accommodation within the SphK2 binding pocket, which appears to tolerate structural variations more readily than that of SphK1, potentially contributing to enhanced isoform selectivity.

To the best of our knowledge, the only reported example of allenic sphingolipids was described by Chun *et al.*,¹⁴ who synthesised allenic ceramide analogues to probe their bioactivity and structure–activity relationships in therapeutic areas such as cancer and Alzheimer's disease. Notably, neither this study nor subsequent work has reported biological evaluation data, highlighting a clear gap in the functional characterization of this class of compounds.

This study investigates the impact of incorporating an allene moiety into sphingosine analogues on SphK inhibitory potency and SphK2/SphK1 selectivity. The designed allenic structures (Fig. 1) preserve the polar headgroup of sphingosine by retaining the essential (2*S*,3*R*)-2-amino-1,3-diol motif required for enzyme recognition. Maintenance of this chiral polar region allows the introduction of a 1,3-disubstituted allene linker as an alkene surrogate, thereby expanding structural diversity through the formation of two diastereomeric series arising from axial chirality. In addition, amino group modifications—such as dimethyl, guanidinium, or methyl-sulfonamide substituents—are expected to enhance polar interactions within the binding site and may also prevent

N-acylation, thereby reducing metabolic diversion into other sphingolipid pathways.

Herein, we report the stereoselective syntheses, SphK1/SphK2 inhibitory activities, and docking studies of axially chiral allenic sphingosine analogues (Fig. 1), featuring diverse derivatizations at the amino group.

Results and discussion

Synthesis of allenic sphingosine analogues

Previous studies of Chun *et al.*¹⁴ employed a directed LiAlH_4 -mediated reduction–elimination sequence from a propargylic alcohol intermediate as a key step for the construction of the allene moiety in their ceramide analogues. However, this transformation also yielded 15–20% of the corresponding allylic alcohol as a by-product, alongside the desired allenic alcohol.

In our synthetic design, access to target compounds **1–4a–b** (Scheme 1) was envisaged through deprotection and late-stage derivatization of common allenic intermediates **5a–b**. Among the various methodologies available for allene synthesis,¹⁵ the allenation of terminal alkynes (ATA reaction)¹⁶ was selected because of its broad functional group tolerance and its proven versatility for the preparation of optically active 1,3-disubstituted allenes through enantioselective protocols based on chiral ligands or secondary amines. This strategy was expected to provide a modular and stereodivergent route to allenic sphingosine analogues while avoiding the competing formation of allylic byproducts associated with reductive elimination approaches.

According to the mechanism proposed (Scheme 2),^{16b} ATA reaction is initiated by π -coordination of the metal salt (MX_n) to the terminal alkyne (**11**), which enhances the acidity of the terminal proton and facilitates its deprotonation by a mild base. Subsequent σ -coordination of the metal generates the 1-alkynyl metal intermediate **12**. This nucleophilic species then undergoes addition to the electrophilic iminium ion **13**—formed *in situ* from the aldehyde and dialkylamine—producing the propargylic amine **14** while regenerating the metal catalyst. Further coordination of MX_n to the alkynyl moiety gives complex **15**, which undergoes a 1,5-hydride shift followed by β -nitrogen elimination through intermediate (**16**), ultimately delivering the allene product **P1** along with the corresponding imine **P2**.

Based on the considerations outlined above, the allene moiety was envisioned to arise from the allenation of chiral

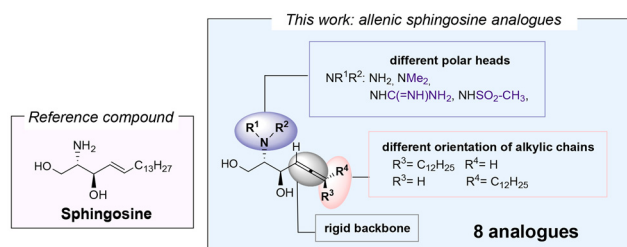
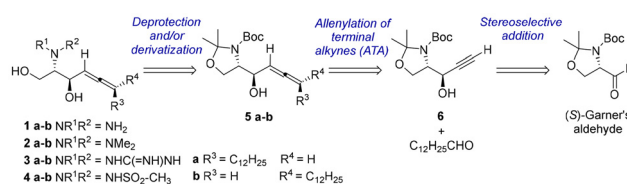
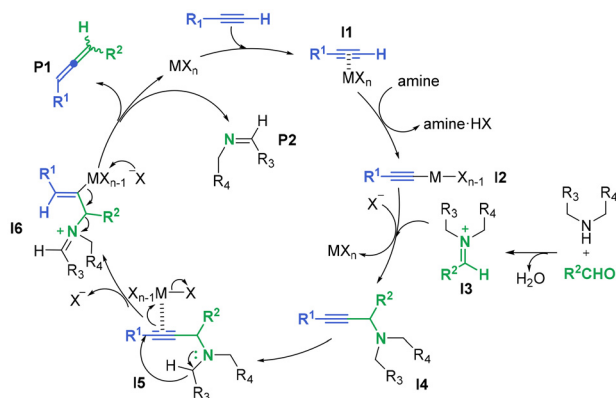


Fig. 1 Sphingosine allenic analogues proposed in this work.



Scheme 1 Retrosynthetic analysis of allenic sphingosine analogues **1–4a–b**.

