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In silico Design, Synthesis, and Screening of Novel Deoxyhypusine Synthase Inhibitors Targeting HIV-1 Replication

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The human enzyme deoxyhypusine synthase (DHS) is an important host cell factor that participates in the post-translational hypusine modification of eukaryotic initiation factor 5A (eIF-5A). Hypusine-modified eIF-5A plays a role in a number of diseases, including HIV infection/AIDS. Thus, DHS represents a novel and attractive drug target. So far, four crystal structures are available, and various substances have been tested for inhibition of human DHS. Among these inhibitors, *N*-1-guanyl-1,7-diaminoheptane (GC7) has been co-crystallized in the active site of DHS. However, despite its potency, GC7 is not selective enough to be used in drug applications. Therefore, new compounds that target DHS are needed. Herein we report the in silico design, chemical synthesis, and biological evaluation of new DHS inhibitors. One of these inhibitors showed dose-dependent inhibition of DHS in vitro, as well as suppression of HIV replication in cell cultures. Furthermore, the compound exhibited no cytotoxic effects at active concentrations. Thus, this designed compound demonstrated proof of principle and represents a promising starting point for the development of new drug candidates to specifically interfere with DHS activity.

Introduction

A key step in HIV chemotherapy was the introduction of combination anti-retroviral therapy (cART) in the mid-1990s, which significantly prolonged patients' expectancy of life.[1] However, to decrease cART-related toxicities[2,3] and the development of potential (multi)drug resistance during long-term cART,[4] it is important to identify new targets for therapy and to develop novel anti-retroviral drugs (that is, low-molecular-weight inhibitors).[5] A general limitation to the development of anti-retroviral drugs that, like most cART regimens, commonly target virus-encoded enzymes is the high mutation rate of retroviruses;[1] these mutations frequently lead to the occurrence of drug-resistant strains.[6] However, a possibility to circumvent these problems is to address host cell components that are essential for virus replication and, because they are of cellular origin, are not subject to virus mutation. Within the HIV replication cycle, various host cell factors play an important role.[7,8] For example, eukaryotic initiation factor 5A (eIF-5A), a cellular protein that primarily promotes the elongation step of translation,[9] particularly during the biosynthesis of polyproline motifs,[10] has also been shown to act as a cellular cofactor of the HIV Rev protein.[11,12] Rev is an essential viral regulator that primarily mediates the nucleocytoplasmic transport and translation of incompletely spliced and unspliced viral transcripts.[13,14]

Activation of eIF-5A involves a unique spermidine-dependent post-translational modification of a specific lysine residue into the unusual amino acid hypusine (Figure 1). This modification is catalyzed by the sequential action of human deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DOHH).[15] Previously, derivatives (that is, polyamine analogues) of the natural DHS substrate spermidine were tested regarding their effect on DHS activity, which showed that *N*-1-guanyl-1,7-diaminoheptane (GC7) is a potent inhibitor.[16,17] However, the potential application of GC7 in vivo is limited, due to its unselective binding properties and high structural similarity to spermidine. This may result in potential undesired side effects, for example, in spermidine biosynthesis and metabolism.[18]

Importantly, other DHS inhibitors, including the spermidine analogue 1,8-diaminooctane, have been shown to significantly suppress virus replication by inhibiting Rev activity in a dose-dependent manner.[19,20] Thus, the targeting of DHS, for example, through the synthesis of improved GC7 derivatives obtained by rational drug design, may be a promising strategy to efficiently block HIV replication, including the replication of viruses that are otherwise resistant to current cART.

Based on the X-ray crystal data for DHS in the Protein Data Bank (PDB)[21] and the known inhibitor GC7, structure- and ligand-based drug design approaches were applied in order to discover novel DHS inhibitors. We report herein the in silico

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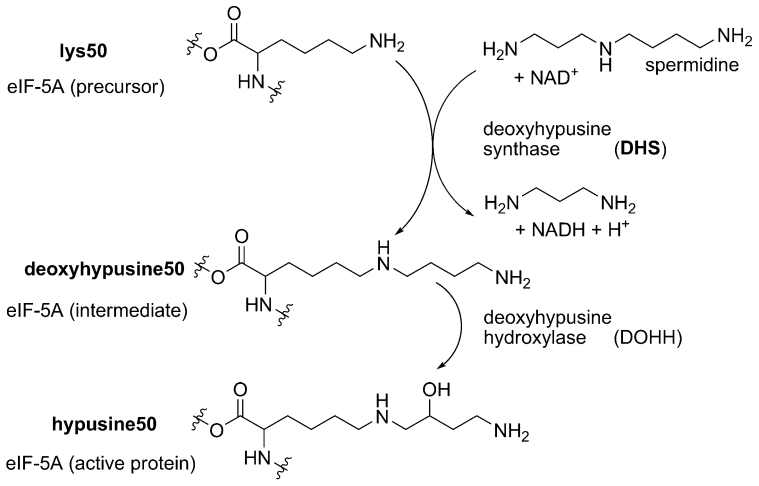


Figure 1. In vivo post-translational activation of eIF-5A catalyzed by DHS and DOHH.

design, synthesis, and biological evaluation of several selected compounds, which were tested for inhibition of DHS in an enzyme assay, for inhibition of HIV-1 replication in vitro, and for potential cytotoxic effects.

Results and Discussion

Virtual screening

To identify new binders to DHS, we combined ligand-based and structure-based drug design approaches. These include large-scale virtual screening (TrixX B-MI),[22] scaffold hopping and ligand decoration (ReCore),[23] combinatorial library design (Loft),[24] virtual screening (LeadIT),[25] and rescoring (HYDE).[26]

The two crystal structures 1RQD and 1ROZ represent the active form of DHS,[27] so these were selected for subsequent docking experiments. However, in these crystal structures, only protein dimers were found, whereas the biologically active enzyme complex is a tetramer.[28] Therefore, the tetrameric protein was built in accordance with the crystal structure parameters by using the Molecular Operating Environment (MOE) software.[29] The two additional amino acid chains were named "C" and "D", respectively (Figure 2). The binding site at the interface of chain A and

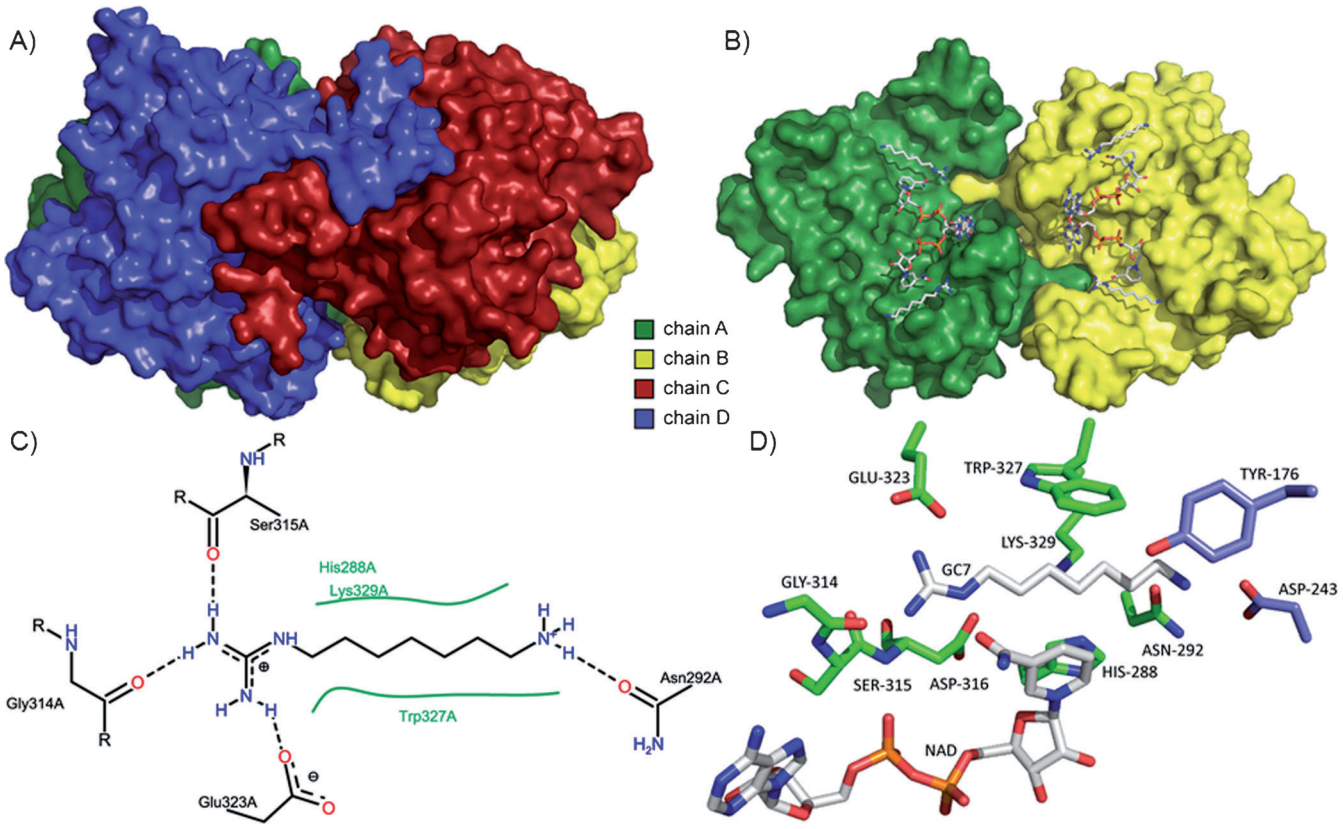


Figure 2. A) Tetrameric form of DHS generated from PDB crystal structure 1RQD. B) Chains A and B with cofactor NAD and inhibitor GC7 in all four binding sites. C) Interactions of GC7 with the DHS binding site. D) Three-dimensional view of the binding site.

chain D involving Asp243 of chain A and Glu323 of chain D was analyzed.

To define the binding site for subsequent docking experiments, the crystal structures (1RQD and 1ROZ) were aligned and superposed according to their α carbon (CA) atoms (root mean square deviation, RMSD = 0.179 Å). The coordinates of the co-crystallized ligand (GC7) and cofactor (nicotinamide adenine dinucleotide, NAD) of crystal structure 1RQD were then transferred into 1ROZ. Two alternative binding sites were defined. The first variant only included amino acids with at least one atom within a distance of less than 6.5 Å from GC7 or NAD. Both the cofactor NAD and the ligand GC7 were removed before docking. The second variant was defined accordingly around GC7 with the cofactor NAD present.

Consequently, the second variant of the binding site is relatively small compared with the first variant. Both variants of the binding site are used with their standard protonation and additional variants of His288. His288 is considered as being either protonated at the NE2 nitrogen (τ nitrogen) atom or rotated by 180° and protonated at the ND1 nitrogen (π nitrogen) atom, which possibly leads to an interaction with the carboxylate group of Asp316.

All ligands that were used for docking were first processed with the CORINA[30] software and subsequently with the NAOMI[31] program to ensure reasonable coordinates and valid valence bond forms. An overview of the development workflow is shown in Figure 3. In a first step, the clean-leads subset of the ZINC database[32] was screened for potential inhibitors of DHS. This dataset contains only lead-like compounds without reactive groups (for example, epoxides). In order to employ the TrixX BMI virtual screening approach, rotamers were generated by using the TrixX conformer generator.[33] The resulting rotamers were subsequently docked into the binding site of 1RQD. Solutions were only considered for further analysis if they had a predicted interaction to the OD2 oxygen atom of Asp243 in chain A and at least one additional interaction to the OE1 or OE2 oxygen atoms of Glu323 in chain D. Thereby, a similar interaction pattern to that of GC7 is ensured. Furthermore, proposed binders with a score above −10 (weak binders) were discarded to filter for medium-to-strong binders.

Based on experimental results, a focused fragment space was designed to match the characteristics of the DHS binding site. Molecules of this fragment space allow systematic testing of combinations of different anchor groups, cores, and linker lengths. Core fragments **c1**–**c20** (Figure 4) were chosen to build the center of generated molecules and to form an additional π–π stacking interaction to Trp327 in chain D of DHS, which cannot be established by the aliphatic compound GC7.

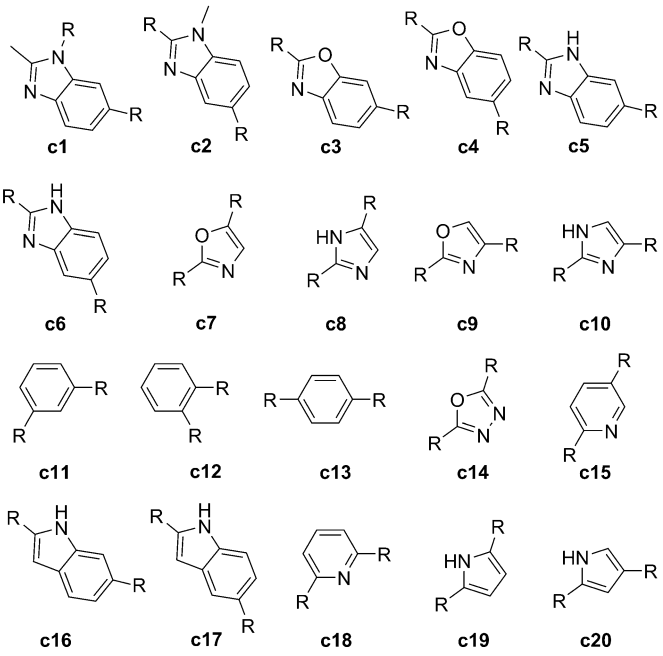


Figure 4. Core fragments designed to interact with Trp327 in chain D of DHS. The linkers (R) are virtually replaced by anchor fragments. Each combination of a single core fragment and two anchor fragments forms a molecule used for subsequent molecular docking calculations.

Anchor fragments **a1**–**a7** (Figure 5) were designed to interact with the hydrogen-bond acceptors of the binding site. All possible combinations of these fragments were enumerated. The resulting molecules were filtered for lead likeness.

Scaffold hopping and ligand decoration were performed with ReCore, by using the standard set of fragments supplied with the software. This set consists of fragments generated by fragmenting the drug-like compounds of the ZINC database according to the BRICS[34] shredding rules. This approach generates further potential lead structures with possibly higher binding affinities and additional interactions with the protein binding site.

In summary, we have followed three strategies. We used large-scale virtual screening of the ZINC database to identify commercially available compounds that can be purchased and tested for inhibition of DHS. This approach does not use any knowledge about already identified binders, so it has the potential to yield completely different inhibitors of DHS. Scaffold hopping was used to directly improve the binding affinity of lead structures and known binders. Only parts of the already known inhibitors are replaced by this

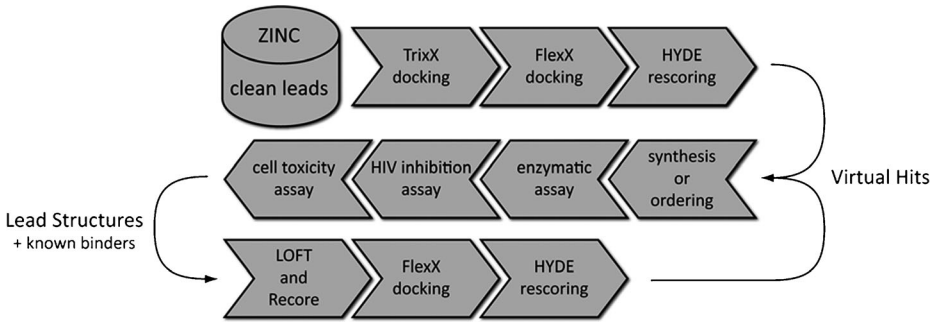


Figure 3. Workflow for the development of new potential DHS inhibitors.

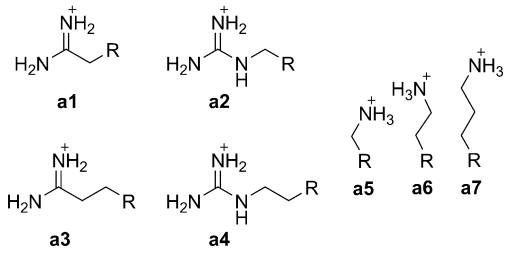


Figure 5. Anchor fragments designed to interact with hydrogen acceptors of the DHS binding site. The linkers (R) are virtually replaced by core fragments. Each combination of a single core fragment and two anchor fragments forms a molecule used for subsequent molecular docking calculations.

approach, so the resulting molecules have a high probability of showing inhibitory activity against DHS. However, molecules designed by this approach, which are not commercially available, have to be individually synthesized. A fragment space was used to systematically investigate a high number of similar molecular structures. Thus, only the most promising representative of a class of similar compounds has to be synthesized and tested in vitro.

All of the molecules selected by the above-mentioned approaches were docked into the binding site of DHS by using the LeadIT docking software (version 2.0.2) based on the FlexX docking approach.[35] The maximum overlap volume of ligand and protein was set to 2 Å^3 and the clash factor was set to 0.7 to limit false-positive docking solutions. All other parameters were kept at the default settings. Furthermore, the same interaction filter was applied as that used for the TrixX BMI virtual screening.

Rescoring was performed with the HYDE scoring function (version 3.25).[26] This scoring function models desolvation effects, so it has a higher accuracy in predicting binding affinities than the standard scoring functions implemented within TrixX or LeadIT, respectively. For every ligand docked with LeadIT, the ten best poses were stochastically optimized and rescored with the HYDE software. All poses with a negative HYDE score were manually inspected for incorrect conformations or wrong protonation states.

Docking studies

Pooled molecules from the initial TrixX BMI screening and structures from scaffold hopping and ligand decoration approaches were docked into the binding site of DHS. The most promising compounds as judged by the FlexX and HYDE scores were further analyzed for synthetic accessibility or commercial availability. Compounds **4** and **6** were commercially available, whereas compounds **1**–**3** and **5** were selected for synthesis (Figure 6).

In addition, substitution of the charged guanidinium group by a urea group should improve the abilities of the molecules to pass through cell membranes relative to the ability of GC7 and resulted in high (negative) FlexX and HYDE scores (Figure 7).

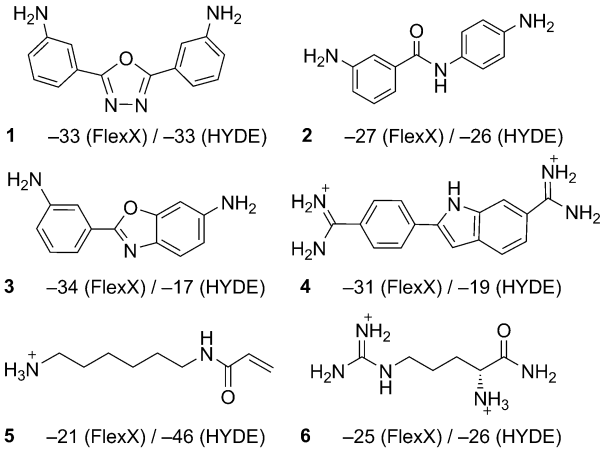


Figure 6. Compounds **1**–**6** were selected as potential DHS inhibitors on the basis of a TrixX BMI screening of the ZINC database.

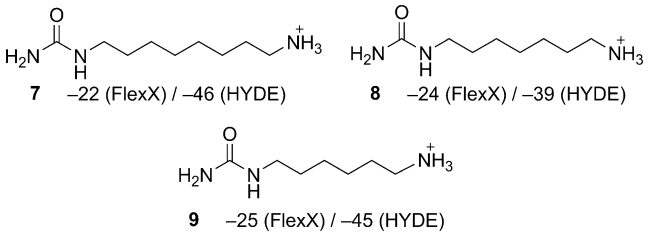


Figure 7. Suggested inhibitors designed on the basis of GC7 with the guanidinium group replaced by a urea group. The shown FlexX and HYDE scores represent the best score of all performed docking experiments for each compound.

Out of the compounds that are shown in Figures 6 and 7, only compound **4** inhibited DHS in an enzymatic assay and the replication of HIV in cell cultures (data not shown). Compound **4** therefore served as a basis for the following inhibitor design. To systematically enhance the inhibitory activity and to decrease the observed cell toxicity of compound **4**, a fragment space was designed as described above. The resulting molecules address the hydrogen-bond acceptors of the terminal parts of the binding site, as well as the aromatic ring of Trp327 in the center of the binding site. Furthermore, the generated molecules were sufficiently flexible to adapt to the binding site of DHS and to avoid intercalation into DNA. They shared predicted interactions with Trp327, Glu323, and Asp243 of DHS. Out of these molecules, compound **10** was selected as a target compound because it had the highest predicted binding affinity (Figure 8). The predicted interactions of compound **10** with

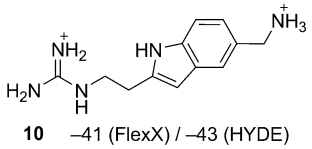


Figure 8. Selected target molecule from a second set of predicted binders generated from focused fragment space with the corresponding FlexX and HYDE scores.