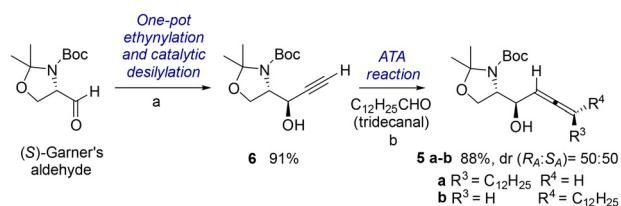


Scheme 2 Proposed mechanism for the metal-catalyzed conversion of terminal alkynes into allenes (ATA reaction).

terminal alkyne **6** with tridecanal (Scheme 1). In this design, the ATA reaction would take place on a stereochemically defined alkynyl substrate, thereby rendering the allenation process diastereoselective. The requisite *anti*-2,3-aminoalcohol framework was planned to be established beforehand through a diastereoselective nucleophilic addition of an alkynylide reagent to (*S*)-Garner's aldehyde, providing enantioenriched propargyl alcohol precursor required for stereocontrolled allene formation.

Compound **6**¹⁷ was then obtained through nucleophilic addition of lithium trimethylsilylacetylide¹⁸ to (*S*)-Garner's aldehyde using our previously developed *one-pot* protocol,¹⁰ⁱ which combines the ethynylation step with a subsequent catalytic desilylation.¹⁹ The addition, performed at $-55\text{ }^{\circ}\text{C}$, proceeded with excellent diastereoselectivity (*dr* = 98 : 2) and after purification, the desired propargylic alcohol **6** was isolated as a single diastereoisomer in 91% yield (Scheme 3). Parallely, tridecanal was obtained by oxidation of tridecan-1-ol with Dess–Martin periodinane, as previously described.²⁰

With both key fragments in hand, the allenation step was then explored using the ATA methodology under the most effective reported conditions to maximize reaction efficiency. A preliminary evaluation of reaction parameters quickly underscored the pronounced sensitivity of this transformation to Cu catalyst choice, reaction temperature and water content.



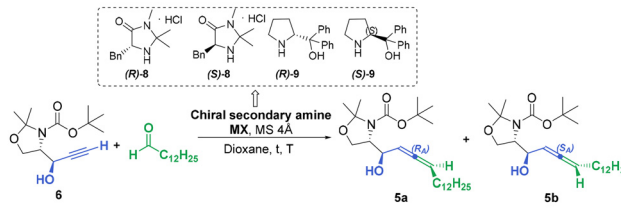
Scheme 3 Synthesis of allenic intermediates **5a–b**. Reagents and conditions: (a) (i) trimethylsilylacetylide, BuLi, HMPA/THF, $-55\text{ }^{\circ}\text{C}$. (ii) TBAF (0.1 equiv.)/THF, r.t., 91%.¹⁰ⁱ (b) Propargyl alcohol **6** (1 equiv.), tridecanal (1.6 equiv.), Bu₂NH (1.4 equiv.), CuI (0.5 equiv.), 4 Å MS (1 g mmol^{−1} starting material).

As summarized in Table S1 (SI), screening of several copper sources, including CuI, CuBr, CuCl and CuBr₂, identified CuI as the optimal catalyst. Among the salts examined, CuI delivered the allenic intermediates **5a** and **5b** in the highest combined yield (88%) and as a 1 : 1 mixture of axial diastereomers (Scheme 3). Under these optimized conditions—CuI (0.5 equiv.), Bu₂NH, dioxane at $150\text{ }^{\circ}\text{C}$ in the presence of 4 Å MS—the reaction consistently outperformed all other copper salts examined, which either resulted in reduced conversion or led to accumulation of the propargylamine intermediate (**14**, 7 in SI), indicating incomplete progression along the catalytic cycle toward the allene product.

To achieve allenation under milder temperature conditions, with the aim of enhancing stereoselectivity, alternative catalytic strategies were next explored. In particular, two-metallic systems have been shown to promote more efficient and selective allene formation at lower temperatures.²¹ These approaches rely on the use of two metal species with different oxidation states, each intended to maximize the efficiency of one of the two key steps of the ATA process. Specifically, metal species in the +1-oxidation state preferentially mediate formation of the propargylamine intermediate **14** (Scheme 2), whereas metal species in the +2-oxidation state efficiently promote its conversion to the allene product **P1** (Scheme 2). In this sense, literature-precedented two-metal systems (CuI/CuBr₂, CuI/ZnBr₂ and CuI/CdI₂) were explicitly evaluated (Table S2, SI); however, they consistently afforded lower allene yields than the corresponding monometallic CuI catalyst under all conditions examined. Thus, at moderate temperatures ($80\text{ }^{\circ}\text{C}$), these systems led predominantly to accumulation of the propargylamine intermediate (**14**, 7 in SI) indicating inefficient progression toward allene formation. Increasing the temperature to $150\text{ }^{\circ}\text{C}$ partially restored the allene pathway; however, isolated yields remained modest (18–38%) and consistently lower than those obtained with CuI alone. Across all the conditions tested under substrate control, a low degree of diastereocontrol was achieved.

With the monometallic CuI system established as the most promising platform, attention then turned to the stereodivergent synthesis of the diastereomers differing in the axial configuration of the allene moiety. Since the reaction under substrate-controlled conditions proceeded with poor diastereocontrol, a double diastereodifferentiation strategy based on the use of chiral secondary amines was envisaged to direct the formation of either allenic diastereomer selectively. In this context, MacMillan-type imidazolidinones (*R*)-**8** and (*S*)-**8**, despite their well-established ability to induce stereocontrol in a wide range of asymmetric transformations,²² resulted in low conversion in the ATA reaction (Table 1, entries 1 and 2).

In contrast, prolinol-derived amines, selected on the basis of their well-documented efficiency in enantioselective ATA (EATA) reactions,^{21b,fg,23} markedly improved reaction performance (Table 1, entries 3–6). In all cases, the products were obtained with excellent diastereoselectivity (*dr* > 95 : 5), dictated by the configuration of the chiral amine, and the yields reported in Table 1 correspond to the isolated diastereomer.