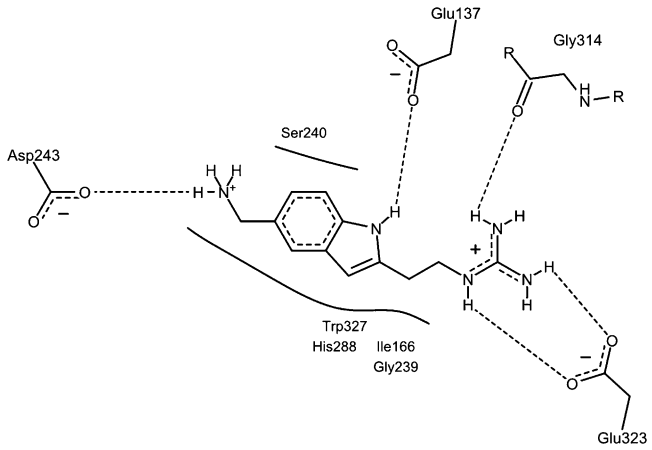


Figure 9. Predicted binding mode of compound **10** to DHS as calculated by the LeadIT 2.0.2 software.

the active site of DHS are shown in Figure 9. Compound **10** has not been described before in the literature.

Chemical synthesis of the in silico designed potential DHS binders

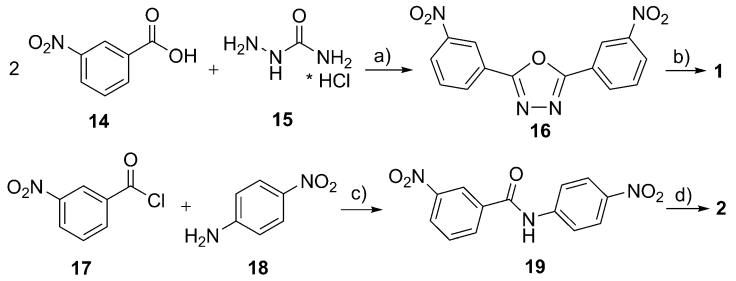
The compounds can be subdivided into three groups: the more rigid and more GC7-unlike compounds **1**–**6**, the flexible and more GC7-like analogues **7**–**9**, and compound **10**, which was derived from the focused fragment space (Figures 6, 7, and 8). It was possible to prepare the proposed compounds from the first two groups in a maximum of two steps by starting from cheap reagents and commercially available starting materials. For the synthesis of the more rigid aromatic compounds **1**–**3**, the synthesis routes are given in Schemes 1 and 2. To avoid additional protection and deprotection steps, the nitro group was chosen as a masked amino group precursor in all cases. The corresponding nitro compounds **16** (for **1**), **19** (for **2**), and **21** (for **3**) were synthesized in yields of 16–91%, despite the fact that the electron-withdrawing nitro group lowers the reactivity of the aromatic system. The substituted 1,3,4-oxadiazole compound **16** was synthesized by cyclization of 3-nitrobenzoic acid with aminourea hydrochloride in polyphosphoric acid in 26% yield.[36] Heating of 3-nitrobenzoyl chloride and 4-nitroaniline in pyridine to reflux, as reported by Hu et al., led to 3-nitro-*N*-(4-nitrophenyl)-benzamide (**19**; in 91% yield).[37]

6-Nitro-2-(3-nitrophenyl)benzoxazole (**21**) was prepared first by applying a copper-catalyzed method reported by Ueda and Nagasawa that led from the benzanilide **19** by way of an oxidative ring closure to the benzoxazole **21** in 27% yield.[38] As shown in Scheme 2, the alternative route described by Hausner et al. allowed the synthesis to be shortened by one step.[39] In this case, benzoxazole **21** was obtained from 2-amino-4-nitrophenol (**20**) and 3-nitrobenzoic acid (**14**) in a nonoptimized yield of 16%.

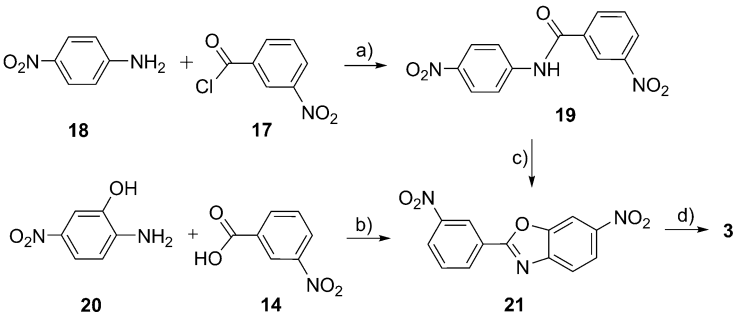
For the last step, different reduction methods were tried to convert the nitro group into the amino group. Among the various procedures, the reduction with tin(II) chloride (Scheme 1) gave the best results for **16** and **19**. However, in case of **21**, the reduction with tin(II) chloride led to a ring opening of the oxazole moiety. Therefore, in this case, hydrogen under pressure and palladium on charcoal were used (Scheme 2).

Beside these rigid aromatic systems, three flexible aliphatic derivatives of GC7 were synthesized (Figure 7 and Scheme 3), in which the guanidine moiety was replaced by a urea function and the alkyl chain length varied from six to eight carbon atoms. Compound **5**, in which the guanidine moiety was substituted with an acryloyl moiety and the alkyl chain length was shortened to six carbon atoms, was also synthesized.

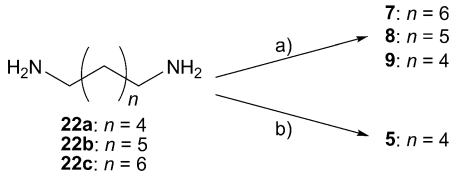
A method reported by Miyagawa et al. led to target compound **5** in 35% yield by adding acryloyl chloride dropwise to a diluted solution of 1,6-diaminohexane in dry methanol.[40] In solvents like pyridine, CH_2Cl_2, or THF with triethylamine as a base, the only isolated product was the disubstituted diamine.[41–43] Compounds **7**–**9** were prepared according to the method of Boden et al. in yields varying from 11 to 39%.[44] The synthesis included washing steps for purification, so parts of the product were also dissolved, which resulted in the poor yields for **7**–**9**.



Scheme 1. Synthesis of the more rigid aromatic compounds **1** and **2** based on the virtual screening results. *Reagents and conditions*: a) P_2O_5, phosphoric acid, reflux, 2 h, 26%; b) $SnCl_2$, conc. HCl, 50 °C, 2 h, 84%; c) pyridine, reflux, 18 h, [N_2], 91%; d) $SnCl_2$, conc. HCl, 50 °C, 2 h, 99%.



Scheme 2. Synthesis of 6-amino-2-(3-aminophenyl)benzoxazole (**3**). *Reagents and conditions*: a) Pyridine, reflux, 18 h, [N_2], 91%; b) $B(OH)_3$, xylene, reflux, 12 h, 16%; c) *o*-dichlorobenzene, copper triflate, reflux, 22 h, 27%; d) Pd/C, H_2, EtOH, RT, 68 h, 25%.

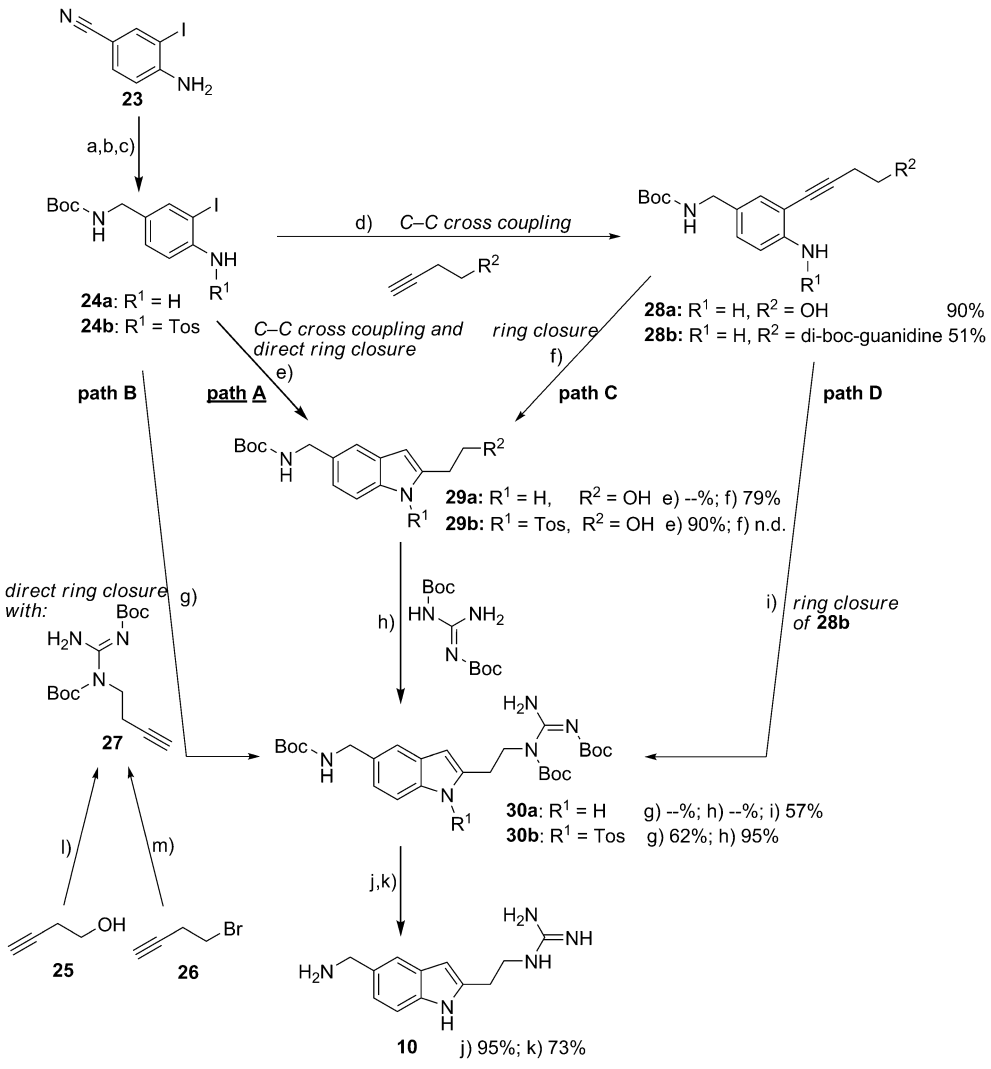


Scheme 3. Overview of the synthesized GC7 analogues **5** and **7**–**9**. *Reagents and conditions*: a) Trimethylsilylisocyanate (0.35 equiv), THF, RT, 4 h, [N_2], 11–39%; b) acryloyl chloride (0.4 equiv), CH_3OH, 0 °C→RT, 16 h, [N_2], 35%.

For compound **10**, the substituted indole moiety is the key structure element. Due to the particular substitution pattern, no suitable indole precursor was commercially available. Thus, the synthesis was started with 4-amino-3-iodobenzonitrile (**23**), which bears a nitrile function as a precursor for the benzyl amine group and an iodine atom in the *ortho* position to the amine (Scheme 4). Different C–C cross-coupling and ring-closure methods with 4-substituted but-1-ynes were tested to yield the indole (paths A–D). Initially, the nitrile group was reduced with BH_3·THF, and this was followed by acidic workup according to the method of Li et al.[45] The following Boc protection was carried out with di-*tert*-butyldicarbonate in 72–80% yield. The in situ coupling and cyclization of **24a** with but-3-yn-1-ol (**25**) was only successful by using the reaction conditions described by Larock et al. (Scheme 4, path A).[46] However, even then, only an inseparable mixture of the 2- and 3-substituted indoles was isolated in a yield of 35%.

Thus, the reactivity of the aromatic amine moiety of **24a** was enhanced by *N*-tosylation with tosyl chloride to give precursor **24b** in 92%.[47,48] A copper iodide/$Pd(PPh_3)_2Cl_2$ mediated reaction with three equivalents of but-3-yn-1-ol yielded the indole **29b** in 90% (Scheme 4, path A).[44] In analogy to known procedures, the hydroxy group of the indole **29b** was converted into the *N*,*N*′-bis-*tert*-butoxycarbonylguanidino group by using the Mitsunobu reaction in 95% yield.[49] The cleavage of the tosyl group gave the best results by using 5 M NaOH in methanol and the product was isolated in 95% yield. The final deprotection step was the acidic cleavage of all of the Boc groups of **30a** by treatment with 2 M HCl in CH_3CN. Purification on reverse-phase (RP) silica gel with H_2O as the eluent and freeze drying gave the pure compound **10** as the hydrochloride salt in 73% yield.

It is noteworthy that a protecting group on the hydroxy function of but-3-yn-1-ol (**25**) was not needed for the cyclization. It actually caused a negative effect, because no product



Scheme 4. Overview of the synthesis of the in silico designed DHS inhibitor **10**. *Reagents and conditions*: a) BH_3·THF (3 equiv), THF, reflux, 4 h, [N_2], then 1 M HCl (3 equiv), reflux, 1 h; b) 1. Et_3N (4 equiv), DMAP (0.2 equiv), 2. di-*tert*-butyldicarbonate (1–7.3 equiv), 0 °C→RT, 5 h, [N_2], 72–80% (over two steps); c) pyridine (3 equiv), TosCl (1.2 equiv), CH_2Cl_2, RT, 42 h, [N_2], 92%; d) DBU (1.5 equiv), Pd EnCat TPP30 (3.5 mol%), alkyne (1.5 equiv), reflux, 17 h, [N_2]; e) alkyne (3 equiv), CuI (0.2 equiv), $Pd(PPh_3)_2Cl_2$ (10 mol%), NEt_3, DMF, 85 °C, 17 h, [N_2]; f) KH (1.2 equiv), $Cu(OAc)_2$ (1.1 equiv), 1,2-dichloroethane, 70 °C, 3–4 d, [N_2], 79%; g) see (e); h) *N*,*N*′-bis-*tert*-butoxycarbonylguanidine (1.5 equiv), PPh_3 (1.5 equiv), DIAD (1.5 equiv), 0 °C→reflux, 3 h, [N_2]; i) see (f); j) 5 M NaOH/CH_3OH, RT, 40 h, [N_2]; k) 2 M HCl/CH_3CN, RT, 28 h; l) see (h), 87%; m) *N*,*N*′-bis-*tert*-butoxycarbonylguanidine (1 equiv), NaH (1 equiv), DMF, [N_2], 10%. Boc: *tert*-butoxycarbonyl; Tos: toluene-4-sulfonyl; n.d.: not determined; DMAP: 4-dimethylaminopyridine; DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene; DMF: *N*,*N*-dimethylformamide; DIAD: diisopropylazodicarboxylate.

was isolated with the methodology of Larock et al. Moreover, for 4-amino-3-(4-*O*-*tert*-butyldimethylsilyl-but-1-yn-1-yl)benzonitrile under the cyclization conditions of Hiroya et al., the 5-cyano-2-vinyl-1*H*-indole was isolated as the main product in 90%.[50] To avoid additional protection steps, the approach of the nitrile reduction after indole synthesis was tested, too. Unfortunately, this was not successful and led to reduction of the indole as a side reaction.[51–53]

The synthesis of *N*,*N*′-bis-*tert*-butoxycarbonylguanidinobut-3-yne (**27**) prior to cross-coupling and cyclization with **24a**/**b** would save a step in the linear synthesis route (Scheme 4, path B), so alkyne **27** was synthesized from but-3-yn-1-ol (**25**) under Mitsunobu reaction conditions[49] (87%) and from 4-bromo-1-butyne (**26**) by nucleophilic substitution[54] (10%). A ring-closure reaction of 4-amino-3-(4-bromobut-1-yn-1-yl)benzonitrile led only to the elimination product 5-cyano-2-vinyl-1*H*-indole in poor yields (14%). Due to the lower yields obtained with *N*,*N*′-bis-*tert*-butoxycarbonylguanidinobut-3-yne (**27**) in the transition-metal-catalyzed cyclizations and the high efficiency of the Mitsunobu reaction conditions, it is more convenient to convert the hydroxy group after the indole synthesis. However, the Mitsunobu reaction with indole **29a** was not successful, possibly due to side reactions of the free indole nitrogen atom (Scheme 4).

To circumvent the tosylation/detosylation but still use the modified "one-pot" indole synthesis from Adachi et al.,[47] we aimed to convert compound **23** directly to the *O*-*tert*-butyl-4-*N*-(*O*-*tert*-butoxycarbonyl)-3-iodobenzylcarbamate. However, even if a large excess of di-*tert*-butyldicarbonate (7.3 equiv) was used, the *bis*-Boc protection was not achieved.

Interestingly, by using a combination of Sonogashira cross-coupling reaction conditions[55,56] and subsequent cyclization according to the method of Hiroya et al., indole **30a** was obtained without the need for a protecting group on the arylamine nitrogen atom (Scheme 4, path D).[50] Although this route was shorter, the yields were significantly lower than those of the first route (path A).

In summary, the most efficient synthetic route yielded, through reduction, Boc protection, tosylation, "one-pot" cyclization with but-3-yn-1-ol, guanidine introduction under Mitsunobu reaction conditions, detosylation, and cleavage of the Boc protecting groups, the target compound **10** in an overall yield of 44% over seven steps (Scheme 4, path A). One advantage of this route is the variability of the starting materials because different hydroxy-substituted alkynes similar to **25** may be suitable and the aromatic precursor (such as **24b**) can also be varied, for example, with different electron-donating substituents and various substitution patterns. Thus, the established synthetic route should enable the preparation of derivatives of compound **10** with, for example, varied alkyl chain lengths.

Inhibition of DHS activity in vitro

In order to investigate the ability of the new compounds **1**–**10** to inhibit DHS, all of the compounds were tested in an enzymatic in vitro DHS assay. The DHS reaction was simulated by employing recombinantly expressed eIF-5A and DHS, together with the substrates NAD and ^{3}H-labeled spermidine. The reaction was performed with the indicated concentrations of derivatives **1**–**10**, GC7 as a reference control, or only dimethylsulfoxide (DMSO) as a negative control, respectively. As a measure for DHS activity, the relative amounts of the tritium-labeled aminobutyl moiety transferred from spermidine to eIF-5A were detected.

Unfortunately, there was no significant inhibition of DHS detected for the in silico designed compounds **1**–**9** of the first two groups (Figures 6 and 7). Compounds **1**–**3** and **6**–**9** showed no inhibition, whereas compounds **4** and **5** showed weak inhibition (11% and 14%, respectively). GC7 showed a 45% inhibition under the assay conditions (80 μM GC7 or the corresponding compound; negative control subtracted from the absolute inhibition). In contrast, the novel designed potential binder **10** (Figure 8) showed significant dose-dependent inhibition of DHS ($IC_{50} \approx 12$ μM; Figure 10a,b).

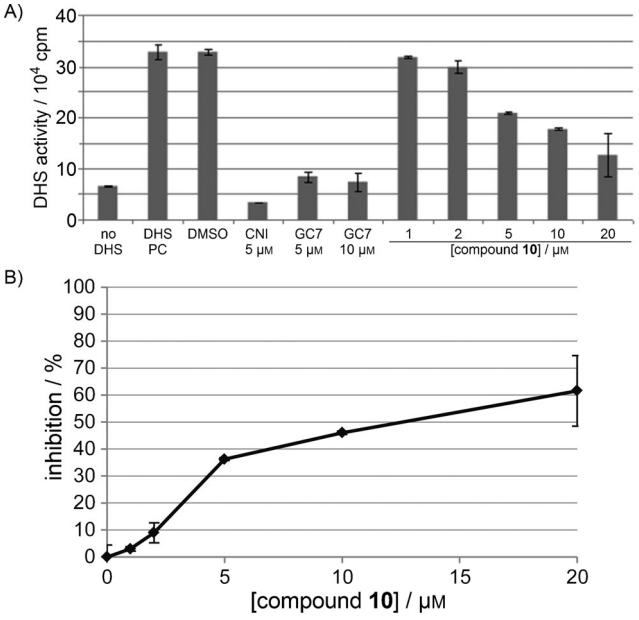


Figure 10. A) DHS inhibition assay with compound **10**. B) Dose-dependent inhibition of DHS by the novel inhibitor **10**.