Table 1 Copper-catalysed allenation of propargyl alcohol **6** with tridecanal using secondary chiral amines^a


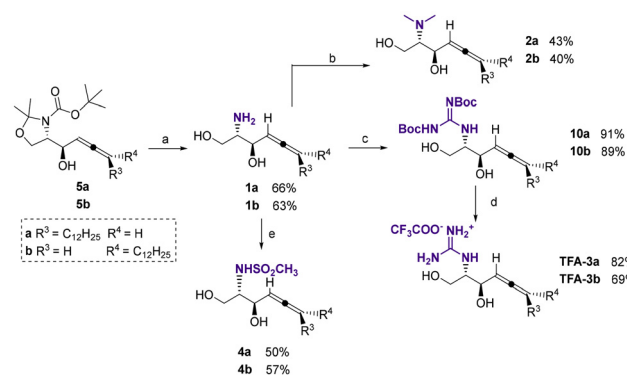
Entry	Chiral amines	MX	T (°C)	t (h)	Yield ^b (%)	
					5a	5b
1	(R)-8	CuI	150	15	—	24
2	(S)-8	CuI	150	15	28	—
3	(R)-9	CuBr ₂	130	15	—	50
4	(S)-9	CuBr ₂	130	15	54	—
5	(R)-9	CuI	150	17	—	78
6	(S)-9	CuI	150	17	30	—
7	(R)-9	CuI + ZnBr ₂	150	15	—	68
8	(S)-9	CuI + ZnBr ₂	150	17	59	—

^a Propargyl alcohol **6** (1 equiv.), tridecanal (1.6 equiv.), chiral secondary amine (1.4 equiv.), Cu salt (0.5 equiv.), ZnBr₂ (0.5 equiv.), 4 Å MS (1 g mmol⁻¹ starting material). ^b Reported yields correspond to the isolated major diastereomer. In all cases, the reactions proceeded with dr >95 : 5.

Notably, the combination of (S)-diphenyl(pyrrolidin-2-yl) methanol, (S)-9, with CuBr₂ at 130 °C provided the (S,R,R_A) allene **5a** in 54% yield (Table 1, entry 4), whereas the use of the enantiomeric (R)-9 with CuI at 150 °C enabled selective formation of the opposite axial (S,R,S_A) isomer **5b** in an high 78% yield.

To explore whether the doubly asymmetric reaction could benefit from a two-metal system, the CuI/ZnBr₂ combination (Table S2, SI), which had previously afforded the most promising results under substrate-control conditions, was selected for further evaluation using the chiral amine (R)-9. Under these conditions, the reaction delivered exclusively diastereomer **5b** in 68% yield (Table 1, entry 7), slightly lower than that obtained with the monometallic CuI system (78%, Table 1, entry 5). In contrast, when (S)-9 was employed, allene derivative **5a** was obtained in 59% yield (Table 1, entry 8), representing a modest improvement compared to the result obtained with CuBr₂ using the same chiral amine (Table 1, entry 4).

The optimized ATA conditions, combined with the stereodivergent chiral-amine approach described above, furnished reliable access to both axially defined allenic intermediates **5a** and **5b**, which constitute the pivotal branching point for the synthesis of the target sphingosine-like analogues. With these key intermediates in hand, attention next turned to unveiling the polar headgroup by deprotecting the 2-amino-1,3-diol motif. Accordingly, treatment of **5a** and **5b** with 1 M HCl in dioxane at 100 °C effected clean and simultaneous cleavage of both the *tert*-butoxycarbonyl and isopropylidene protecting groups, affording the unprotected aminodiols **1a** and **1b** in 66% and 63% isolated yield, respectively (Scheme 4). The free



Scheme 4 Synthesis of allenic sphingosine analogues **1–4a–b**. Reagents and conditions: (a) HCl 1 M, dioxane, 1 h, 100 °C. (b) *p*-HCHO (5 equiv.), NaBH₃CN (6 equiv.), MeOH, 0 °C–rt., 2.5 h. (c) *N,N'*-di-Boc-1*H*-pyrazole-1-carboxamidine, Et₃N, CH₂Cl₂:DME (1 : 1), rt, 18 h. (d) CF₃COOH, CH₂Cl₂, rt, 1 h. (e) RSO₂Cl (1.1 equiv.), DIPEA (1.1 equiv.), CH₂Cl₂, 16 h.

amino groups in **1a** and **1b** then served as versatile handles for subsequent diversification of the polar head region. Accordingly, exposure of the aminodiols to paraformaldehyde and sodium cyanoborohydride furnished the corresponding *N,N*-dimethylated sphingoid analogues **2a** and **2b** in 43% and 40% yield, respectively (Scheme 4).

In parallel, a second derivatization was carried out to introduce a guanidinium head group, motivated by the selective SphK2 inhibition previously observed for guanidinium-containing sphingolipid analogues in our group.¹¹ Reaction of aminodiols **1a** and **1b** with *N,N'*-di-Boc-1*H*-pyrazole-1-carboxamidine provided the corresponding guanidine derivatives **10a** and **10b** in excellent yields (91% and 89%, respectively). Subsequent Boc removal under standard TFA/CH₂Cl₂ conditions cleanly generated the guanidinium salts **TFA-3a** and **TFA-3b** in 82% and 69% yield, respectively.

Sulfonylation of the allene-based aminodiols **1a** and **1b** offered an alternative route for late-stage diversification of the sphingosine polar head, enabling incorporation of methyl sulfonamide groups (Scheme 4). Treatment with methanesulfonyl chloride under mild conditions afforded **4a** and **4b** in moderate yields (50% and 57%, respectively).