Inhibition of HIV-1 replication in vitro

It has been previously demonstrated that the eIF-5A-modifying enzyme DHS may serve as a novel target for anti-retroviral therapy.[19,20] Therefore, the potential inhibitory effect on the replication of HIV-1 in tissue cultures was analyzed. For this purpose, PM1 lymphocytes were incubated for seven days, either in the presence of compound **10**, the established DHS inhibitor GC7 (positive control), or culture medium alone (negative control). Subsequently, the respective cultures were infected with HIV-1_{BaL} and culturing was continued for another week, at which time the p24 antigen levels in the culture supernatants were determined. Both compounds moderately inhibited the formation of HIV-1 progeny in a dose-dependent

manner (Figure 11). Clearly, GC7 was about twice as active as the in silico designed compound **10**. Compound **10** at a concentration of 2 μM achieved a moderate HIV-1 inhibition rate of 14%. Cell viability testing (by alamarBlue Assay) in the respective cultures failed to detect drug-induced cytotoxicity (data not shown).

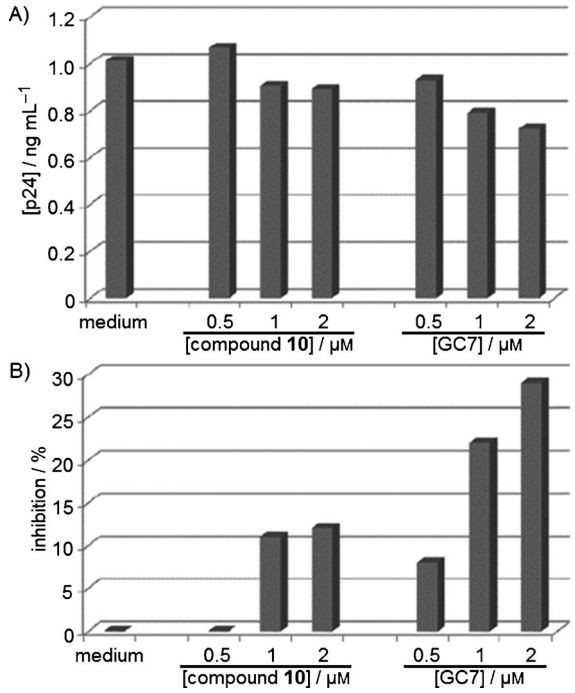


Figure 11. Antiviral effects of DHS inhibitors. PM1 cell cultures were incubated in the presence of the indicated concentrations of compound **10**, GC7, or in medium alone for seven days, infected with the CCR5-tropic HIV-1 isolate BaL, and further cultivated for another week. A) Release of viral particles was determined by an HIV-1 p24 antigen-specific enzyme-linked immunosorbent assay at day seven post-infection. B) The percentage of inhibition of virus replication relative to the replication in the control culture without drugs (medium), which was arbitrarily set at 100%.

Conclusions

In the presented approach, we have described a way of designing a new small-molecule inhibitor of deoxyhypusine synthase. A compound was successfully designed, synthesized, and validated to show dose-dependent inhibition of DHS in vitro and suppression of HIV replication in cell cultures. Thereby, we have demonstrated that the in silico design of DHS inhibitors may serve as a starting point to develop new drugs against diseases, such as HIV infection, in which the hypusine-containing protein eIF-5A plays a critical role. Although the observed antiviral activity for compound **10** is not very high, the data found here can be used now for a further development of the concept. The synthesis pathways described for compound **10** are flexible enough to prepare more structurally diverse molecules. Variations of compound **10** are currently being synthesized and will soon be analyzed with respect to their inhibition of DHS activity and HIV replication. Moreover, compound **10** is being used as a hit structure for further virtual screening optimization and also co-crystallization with DHS.

Experimental Section

Chemistry

All air- or water-sensitive reactions were performed in flame-dried glassware under a nitrogen atmosphere. Compounds **4** and **6** were obtained from Sigma–Aldrich. Commercially available solvents and reagents were used without further purification with the following exceptions: dichloromethane (CH_2Cl_2) was distilled from calcium hydride and stored over activated molecular sieves. THF was dried over potassium/benzophenone, distilled under nitrogen, and stored over activated molecular sieves. Petroleum ether 50–70, EtOAc, CH_2Cl_2, and CH_3OH employed in chromatography were distilled before use. For column chromatography, silica gel 60 (230–400 mesh) was used. For thin layer chromatography (TLC), precoated aluminum 60 F_{254} plates with a 0.2 mm layer of silica gel containing a fluorescence indicator were used. NMR spectra were recorded on 400 or 500 MHz spectrometers (Bruker AMX 400, Bruker AV 400, or Bruker DRX 500). All ^1H and ^{13}C NMR chemical shifts (δ) are quoted in parts per million (ppm) and were calibrated against solvent signals. High-resolution (HR) mass spectra were obtained with a VG Analytical VG/70–250F spectrometer (FAB; matrix was *m*-nitrobenzyl alcohol). HR ESI mass spectra were obtained with an Agilent Technologies ESI-TOF 6224 spectrometer. IR spectra were acquired by using a Bruker Alpha-P IR spectrometer within the 400–4000 cm^{-1} range in the attenuated total reflection (ATR) mode. Analytical HPLC was carried out on a VWR-Hitachi LaChromElite HPLC system, which consisted of a VWR-Hitachi L-2130 pump, an L-2200 autosampler, and an L-2455diode array detector. The column used was a Nucleodur C18 Gravity, 5 μm (Macherey–Nagel). Elution was performed with a 100 mM ammonium formiate buffer (pH 3.0)/acetonitrile (Sigma–Aldrich, HPLC grade) eluent, 5–44% CH_3CN (0–12 min), a flow rate of 1.3 mL min^{-1}, and UV detection at 264 nm.

General procedure 1: **Mitsunobu introduction of the guanidine**: The reaction was carried out under a nitrogen atmosphere. A solution of the corresponding alcohol, PPh_3 (1.5 equiv), and *N*,*N*′-bis-*tert*-butoxycarbonylguanidine (1.5 equiv) in dry THF (15 mL $mmol^{-1}$ alcohol) was cooled down to 0 °C. This was followed by dropwise addition of DIAD (1.5 equiv). After that, the reaction mixture was heated at reflux for 3 h. The solvent was removed in vacuo and the crude product was purified by column chromatography on silica gel (petroleum ether 50–70/EtOAc, 4:1→1:1).

General procedure 2: **Synthesis of the indole**: The reaction was carried out under a nitrogen atmosphere. Dry triethylamine (30 equiv) was added to a suspension of *O*-*tert*-butyl-3-iodo-4-(4-methylphenylsulfonylamido)benzylcarbamate (**24b**), CuI (0.2 equiv), and $Pd(PPh_3)_2Cl_2$ (0.1 equiv) in dry DMF (10–15 mL $mmol^{-1}$). This was followed by dropwise addition of the substituted alkyne (3 equiv). After being stirred for 17 h at 85 °C, the suspension was diluted with ethyl acetate and washed with H_2O (3×). The organic layer was dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was then purified by column chromatography on silica gel.

2,5-Di-(3-nitrophenyl)-1,3,4-oxadiazole (16): For the polyphosphoric acid solution, P_2O_5 (15 g) was added to phosphoric acid (10 mL). 3-Nitrobenzoic acid (**14**; 1.00 g, 5.99 mmol) and aminourea hydrochloride (**15**; 0.67 g, 5.99 mmol) were added to this solution. After being stirred at 170 °C for 2 h, the reaction mixture was

poured into ice/H_2O. The precipitated product was filtered and washed with H_2O to yield a colorless solid (482 mg, 1.55 mmol, 26%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 9.00$ (dd, $J = 1.7$, 1.7 Hz, 2H; H2), 8.64 (dt, $J = 7.9$, 1.4 Hz, 2H; H6), 8.51 (ddd, $J = 8.2$, 1.0, 1.0 Hz, 2H; H4), 7.96 ppm (dd, $J = 8.0$, 8.0 Hz, 2H; H5); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 163.6$ (oxadiazole C), 148.9 (aryl CNO_2), 132.8 (aryl C6), 130.8 (aryl C5), 126.8 (aryl C4), 125.2 (C1), 122.2 ppm (aryl C2); TLC: $R_f = 0.82$ (CH_2Cl_2/CH_3OH, 9:1); IR (neat): $\tilde{\nu} = 3093$, 1517, 1350, 1062, 912, 713, 667 cm^{-1}; HRMS (FAB): *m/z* calcd: 313.0572 $[M+H]^+$; found: 313.0576.

3-Nitro-*N*-(4-nitrophenyl)benzamide (19): The reaction was carried out under a nitrogen atmosphere. 4-Nitroaniline (**18**; 1.00 g, 7.24 mmol) was dissolved in dry pyridine (24 mL) and then 3-nitrobenzoyl chloride (**17**; 1.61 g, 8.69 mmol, 1.2 equiv) was added. After that, the reaction mixture was heated at reflux for 18 h and then poured into ice/H_2O. The precipitated product was filtered and washed with H_2O to yield a yellow solid (1.90 g, 6.61 mmol, 91%). ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 11.10$ (s, 1H; amide NH), 8.82 (dd, $J = 1.8$, 1.8 Hz, 1H; H3), 8.48 (ddd, $J = 8.2$, 1.5, 1.5 Hz, 1H; H7), 8.43 (ddd, $J = 8.0$, 1.2, 1.2 Hz, 1H; H5), 8.30 (d, $J = 9.1$ Hz, 2H; H10), 8.07 (d, $J = 9.2$ Hz, 2H; H9), 7.88 ppm (dd, $J = 8.1$, 8.1 Hz, 1H; H6); ^{13}C NMR (101 MHz, [D$_6$]DMSO): $\delta = 164.1$ (carbonyl C), 147.6 (C4), 145.0 (C8), 142.6 (C11), 135.6 (C2), 134.4 (C5), 130.3 (C6), 126.6 (C7), 124.8 (C10), 122.6 (C3), 120.2 ppm (C9); TLC: $R_f = 0.85$ (CH_2Cl_2/CH_3OH, 9:1); IR (neat): $\tilde{\nu} = 3367$, 3082, 1686, 1525, 1347, 1299, 1109, 848, 704 cm^{-1}; HRMS (FAB): *m/z* calcd: 288.0620 $[M+H]^+$; found: 288.0610.

6-Nitro-2-(3-nitrophenyl)benzoxazole (21): Method a: 2-Amino-5-nitrophenol (**20**; 771 mg, 5.00 mmol) and 3-nitrobenzoic acid (**14**; 836 mg, 5.00 mmol) were suspended in xylene (15 mL). After addition of boronic acid (340 mg, 5.50 mmol, 1.1 equiv), the reaction mixture was heated at reflux for 8 h and then the solvent was removed in vacuo. The crude product was purified by column chromatography on silica gel (CH_2Cl_2/CH_3OH, 99:1) to yield a pale-rose solid (229 mg, 0.803 mmol, 16%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta =$ 9.15 (dd, $J = 1.8$, 1.8 Hz, 1H; H9), 8.63 (ddd, $J = 7.7$, 1.6, 1.1 Hz, 1H; H13), 8.57 (dd, 1H, $J = 2.3$, 0.4 Hz; H6), 8.48 (ddd, $J = 8.3$, 2.3, 1.1 Hz, 1H; H11), 8.39 (dd, $J = 8.8$, 2.2 Hz, 1H; H4), 7.93 (dd, $J = 8.8$, 0.3 Hz, 1H; H3), 7.81 ppm (t, $J = 8.0$ Hz, 1H; H12); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 149.7$ (C7), 148.5 (C2), 146.4 (C1), 145.5 (C10), 133.5 (C13), 130.5 (C12), 127.0 (C11), 123.2 (C8), 122.7 (C3), 122.2 (C9), 121.5 (C5), 121.2 (C4), 107.6 ppm (C6); TLC: $R_f = 0.41$ (petroleum ether 50–70/EtOAc, 4:1); IR (neat): $\tilde{\nu} = 3104$, 2922, 2852, 1521, 1345, 1061, 815, 733, 707 cm^{-1}; HRMS (FAB): *m/z* calcd: 286.0386 $[M+H]^+$; found: 286.0463.

Method b: In a round-bottomed flask, 3-nitro-*N*-(4-nitrophenyl)benzamide (**19**; 104 mg, 362 μmol) was dissolved in *o*-dichlorobenzene (1.2 mL). This was followed by addition of copper triflate (30 mg, 83 μmol, 0.2 equiv). After that, the reaction mixture was heated at reflux for 22 h and then the solvent was removed in vacuo. The crude product was purified by column chromatography on silica gel (petroleum ether 50–70/EtOAc, 6:1→1:1) to yield a pale-rose solid (28 mg, 99 μmol, 27%). The analytical data were identical to those reported above.

6-Amino-2-(3-aminophenyl)benzoxazole (3): The reaction was carried out under a hydrogen atmosphere. 6-Nitro-2-(3-nitrophenyl)benzoxazole (**21**; 75 mg, 33 μmol) and Pd/C (10 mg) were suspended in dry ethanol (8 mL). This was followed by activation with ultrasound for 30 s. The reaction mixture was then stirred at room temperature under hydrogen pressure for 68 h, before being filtered and extracted with methanol. The crude product was purified by column chromatography on silica gel (CH_2Cl_2/CH_3OH, 97:3) to yield a colorless solid (18 mg, 80 μmol, 25%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.37$ (d, $J = 8.2$ Hz, 1H; H3), 7.31 (s, 1H; H9), 7.24–7.14 (m, 2H; H12, H13), 6.79 (s, 1H; H6), 6.70 (d, $J = 7.4$ Hz, 1H; H11), 6.62 (d, $J = 8.4$ Hz, 1H; H4), 5.38 ppm (2×s, 4H; 2×NH_2); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 159.8$ (C1), 151.7 (C2), 149.2 (C8), 147.8 (C7), 131.9 (C5), 129.5 (C12), 127.6 (C10), 119.5 (C3), 116.2 (C11), 113.9 (C13), 112.3 (C4), 111.3 (C9), 94.2 ppm (C6); TLC: $R_f = 0.47$ (CH_2Cl_2/CH_3OH, 9:1); IR (neat): $\tilde{\nu} = 3413$, 3314, 3205, 1628, 1489, 1354, 1146, 811, 717, 622 cm^{-1}; HRMS (ESI$^+$): *m/z* calcd: 226.0902 $[M+H]^+$; found: 226.0974.

2,5-Di-(3-aminophenyl)-1,3,4-oxadiazole (1): In a 25 mL round-bottomed flask, tin(II) chloride (608 mg, 3.20 mmol, 10 equiv) was dissolved in concentrated HCl (8 mL) and warmed up to 50 °C. This was followed by slow addition of 2,5-di-(3-nitrophenyl)-1,3,4-oxadiazole (**16**; 100 mg, 0.320 mmol). Afterward, the solution was stirred for a further 1.5 h and then poured, under gas formation, into a mixture of potassium carbonate (10 g) and ice/H_2O (100 mL). The product was extracted with EtOAc (3×) and the combined organic layers were dried over sodium sulfate. Finally, the solvent was removed in vacuo to yield a pale-yellow solid (68 mg, 0.27 mmol, 84%). ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 7.29$ (dd, $J =$ 1.8, 1.8 Hz, 2H; H2), 7.26–7.21 (m, 2H; H5), 7.21–7.18 (m, 2H; H6), 6.79 (ddd, $J = 7.8$, 2.3, 1.3 Hz, 2H; H4), 5.52 ppm (s, 4H; NH_2); ^{13}C NMR (101 MHz, [D$_6$]DMSO): $\delta = 164.2$ (oxadiazole C), 149.4 (C1), 129.8 (C5), 123.5 (C3), 117.2 (C4), 113.7 (C6), 111.0 ppm (C2); TLC: $R_f = 0.61$ (CH_2Cl_2/CH_3OH, 9:1); IR (neat): $\tilde{\nu} = 3207$, 1592, 1468, 1317, 781, 678 cm^{-1}; HRMS (FAB): *m/z* calcd: 253.1083 $[M+H]^+$; found: 253.1089.

3-Amino-*N*-(4-aminophenyl)benzamide (2): In a 25 mL round-bottomed flask, tin(II) chloride (727 mg, 3.83 mmol, 11 equiv) was dissolved in concentrated HCl (7 mL) and warmed up to 50 °C. This was followed by slow addition of 3-nitro-*N*-(4-nitrophenyl)benzamide (**19**; 100 mg, 0.348 mmol). Next, the solution was stirred for a further 2 h and then poured, under gas formation, into a mixture of potassium carbonate (8.25 g) and ice/H_2O (100 mL). The product was extracted with EtOAc (3×) and the combined organic layers were dried over sodium sulfate. Finally, the solvent was removed in vacuo to yield a colorless solid (78 mg, 0.345 mmol, 99%). ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 9.64$ (s, 1H; amide NH), 7.35 (dd, $J = 6.8$, 1.9 Hz, 2H; H9), 7.12–7.00 (m, 3H; H2, H5, H6), 6.70 (ddd, $J = 7.9$, 2.3, 1.0 Hz, 1H; H4), 6.52 (dd, $J = 6.7$, 2.1 Hz, 2H; H10), 5.23 (s, 2H; NH_2), 4.87 ppm (s, 2H; NH_2); ^{13}C NMR (101 MHz, [D$_6$]DMSO): $\delta =$ 165.3 (amide C), 148.4 (C3), 144.6 (C11), 136.1 (C1), 128.4 (C5), 128.2 (C8), 122.0 (C9), 116.1 (C4), 114.3 (C6), 113.4 (C10), 112.7 ppm (C2); TLC: $R_f = 0.52$ (CH_2Cl_2/CH_3OH, 9:1); IR (neat): $\tilde{\nu} = 3325$, 3218, 1597, 1583, 1511, 1320, 1246, 817, 505 cm^{-1}; HRMS (FAB): *m/z* calcd: 228.1136 $[M+H]^+$; found: 228.1132.

(6-Aminohexyl)urea (9): The reaction was carried out under a nitrogen atmosphere. A solution of trimethylsilylisocyanate (0.20 mL, 1.5 mmol, 0.35 equiv) in dry THF (45 mL) was added dropwise to a solution of 1,6-diaminohexane (**22a**; 500 mg, 4.30 mmol) in dry THF (15 mL) over a period of 3 h. The reaction mixture was then stirred for 2 h at room temperature. This was followed by addition of H_2O (6 mL) and additional stirring for 2 h. Next, the solvent was removed in vacuo and the residue was suspended in hot EtOAc and filtered. The remaining solid was washed with H_2O (10 mL). The product was isolated by freeze drying of the aqueous phase to yield a colorless foam (68 mg, 0.43 mmol, 28%). ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 5.92$ (t, $J = 6.3$ Hz, 1H; urea NH), 5.34 (s, 2H; urea NH_2), 3.44 (brs, 2H; NH_2), 2.93 (q, $J = 6.6$ Hz, 2H; H1), 2.53 (t, $J =$ 6.7 Hz, 2H; H6), 1.39–1.20 ppm (m, 8H; alkyl H); ^{13}C NMR (101 MHz,

[D_6]DMSO): $\delta = 158.6$ (urea C), 41.1 (C6), 38.9 (C1), 30.0, 26.2, 26.1, 26.0 ppm (alkyl C); IR (neat): $\tilde{\nu} = 3301$, 2932, 2854, 1643, 1556, 1356, 1228, 587 cm^{-1}; HRMS (FAB): *m/z* calcd: 160.1449 $[M+H]^+$; found: 160.1451.

(7-Aminoheptyl)urea (8): The reaction was carried out under a nitrogen atmosphere. A solution of trimethylsilylisocyanate (0.18 mL, 1.4 mmol, 0.35 equiv) in dry THF (39 mL) was added dropwise to a solution of 1,7-diaminoheptane (**22b**; 500 mg, 3.85 mmol) in dry THF (13 mL) over a period of 3 h. The reaction mixture was stirred for 2 h at room temperature. This was followed by addition of H_2O (6 mL) and additional stirring for 2 h. The solvent was then removed in vacuo and the residue was suspended in hot EtOAc and filtered. The remaining solid was washed with H_2O (10 mL). The product was isolated by freeze drying of the aqueous phase to yield a colorless foam (24 mg, 0.14 mmol, 11%). ^{1}H NMR (400 MHz, [D_6]DMSO): $\delta = 5.92$ (t, $J = 6.4$ Hz, 1H; urea NH), 5.34 (s, 2H; urea NH_2), 3.78 (br s, 2H; NH_2), 2.93 (q, $J = 6.6$ Hz, 2H; H1), 2.59 (t, $J = 7.1$ Hz, 2H; H7), 1.42–1.21 ppm (m, 10H; alkyl H); ^{13}C NMR (101 MHz, [D_6]DMSO): $\delta = 158.6$ (urea C), 40.2 (C7), 38.7 (C1), 31.0, 29.9, 28.5, 26.3, 26.2 ppm (alkyl C); IR (neat): $\tilde{\nu} = 3349$, 2931, 2852, 1651, 1596, 1553, 1262, 1225 cm^{-1}; HRMS (FAB): *m/z* calcd: 174.1606 $[M+H]^+$; found: 174.1614.