Importantly, all transformations proceeded without detectable erosion of stereochemical integrity. The absolute configuration of **4a**, confirmed by X-ray diffraction (Fig. 2), validates the robustness of the allene scaffold under sulfonamidation conditions and confirms the stereochemistry of the allenic motif in the synthesized sphingosine analogues.

In vitro sphingosine kinase assays

The inhibitory potency of compounds **1–4a–b** against SphK was evaluated *in vitro* using a time-resolved fluorescence resonance energy transfer (TR-FRET) assay based on the immunodetection of adenosine diphosphate (ADP).^{24,25} The half maximal inhibitory concentration of synthesized allenic sphingosine analogues were determined independently for SphK1

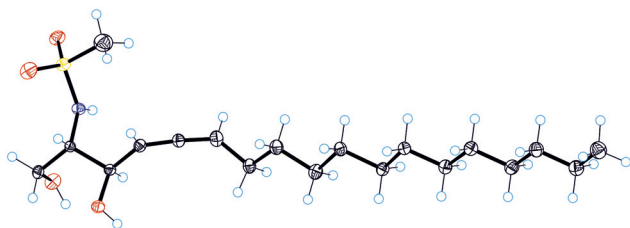


Fig. 2 ORTEP diagram of the unit cell of compound **4a** with 50% probability ellipsoid. Black = carbon, red = oxygen, blue = hydrogen, yellow = sulfur.

and SphK2 using dimethyl sphingosine (DMS) as a sphingosine-type reference inhibitor (Table 2).

Among the allenic derivatives tested, compounds **1a**, **1b**, **3a**, and **3b**, exhibited measurable inhibition of sphingosine kinase activity, with IC_{50} values in the low micromolar range and activities comparable to the reference inhibitor DMS. These results indicate that replacement of the native *trans*-olefin by an allene moiety is well tolerated in these *in vitro* assays and appears to preserve the bioactivity associated with the sphingoid backbone under the conditions evaluated in this study. The elongated and rigid geometry of the allene linker may therefore be compatible with the spatial requirements for activity.

Notably, all active compounds showed dual inhibition with a preference for SphK2, consistent with the original design hypothesis aimed at exploiting the expanded hydrophobic binding site (toe region) of SphK2 relative to that of SphK1. Thus, elongated and rigid geometry imposed by the allene moiety appears to be well suited to the extended J-channel of SphK2, which is known to be larger and more permissive than that of SphK1. In contrast, the more restrictive topology of SphK1 may disfavor binding of such elongated scaffolds, consistent with the lower inhibitory potency observed for this isoform.

However, compounds bearing dimethylated amino or methyl sulfonamide groups at the polar head were essentially

inactive ($IC_{50} > 120 \mu M$ for both isoforms), highlighting the stringent requirements of the enzyme with respect to polar head functionality.

The most potent inhibitors in the series are the guanidino derivatives **3a** and **3b**. Analogues bearing a free primary amine **1a** and **1b** also displayed inhibitory activity, albeit with lower potency than their guanidino counterparts. Notably, compound **1b** emerged as the most selective analogue, showing a SphK2/SphK1 selectivity ratio of approximately 3.7.

To the best of our knowledge, no sphingosine kinase inhibition data have been reported for the corresponding guanidinium or *N*-sulfonyl analogues of sphingosine, precluding a direct comparison with the biological activities observed for compounds **3a–b** and **4a–b**.

Interestingly, comparison of diastereomeric pairs (**a** versus **b**) within the series revealed no significant dependence of inhibitory activity on the axial chirality of the allene unit. This observation suggests that the flexible aliphatic chain may adapt to the hydrophobic region of the binding pocket regardless of the axial configuration of the allene.

Docking studies

To rationalize the inhibitory activity and isoform selectivity observed for the synthesized allenic sphingosine analogues, molecular docking studies were performed on both SphK1 and SphK2. Representative compounds were selected to elucidate the structure–activity relationships governing their interaction with the catalytic site.

As a preliminary step, the suitability of AlphaFold3 (AF3)²⁶ structural models of SphK2 for docking applications was assessed. Although the AF3 models displayed high global confidence values (Fig. S35, SI) (ipTM ≈ 0.74 , increasing to 0.92 upon removal of flexible regions), inspection of the orthosteric pocket revealed a consistent inward orientation of residue Phe584 (Fig. S36, SI). This conformation substantially narrows the J-shaped cavity and would hinder accommodation of

Table 2 IC_{50} values of compounds **1–4a–b** and the reference inhibitor (DMS) against SphK1 and SphK2 inhibition

Inh.	IC_{50}^a SphK1	IC_{50}^a SphK2	Sel. ^b	Inh.	IC_{50}^a SphK1	IC_{50}^a SphK2	Sel. ^b
1a	50.1 $\pm$ 5.1	19.7 $\pm$ 1.4	2.5	1b	65.6 $\pm$ 11.7	17.8 $\pm$ 2.1	3.7
2a	>120	>120	—	2b	>120	>120	—
3a	24.1 $\pm$ 3.2	10.1 $\pm$ 0.4	2.4	3b	27.9 $\pm$ N/A ^c	11.1 $\pm$ 0.8	2.5
4a	>120	>120	—	4b	>120	>120	—
DMS	45.9 $\pm$ 4.9	16.9 $\pm$ 1.8	2.7				

^a IC_{50} values are expressed in μM and are reported as $IC_{50} \pm$ asymptotic standard error (SE). ^b Sel. refers to SphK2/SphK1 selectivity ratio ($IC_{50}SphK1/IC_{50}SphK2$). ^c See the SI for further details.

sphingosine-like ligands. Notably, this feature was observed across high-confidence AF3 models, suggesting that it reflects a stable conformational state. This observation may reflect conformational variability of the binding site or capture of a different functional state for the enzyme. However, this topology is not consistent with previously reported binding modes derived from homology models of SphK2 on the crystal structure for SphK1, and precludes productive ligand docking. Therefore, the AF3 structures were not further considered, and the previously validated homology model^{11b} was retained for subsequent docking studies, enabling direct comparison with earlier work from our group.

Active compounds **1a–b** adopted well-defined binding poses within the SphK2 pocket. In these conformations, the polar head group engages in strong ionic and hydrogen-bonding interactions with key residues located in the polar region of the enzyme. In particular, the amino functionality interacts with Asp344, while the primary hydroxyl group forms a hydrogen bond with Ser334 (Fig. S37, SI).

For guanidino derivatives **3a–b**, the positioning of the polar head within SphK2 enables the formation of multiple hydrogen bonds with residues Ser334, Leu549 and Asp334, in addition to interactions with the deeply buried residue Asp247 (Fig. 3). Furthermore, the formation of an ionic interaction between Asp247 and the guanidino group likely contributes further to binding stabilization. These extensive polar interactions may explain the affinity of these molecules toward SphK2.

Consistent with compounds **1a** and **1b**, the allene diastereomeric pair establishes comparable polar interactions. Accordingly, and in agreement with the biochemical data, the axial chirality of the allene exerts a negligible influence on the binding mode, highlighting the adaptability of the flexible aliphatic chain within the hydrophobic channel. This can be readily visualized by comparing the tail orientations in the most probable binding poses of the diastereomeric guanidino compounds **3a** and **3b** within the SphK2 active site (Fig. S38, SI).

On the other hand, docking into SphK1 revealed less favorable binding geometries for compounds **1a** and the most selec-

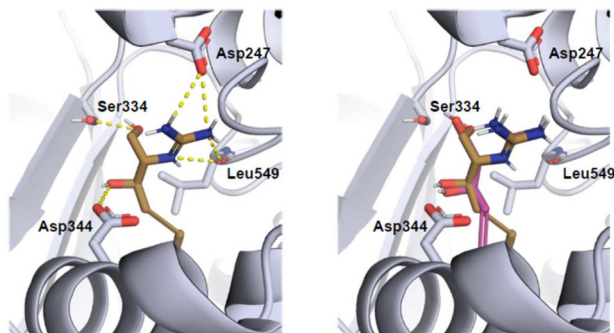


Fig. 3 Most probable binding poses and main polar interactions of guanidino compounds **3a** (in brown) and **3b** (in pink) in SphK2.

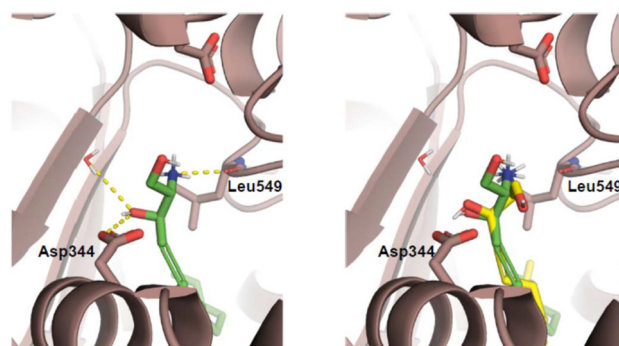


Fig. 4 Most probable binding poses and main polar interactions of amino compounds **1a** (in green) and the most selective derivative **1b** (in yellow) in SphK1 (brown).

tive compound **1b**. In this isoform, the ligands adopt deeper positions within the polar region of the pocket. In their most probable pose, both compounds form two hydrogen bonds: the amino group interacts with Leu549, while the secondary alcohol engages Asp344. Additionally, this hydroxyl group also interacts with a crystallographic water molecule present in SphK1 (Fig. 4). Moreover, the distinct positioning of Phe584 at the neck of the binding pocket in the two isoforms appears to affect the orientation of the allene moiety, potentially inducing subtle changes in the positioning of the polar head group that might contribute to the differential activity observed for **1a** (Fig. S39, SI). A similar effect cannot be excluded for the other allene analogues **1b**, **3a**, and **3b**.

The presence of a water molecule interacting with Ser334, together with Ala339, alters the shape and size of the polar pocket in SphK1, resulting in a reorganization of interactions. The higher IC₅₀ values observed for both compounds against SphK1 may therefore result from the loss of the hydrogen bond with Ser334 and the ionic interaction with Asp344, which are not fully compensated by the alternative interaction pattern.

Furthermore, docking studies rationalized the loss of activity observed upon dialkylation or sulfonylation of the amino group. In particular, the introduction of methyl substituents in compounds **2a–b** perturbs the orientation of the adjacent hydroxyl group, thereby disrupting critical hydrogen-bonding interactions. For sulfonamide derivative **4a**, this functionality leads to the loss of both hydrogen-bonding and ionic interactions (Fig. S40, SI).

Conclusions

In this study, we have developed a concise and stereodivergent synthetic strategy that enables efficient access to axially chiral allenic sphingosine analogues, incorporating diverse and late-stage modifications at the polar head group. Central to this approach is the copper-promoted allenation of terminal alkynes, which, in combination with chiral secondary amines, provides reliable control over the axial configuration of the

allene moiety while preserving the stereochemical integrity of the sphingoid backbone.

In vitro inhibitory assays demonstrated that replacement of the native *trans*-olefin by an allene moiety is well tolerated, affording inhibitors with low micromolar SphK inhibitory potency and a consistent preference for SphK2 over SphK1. Guanidinium-containing analogues emerged as the most potent compounds, whereas *N*-alkylation or sulfonylation of the amino group resulted in loss of activity, underscoring the critical role of polar head interactions in the active site of the enzyme. No significant activity differences were observed between diastereomeric pairs, consistent with flexible accommodation of the aliphatic chain in the hydrophobic binding region.