(8-Aminooctyl)urea (7): The reaction was carried out under a nitrogen atmosphere. A solution of trimethylsilylisocyanate (0.17 mL, 1.2 mmol, 0.35 equiv) in dry THF (37 mL) was added dropwise to a solution of 1,8-diaminooctane (**22c**; 500 mg, 3.5 mmol) in dry THF (13 mL) over a period of 2 h. The reaction mixture was then stirred for 2 h at room temperature. This was followed by addition of H_2O (6 mL) and additional stirring for 2 h. Next, the solvent was removed in vacuo and the residue was suspended in hot EtOAc and filtered. The remaining solid was washed with H_2O (10 mL). The product was isolated by freeze drying of the aqueous phase to yield a colorless foam (90 mg, 0.48 mmol, 39%). ^{1}H NMR (400 MHz, [D_6]DMSO): $\delta = 5.93$ (t, $J = 6.0$ Hz, 1H; urea NH), 5.35 (s, 2H; urea NH_2), 3.60 (br s, 2H; NH_2), 2.92 (q, $J = 6.6$ Hz, 2H; H1), 2.56 (t, $J = 7.2$ Hz, 2H; H8), 1.40–1.30 (m, 4H; H2, H7), 1.27–1.22 ppm (m, 8H; alkyl H); ^{13}C NMR (101 MHz, [D_6]DMSO): $\delta = 158.7$ (urea C), 40.8 (C8), 38.9 (C1), 31.6, 30.0, 28.9, 28.7, 26.4, 26.2 ppm (alkyl C); IR (neat): $\tilde{\nu} = 3393$, 1928, 2851, 1655, 1594, 1558, 1404, 655, 572 cm^{-1}; HRMS (FAB): *m/z* calcd: 188.1762 $[M+H]^+$; found: 188.1763.

***N*-(6-Aminohexyl)prop-2-enamide (5)**: The reaction was carried out under a nitrogen atmosphere. A solution of 1,6-diaminohexane (**22a**; 500 mg, 4.30 mmol) in dry methanol (20 mL) was cooled to 0 °C. This was followed by dropwise addition of acryloyl chloride (0.35 mL, 4.3 mmol) over 1 h. The reaction mixture was then allowed to warm up to room temperature and stirred for a further 30 min. The solvent was removed in vacuo and the crude product was purified by column chromatography on silica gel (CH_2Cl_2/CH_3OH/Et_3N, 80:18:2) to yield a colorless solid (253 mg, 1.49 mmol, 35%). ^{1}H NMR (400 MHz, [D_6]DMSO): $\delta = 8.05$ (s, 1H; amide NH), 6.20 (dd, $J = 17.1$, 10.3 Hz, 1H; H2), 6.05 (dd, $J = 17.1$, 2.3 Hz, 1H; H1a), 5.55 (dd, $J = 10.0$, 2.3 Hz, 1H; H1b), 3.25 (br s, 2H; NH_2), 3.10 (q, $J = 6.8$ Hz, 2H; H4), 2.53–2.51 (m, 2H; H9), 1.44–1.22 ppm (m, 8H; alkyl H); ^{13}C NMR (101 MHz, [D_6]DMSO): $\delta = 164.4$ (amide C), 131.9 (C2), 124.6 (C3), 41.4 (C9), 38.5 (C4), 33.0, 29.1, 26.4, 26.0 ppm (alkylC); IR (neat): $\tilde{\nu} = 3296$, 3057, 2930, 2856, 1653, 1542, 1239, 951 cm^{-1}; HRMS (FAB): *m/z* calcd: 171.1497 $[M+H]^+$; found: 171.1499.

4-Amino-3-iodo-*N*-benzylamine hydrochloride (31): The reaction was carried out under a nitrogen atmosphere. 1 M BH_3·THF solution (60 mL, 60 mmol, 3 equiv) was added dropwise to a solution of 4-amino-3-iodo-benzonitrile (**23**; 4.86 g, 20.0 mmol) in dry THF (60 mL). The reaction mixture was then heated at reflux for 4 h. After the reaction mixture had cooled down to room temperature, 2 M HCl (30 mL, 60 mmol, 3 equiv) was added. This was followed by further heating at reflux for 1 h. The solvent was removed in vacuo to yield a yellowish solid, which was used without further purification.

***O-tert*-Butyl-4-amino-3-iodobenzylcarbamate (24a)**: The reaction was carried out under a nitrogen atmosphere. A suspension of dry triethylamine (11 mL, 80 mmol, 4 equiv) and **31** (5.68 g, 20.0 mmol) in dry CH_2Cl_2 (90 mL) was cooled to 0 °C. After that, DMAP (487 mg, 3.99 mmol, 0.2 equiv) and di-*tert*-butyldicarbonate (9.20 g, 42.1 mmol, 2.1 equiv) were added. The reaction mixture was stirred at room temperature for 5 h. The solution was washed with H_2O (3×) and the aqueous phase was re-extracted with H_2O. The combined organic layers were dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (petroleum ether 50–70/EtOAc, 2:1) to yield a pale-orange oil (4.99 g, 14.4 mmol, 72%). ^{1}H NMR (400 MHz, [D_6]DMSO): $\delta = 7.41$ (s, 1H; H2), 7.24–7.21 (t, $J = 5.8$ Hz 1H; NH), 6.95 (dd, $J = 8.2$, 1.9 Hz, 1H; H6), 6.68 (d, $J = 8.2$ Hz, 1H; H5), 5.09 (s, 2H; NH_2), 3.91 (d, $J = 6.4$ Hz, 2H; $BnCH_2$), 1.37 ppm (s, 9H; 3×$BocCH_3$); ^{13}C NMR (101 MHz, [D_6]DMSO): $\delta = 155.6$ (Boc C=O), 147.2 (C1), 137.1 (C2), 129.8 (C3), 128.2 (C6), 114.1 (C5), 82.8 (C4), 77.6 (Boc $C(CH_3)_3$), 42.2 ($BnCH_2$), 28.2 ppm (Boc $C(CH_3)_3$); TLC: $R_f = 0.55$ (petroleum ether 50–70/EtOAc, 2:1); IR (neat): $\tilde{\nu} = 3342$, 2975, 1686, 1614, 1496, 1365, 1247, 1154, 1027, 783 cm^{-1}; HRMS (ESI$^+$): *m/z* calcd: 349.0413 $[M+H]^+$; found: 349.0406.

***O-tert*-Butyl-3-iodo-4-(4-methylphenylsulfonylamido)benzylcarbamate (24b)**: The reaction was carried out under a nitrogen atmosphere. Dry pyridine (2.0 mL, 26 mmol, 3 equiv) and *p*-tosyl chloride (1.97 g, 10.3 mmol, 1.2 equiv) were added to a solution of **24a** (3.00 g, 8.61 mmol) in dry CH_2Cl_2 (36 mL). After being stirred at room temperature for 42 h, the reaction mixture was diluted with CH_2Cl_2 and extracted with H_2O (3×). The combined organic layers were dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (petroleum ether 50–70/EtOAc, 2:1) to yield an orange oil (3.99 g, 7.93 mmol, 92%). ^{1}H NMR (400 MHz, [D_6]DMSO): $\delta = 9.61$ (s, 1H; Tos NH), 7.67 (s, 1H; H2), 7.58 (d, $J = 7.8$ Hz, 2H; H2′, H6′), 7.36 (d, $J = 7.8$ Hz, 3H; H3′, H5′, Boc NH), 7.13 (d, $J = 8.4$ Hz, 1H; H5), 6.90 (d, $J = 8.4$ Hz, 1H; H6), 4.07–3.99 (m, 2H; Bn CH_2), 2.38 (s, 3H; Tos CH_3), 1.38 ppm (s, 9H; 3×Boc CH_3); ^{13}C NMR (101 MHz, [D_6]DMSO): $\delta = 156.8$ (Boc C=O), 143.5 (C4′), 141.4 (C1), 138.2 (C3), 138.0 (C2), 129.8 (C3′, C5′), 128.5 (C1′), 127.7 (C5), 127.3 (C6), 127.1 (C2′, C6′), 99.8 (C4), 78.6 (Boc $C(CH_3)_3$), 42.6 (Bn CH_2), 28.4 (Boc $C(CH_3)_3$), 21.4 ppm (Tos CH_3); TLC: $R_f = 0.43$ (petroleum ether 50–70/EtOAc, 2:1); IR (neat): $\tilde{\nu} = 3325$, 2976, 2930, 2217, 1688, 1487, 1332, 1246, 1157, 664, 548 cm^{-1}; HRMS (ESI$^+$): *m/z* calcd: 525.0321 $[M+Na]^+$; found: 525.0322.