Docking studies provided a structural rationale for the observed rationalized inhibition profiles, highlighting favorable polar interactions and good accommodation of the allene scaffold within the SphK2 binding pocket. Collectively, these findings identify allenic sphingoid architectures as viable scaffolds for SphK2 inhibition and provide a foundation for the future design of more potent and selective inhibitors.

Author contributions

Eric Miró: investigation. Javier Jaureguizar: investigation. Varbina Ivanova: investigation. Raúl Beltrán-Debón: investigation. Jordi Juárez-Jiménez: investigation, supervision. Omar Boutureira: investigation, supervision, writing – review & editing, funding acquisition. Yolanda Díaz: conceptualization, investigation, methodology, supervision, writing – review & editing. M. Isabel Matheu: conceptualization, investigation, methodology, supervision, writing – original draft, writing – review & editing, visualization, project administration, funding acquisition.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included as part of the supplementary information (SI). Supplementary information: experimental details, NMR spectra, additional docking figures, inhibition curves and X-ray crystallographic data. See DOI: <https://doi.org/10.1039/d6ob01005a>.

CCDC 2559406 contains the supplementary crystallographic data for this paper.²⁷

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References

- (a) H. Chen, N. Haddadi, X. Zhu, D. Hatoum, S. Chen, N. T. Nassif, Y. Lin and E. M. McGowan, *J. Oncol.*, 2022, 2250407; (b) Y. Luo, H. Xue, Y. Gao, G. Ji and T. Wu, *Int. J. Biol. Macromol.*, 2025, **306**, 141392; (c) O. A. Sukocheva, H. Furuya, M. L. Ng, M. Friedemann, M. Menschikowski, V. V. Tarasov, V. N. Chubarev, S. G. Klochkov, M. E. Neganova, A. A. Mangoni, G. Aliev and A. Bishayee, *Pharmacol. Ther.*, 2020, **207**, 107464.
- (a) O. A. Sukocheva, M. E. Neganova, Y. Aleksandrova, J. T. Burcher, E. Chugunova, R. Fan, E. Tse, G. Sethi, A. Bishayee and J. Liu, *Cell Commun. Signaling*, 2024, **22**, 251; (b) H. Alshaker, H. Thrower and D. Pchejetski, *Front. Oncol.*, 2020, **10**, 289; (c) X. Wang, Y. Yang, W.-Q. Cai and Y. Lu, *Front. Immunol.*, 2020, **11**, 574990.
- (a) S. S. Alkafaas, M. I. Elsalahaty, D. F. Ismail, M. A. Radwan, S. S. Elkafas, S. A. Loutfy, R. M. Elshazli, N. Baazaoui, A. E. Ahmed, W. Hafez, M. Diab, M. Sakran, M. T. El-Saadony, K. A. El-Tarabily, H. K. Kamal and M. Hessien, *Cancer Cell Int.*, 2024, **24**, 89; (b) W. Li, C. P. Yu, J. T. Xia, L. Zhang, G. X. Weng, H.-Q. Zheng, Q. L. Kong, L.-J. Hu, M.-S. Zeng, Y.-X. Zeng, M. Li, J. Li and L. B. Song, *Clin. Cancer Res.*, 2009, **15**, 1393; (c) M. Bataller, A. Sanchez-Garcia, Y. Garcia-Mayea, C. Mir, I. Rodriguez and M. E. Lleonaart, *Front. Oncol.*, 2021, **11**, 807636.
- (a) R. D. Escarcega, L. D. McCullough and A. S. Tsvetkov, *Front. Mol. Biosci.*, 2021, **8**, 663767; (b) D. Wang, L. Sun, X. Niu, L. Ren and X. Yang, *Biochem. Biophys. Res. Commun.*, 2025, **751**, 151396; (c) X. Li, Y. Chen, Y. Liang and W. Shi, *BMC Cancer*, 2024, **24**, 983; (d) H. Janakiraman, Z. Gao, Y. Zhu, J. Dong, S. A. Becker, A. Janneh, B. Ogretmen and E. R. Camp, *World J. Oncol.*, 2024, **15**, 744; (e) L. B. Fugio, G. Silva, C. L. Ferraz, G. L. Trevisan, F. B. Coeli-Lacchini, C. B. Garcia, L. O. Sousa, T. M. Malta, C. D. Gil and A. M. Leopoldino, *Biochim. Biophys. Acta, Mol. Cell Res.*, 2024, **1871**, 119616.
- (a) D. J. Gustin, Y. Li, M. L. Brown, X. Min, M. J. Schmitt, M. Wanska, X. Wang, R. Connors, S. Johnstone, M. Cardozo, A. C. Cheng, S. Jeffries, B. Franks, S. Li, S. Shen, M. Wong, H. Wesche, G. Xu, T. J. Carlson, M. Plant, K. Morgenstern, K. Rex, J. Schmitt, A. Coxon, N. Walker, F. Kayser and Z. Wang, *Bioorg. Med. Chem. Lett.*, 2013, **23**, 4608; Some recent reviews are: (b) X. Yi, X. Tang, T. Li, L. Chen, H. He, X. Wu, C. Xiang, M. Cao, Z. Wang,

- Y. Wang, Y. Wang and X. Huang, *Biomed. Pharmacother.*, 2023, **163**, 114401; (c) T. Ding, Y. Zhi, W. Xie, Q. Yao and B. Liu, *Eur. J. Med. Chem.*, 2021, **213**, 113164; (d) D. R. Adams, S. Pyne and N. J. Pyne, *Cell Signalling*, 2020, **76**, 109806; (e) E. Magli, A. Corvino, F. Fiorino, F. Frecentese, E. Perissutti, I. Saccone, V. Santagada, G. Caliendo and B. Severino, *Curr. Pharm. Des.*, 2019, **25**, 956; (f) M. Cao, C. Ji, Y. Zhou, W. Huang, W. Ni, X. Tong and J.-F. Wei, *Int. J. Mol. Med.*, 2018, **41**, 2450.
- 6 D. Hatoum, N. Haddadi, Y. Lin, N. T. Nassif and E. M. McGowan, *Oncotarget*, 2017, **8**, 36898.
- 7 Z. Wang, X. Min, S.-H. Xiao, S. Johnstone, W. Romanow, D. Meininger, H. Xu, J. Liu, J. Dai, S. An, S. Thibault and N. Walker, *Structure*, 2013, **21**, 798.
- 8 J. Zhang, M. Zhang, J. Yu, Y. Shang, K. Jiang, Y. Jia, J. Wang and K. Yang, *J. Mol. Struct.*, 2020, **1208**, 127900.
- 9 D. R. Adams, S. Tawati, G. Berretta, P. Lopez-Rivas, J. Baiget, Z. Jiang, A. Alsouk, S. P. Mackay, N. J. Pyne and S. Pyne, *J. Med. Chem.*, 2019, **62**, 3658.
- 10 (a) J. Guasch, I. Giménez-Nueno, I. Funes-Ardoiz, M. Bernús, M. I. Matheu, F. Maseras, S. Castellón and Y. Díaz, *Chem. – Eur. J.*, 2018, **24**, 4635; (b) J. Llaveria, A. Beltrán, W. M. C. Sameera, A. Locati, M. M. Díaz-Requejo, M. I. Matheu, S. Castellón, F. Maseras and P. J. Pérez, *J. Am. Chem. Soc.*, 2014, **136**, 5342; (c) J. Guasch, Y. Díaz, M. I. Matheu and S. Castellón, *Chem. Commun.*, 2014, **50**, 7344; (d) J. A. Morales-Serna, J. Llaveria, Y. Díaz, M. I. Matheu and S. Castellón, *Curr. Org. Chem.*, 2010, **14**, 2483; (e) J. Llaveria, A. Beltrán, M. M. Díaz-Requejo, M. I. Matheu, S. Castellón and P. J. Pérez, *Angew. Chem., Int. Ed.*, 2010, **49**, 7092; (f) J. A. Morales-Serna, Y. Díaz, M. I. Matheu and S. Castellón, *Synthesis*, 2009, 710; (g) J. Llaveria, Y. Díaz, M. I. Matheu and S. Castellón, *Org. Lett.*, 2009, **11**, 205; (h) J. A. Morales-Serna, J. Llaveria, Y. Díaz, M. I. Matheu and S. Castellón, *Org. Biomol. Chem.*, 2008, **6**, 4502; (i) P. Rivero, V. Ivanova, X. Barril, M. Casampere, J. Casas, G. Fabriàs, Y. Díaz and M. I. Matheu, *Bioorg. Chem.*, 2024, **145**, 107233.
- 11 (a) M. Escudero-Casao, A. Cardona, R. Beltrán-Debón, Y. Díaz, M. I. Matheu and S. Castellón, *Org. Biomol. Chem.*, 2018, **16**, 7230; (b) M. Corro-Morón, A. Granell, V. Ivanova, E. Domingo, R. Beltrán-Debón, X. Barril, M.-J. Sanz, M. I. Matheu, S. Castellón and Y. Díaz, *Bioorg. Chem.*, 2022, **121**, 105668; (c) A. Cardona, V. Ivanova, R. Beltrán-Debón, X. Barril, S. Castellón, Y. Díaz and M. I. Matheu, *Org. Biomol. Chem.*, 2025, **23**, 1104.
- 12 (a) A. Hoffmann-Röder and N. Krause, *Angew. Chem., Int. Ed.*, 2004, **43**, 1196; (b) S. Yu and S. Ma, *Angew. Chem., Int. Ed.*, 2012, **51**, 3074; (c) H. Wu, Z. Zheng, K. Zhang, J. Kajanus, M. J. Johansson, A. Córdova and J.-E. Bäckvall, *Angew. Chem., Int. Ed.*, 2023, **62**, e202314512.
- 13 B. Alcaide and P. Almendros, *Chem. Soc. Rev.*, 2014, **43**, 2886.
- 14 J. Chun, J. Sun and W. Deng, *Synth. Commun.*, 2020, **50**, 3149.
- 15 Some representative references of the most established methodologies for allene synthesis are: (a) S. Yua and S. Ma, *Chem. Commun.*, 2011, **47**, 5384; (b) W.-D. Chu, Y. Zhang and J. Wang, *Catal. Sci. Technol.*, 2017, **7**, 4570; (c) H. H. A. M. Hassan, *Curr. Org. Synth.*, 2007, **4**, 413; (d) R. K. Neff and D. E. Frantz, *ACS Catal.*, 2014, **4**, 519; (e) J. Singh, B. Saxena and A. Sharma, *Catal. Sci. Technol.*, 2024, **14**, 5143; (f) X. Wang, X. Chen, W. Lin, P. Li and W. Li, *Adv. Synth. Catal.*, 2022, **364**, 1212; (g) W. Xiao and J. Wu, *Org. Chem. Front.*, 2022, **9**, 5053.
- 16 (a) P. Crabbé, D. André and H. Fillion, *Tetrahedron Lett.*, 1979, **20**, 893; (b) X. Huang and S. Ma, *Acc. Chem. Res.*, 2019, **52**, 1301; (c) J. Kuang and S. Ma, *J. Org. Chem.*, 2009, **74**, 1763–1765.
- 17 P. Herold, *Helv. Chim. Acta*, 1988, **71**, 354.
- 18 S. Pabbaraja, N. Gantasala, S. Ydhyam, H. K. Namballa, S. Gundeboina, M. R. Lambu, S. Meena and D. Datta, *Tetrahedron Lett.*, 2018, **59**, 2570.
- 19 F. F. Wong, S. H. Chuang, S.-C. Yang, Y.-H. Lin, W.-C. Tseng, S.-K. Lin and J.-J. Huang, *Tetrahedron*, 2010, **66**, 4068.
- 20 C. M. J. Ouairy, M. J. Ferraz, R. G. Boot, M. P. Baggelaar, M. van der Stelt, M. Appelman, G. A. van der Marel, B. I. Florea, J. M. F. G. Aerts and H. S. Overkleeft, *Chem. Commun.*, 2015, **51**, 6161–6163.
- 21 (a) X. Tang, C. Zhu, T. Cao, J. Kuang, W. Lin, S. Ni, J. Zhang and S. Ma, *Nat. Commun.*, 2013, **4**, 2450; (b) J. Ye, S. Li, B. Chen, W. Fan, J. Kuang, J. Liu, Y. Liu, B. Miao, B. Wan, Y. Wang, X. Xie, Q. Yu, W. Yuan and S. Ma, *Org. Lett.*, 2012, **14**, 1346; (c) J. Kuang, X. Tang and S. Ma, *Org. Chem. Front.*, 2015, **2**, 470; (d) Q. Liu, X. Tang, Y. Cai and S. Ma, *Org. Lett.*, 2017, **19**, 5174; (e) J. Zhang, J. Ye and S. Ma, *Org. Biomol. Chem.*, 2015, **13**, 4080; (f) R. Gurubrahmam and M. Periasamy, *J. Org. Chem.*, 2013, **78**, 1463; (g) R. Lü, J. Ye, T. Cao, B. Chen, W. Fan, W. Lin, J. Liu, H. Luo, B. Miao, S. Ni, X. Tang, N. Wang, Y. Wang, X. Xie, Q. Yu, W. Yuan, W. Zhang, C. Zhu and S. Ma, *Org. Lett.*, 2013, **15**, 2254.
- 22 Some examples are: (a) B. Simmons, A. M. Walji and D. W. C. MacMillan, *Angew. Chem., Int. Ed.*, 2009, **48**, 4349; (b) J. B. Brazier, T. J. K. Gibbs, J. H. Rowley, L. Samulis, S. C. Yau, A. R. Kennedy, J. A. Platts and N. C. O. Tomkinson, *Org. Biomol. Chem.*, 2015, **13**, 133; (c) J. F. Austin and D. W. C. MacMillan, *J. Am. Chem. Soc.*, 2002, **124**, 1172; (d) Y. K. Chen, M. Yoshida and D. W. C. MacMillan, *J. Am. Chem. Soc.*, 2006, **128**, 9328.
- 23 (a) J. Ye, R. Lü, W. Fan and S. Ma, *Tetrahedron*, 2013, **69**, 8959; (b) X. Huang, T. Cao, Y. Han, X. Jiang, W. Lin, J. Zhang and S. Ma, *Chem. Commun.*, 2015, **51**, 6956; (c) X. Tang, X. Huang, T. Cao, Y. Han, X. Jiang, W. Lin, Y. Tang, J. Zhang, Q. Yu, C. Fu and S. Ma, *Org. Chem. Front.*, 2015, **2**, 688; (d) X. Huang, C. Xue, C. Fu and S. Ma, *Org. Chem. Front.*, 2015, **2**, 1040.
- 24 M. A. Ayoub, J. Trebaux, J. Vallaghe, F. Charrier-Savournin, K. Al-Hosaini, A. Gonzalez Moya, J.-P. Pin, K. D. G. Pfleger and E. Trinquet, *Front. Endocrinol.*, 2014, **5**, 1.
- 25 (a) T. A. Klink, K. M. Kleman-Leyer, A. Kopp, T. A. Westermeyer and R. G. Lowery, *J. Biomol. Screening*, 2008, **13**, 476; (b) Adapta Universal Kinase Assay User

- Guide. Protocol part PV5099. Rev. 14 February 2008. Invitrogen.
- 26 J. Abramson, J. Adler, J. Dunger, R. Evans, T. Green, A. Pritzel, O. Ronneberger, L. Willmore, A. J. Ballard, J. Bambrick, S. W. Bodenstein, D. A. Evans, C.-C. Hung, M. O'Neill, D. Reiman, K. Tunyasuvunakool, Z. Wu, A. Žemgulytė, E. Arvaniti, C. Beattie, O. Bertolli, A. Bridgland, A. Cherepanov, M. Congreve, A. I. Cowen-Rivers, A. Cowie, M. Figurnov, F. B. Fuchs, H. Gladman, R. Jain, Y. A. Khan, C. M. R. Low, K. Perlin, A. Potapenko, P. Savy, S. Singh, A. Stecula, A. Thillaisundaram, C. Tong, S. Yakneen, E. D. Zhong, M. Zielinski, A. Židek, V. Bapst, P. Kohli, M. Jaderberg, D. Hassabis and J. M. Jumper, *Nature*, 2024, **630**, 493.
- 27 CCDC 2559406: Experimental Crystal Structure Determination, 2026, DOI: [10.5517/ccdc.csd.cc2rx8hg](https://doi.org/10.5517/ccdc.csd.cc2rx8hg).