***O-tert*-Butyl-4-amino-3-(4-*N*,*N*′-bis-*tert*-butoxycarbonylguanidino-but-1-yne-1-yl)benzylcarbamate (28b)**: The reaction was carried out under a nitrogen atmosphere. DBU (0.21 mL, 1.37 mmol, 1.5 equiv) was added to a suspension of **24a** (306 mg, 0.878 mmol) and Pd EnCat TPP30 (77 mg, 3.5 mol%; 0.4 mmol Pd per 1 g) in dry CH_3CN (8 mL), followed by addition of **27** (410 mg, 1.37 mmol, 1.5 equiv). The reaction mixture was heated at reflux for 17 h and, after cooling down to room temperature, was filtered. The residue was washed with CH_2Cl_2 and CH_3OH. The filtrate was concentrated in vacuo and the crude product was purified by column chroma-

tography on silica gel (CH_2Cl_2/CH_3OH, 99:1→19:1) to yield a colorless solid (237 mg, 0.446 mmol, 51%). ^1H NMR (400 MHz, $CDCl_3$): $\delta = 9.08$ (s, 2H; guanidine NH_2), 7.17 (t, $J = 5.9$ Hz, 1H; NH), 6.96 (s, 1H; H2), 6.89 (d, $J = 8.2$ Hz, 1H; H6), 6.60 (d, $J = 8.2$ Hz, 1H; H5), 5.13 (s, 2H; NH_2), 4.01 (t, $J = 7.7$ Hz, 2H; H10), 3.90 (d, $J = 5.9$ Hz, 2H; Bn CH_2), 2.74 (t, $J = 7.0$ Hz, 2H; H9), 1.49 (s, 9H; 3×Boc CH_3), 1.41 (s, 9H; 3×Boc CH_3), 1.37 ppm (s, 9H; 3×Boc CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 162.8$ (C11), 159.4 (Boc C=O), 155.6 (Boc C=O), 153.9 (Boc C=O), 148.1 (C4), 130.4 (C2), 128.3 (C6), 127.3 (C1), 113.6 (C5), 106.0 (C3), 91.4 (C8), 83.7 (Boc $C(CH_3)$), 79.0 (C7), 77.8 (Boc $C(CH_3)$), 77.6 (Boc $C(CH_3)$), 43.2 (C10), 42.7 (Bn CH_2), 28.3 (Boc $C(CH_3)$), 28.0 (Boc $C(CH_3)$), 27.5 (Boc $C(CH_3)$), 19.2 ppm (C9); TLC: $R_f = 0.55$ (CH_2Cl_2/CH_3OH, 9:1); IR (neat): $\tilde{\nu} = 3373$, 2976, 1709, 1608, 1502, 1365, 1245, 1141, 1004, 744 cm^{-1}; HRMS (ESI^+): *m/z* calcd: 532.3135 $[M+H]^+$; found: 532.3136.

5-(*O*-*tert*-Butoxycarbonyl)aminomethyl-2-(2-hydroxyethyl)-1-*N*-(4-methylphenylsulfonyl)indole (29b): The reaction was performed according to general procedure 2 by using **24b** (500 mg, 0.995 mmol), **25** (3 equiv), and dry DMF (10 mL). A CH_2Cl_2/CH_3OH gradient from 99:1 to 19:1 was used for the column chromatography to yield a pale-yellow oil (399 mg, 0.898 mmol, 90%). ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.94$ (d, $J = 8.2$ Hz, 1H; H7), 7.66 (d, $J = 8.2$ Hz, 2H; H2′, H6′), 7.34 (d, $J = 8.1$ Hz, 3H; H3′, H5′, NH), 7.30 (s, 1H; H4), 7.14 (d, $J = 8.7$ Hz, 1H; H6), 6.59 (s, 1H; H3), 4.77 (t, $J = 5.5$ Hz, 1H; OH), 4.15 (d, $J = 5.9$ Hz, 2H; Bn CH_2), 3.76 (dt, $J = 6.4$, 9.5 Hz, 2H; H9), 3.14 (t, $J = 6.6$ Hz, 2H; H8), 2.30 (s, 3H; Tos CH_3), 1.38 ppm (s, 9H; 3×Boc CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 156.1$ (Boc C=O), 144.9 (C4′), 138.9 (C2), 134.4 (C7a), 130.4 (C3′, C5′), 129.4 (C3a), 126.0 (C2′, C6′), 122.8 (C5), 119.2 (C4), 110.1 (C3), 75.2 (Boc $C(CH_3)_3$), 59.5 (C9), 43.0 (Bn CH_2), 32.2 (C8), 28.4 (Boc $C(CH_3)_3$), 21.3 ppm (Tos CH_3); TLC: $R_f = 0.37$ (CH_2Cl_2/CH_3OH, 19:1); IR (neat): $\tilde{\nu} = 3400$, 3054, 2977, 2930, 1510, 1469, 1364, 1090, 961, 670 cm^{-1}; HRMS (ESI^+): *m/z* calcd: 467.1617 $[M+Na]^+$; found: 467.1615.

***N*,*N*′-Bis-*tert*-Butoxycarbonylguanidinobut-3-yne (27)**: The reaction was performed according to general procedure 1 by using **25** (0.38 mL, 5.0 mmol) to yield a colorless solid (1.36 g, 4.37 mmol, 87%). ^1H NMR (400 MHz, $CDCl_3$): $\delta = 9.06$ (s, 2H; NH_2), 3.90 (t, $J = 8.3$ Hz, 2H; H1), 2.89 (t, $J = 2.5$ Hz, 1H; H4), 2.44 (dt, $J = 8.1$, 2.9 Hz, 2H; H2), 1.50 (s, 9H; 3×Boc CH_3), 1.41 ppm (s, 9H; 3×Boc CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 160.9$ (C5), 154.8 (2×Boc C=O), 83.0 (C3), 80.0 (2×Boc $C(CH_3)_3$), 73.3 (C4), 43.4 (C1), 28.4 (2×Boc $C(CH_3)_3$), 18.3 ppm (C2); TLC: $R_f = 0.42$ (petroleum ether 50–70/EtOAc, 2:1); IR (neat): $\tilde{\nu} = 3376$, 3197, 2984, 2965, 1637, 1512, 1450, 1308, 1120, 1003, 597 cm^{-1}; MS (FAB): *m/z* calcd: 312.2 $[M+H]^+$; found: 312.3.

5-(*O*-*tert*-Butoxycarbonyl)aminomethyl-2-(2-*N*,*N*′-bis-*tert*-butoxycarbonylguanidinoethyl)-1-*N*-(4-methylphenylsulfonyl)indole (30b): Method a: The reaction was performed according to general procedure 2 by using **24b** (254 mg, 0.506 mmol), **27** (3 equiv), and dry DMF (7 mL). A petroleum ether 50–70/EtOAc gradient from 4:1 to 1:1 was used for the column chromatography to yield a pale-yellow oil (212 mg, 0.309 mmol, 62%). ^1H NMR (400 MHz, $CDCl_3$): $\delta = 9.12$ (s, 2H; NH_2), 8.01 (d, $J = 8.6$ Hz, 1H; H7), 7.71 (d, $J = 8.2$ Hz, 2H; H2′, H6′), 7.37–7.30 (m, 3H; H3′, H5′, NH), 7.29 (s, 1H; H4), 7.18 (dd, $J = 8.8$, 1.2 Hz, 1H; H6), 6.50 (s, 1H; H3), 4.20 (t, $J = 6.3$ Hz, 2H; H9), 4.14 (d, $J = 5.9$ Hz, 2H; Bn CH_2), 3.23 (t, $J = 5.8$ Hz, 2H; H8), 2.30 (s, 3H; Tos CH_3), 1.41 (s, 9H; 3×Boc CH_3), 1.38 (s, 9H; 3×Boc CH_3), 1.19 ppm (s, 9H; 3×Boc CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 160.0$ (Boc C=O), 156.1 (Boc C=O), 154.5 (C10), 145.6 (C4′), 138.8 (C2), 135.5 (C3a), 135.5 (C1′), 130.5 (C3′, C5′), 130.0 (C7a), 129.9 (C5), 126.3 (C2′, C6′), 123.9 (C6), 119.0 (C4), 114.3 (C7), 110.8 (C3), 84.7 (Boc $C(CH_3)_3$), 83.9 (Boc $C(CH_3)_3$), 78.3 (Boc $C(CH_3)_3$), 43.6 (Bn CH_2). 44.2 (C9), 28.5 (Boc $C(CH_3)_3$), 28.1 (C8), 27.5 (Boc $C(CH_3)_3$), 21.4 ppm (Tos CH_3); TLC: $R_f = 0.42$ (petroleum ether 50–70/EtOAc, 2:1); IR (neat): $\tilde{\nu} = 3378$, 2931, 1709, 1607, 1504, 1471, 1443, 1365, 1282, 1162, 811, 545 cm^{-1}; HRMS (FAB): *m/z* calcd: 686.3 $[M+H]^+$; found: 686.5.

Method b: The reaction was performed according to general procedure 1 by using **29b** (399 mg, 0.897 mmol) to yield a pale-yellow oil (585 mg, 0.853 mmol, 95%). The analytical data were identical to those reported above.

5-(*O*-*tert*-Butoxycarbonyl)aminomethyl-2-(2-*N*,*N*′-bis-*tert*-butoxycarbonylguanidinoethyl)-1*H*-indole (30a): Method a: The reaction was carried out under a nitrogen atmosphere. A solution of **30b** (59 mg, 86 μmol) in dry methanol (3 mL) was mixed with NaOH (600 mg, 15 mmol). The viscose reaction mixture was stirred at room temperature for 40 h. After dilution with EtOAc, the solution was washed with H_2O (3×). The aqueous phase was extracted with EtOAc and the combined organic layers were dried over sodium sulfate. After removal of the solvent, the crude product was purified by column chromatography on silica gel (CH_2Cl_2/CH_3OH, 19:1→9:1) to yield a colorless solid (43 mg, 82 μmol, 95%). ^1H NMR (400 MHz, $CDCl_3$): $\delta = 10.94$ (s, 1H; NH1), 7.27 (br s, 2H; H4, Boc NH), 7.22 (d, $J = 8.2$ Hz, 1H; H7), 6.92 (d, $J = 8.2$ Hz, 1H; H6), 6.17 (s, 1H; H3), 4.15 (d, $J = 6.3$ Hz, 1H; Bn CH_2), 3.52 (t, $J = 7.9$ Hz, 2H; H9), 2.90 (br s, 2H; H8), 1.42 (s, 9H; 3×Boc CH_3), 1.38 (s, 9H; 3×Boc CH_3), 1.29 ppm (s, 9H; 3×Boc CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 160.9$ (C10), 155.7 (2×Boc C=O), 136.9 (C2), 135.1 (C7a), 130.4 (C5), 128.1 (C3a), 120.2 (C6), 117.7 (C4), 110.4 (C7), 99.0 (C3), 77.5 (C9), 44.0 (Bn CH_2), 28.3 (2×Boc $C(CH_3)$), 28.1 ppm (C8); TLC: $R_f = 0.28$ (CH_2Cl_2/CH_3OH, 9:1); IR (neat): $\tilde{\nu} = 3281$, 2930, 1683, 1632, 1486, 1390, 1146, 966, 776 cm^{-1}; HRMS (FAB): *m/z* calcd: 532.3135 $[M+H]^+$; found: 532.3122.

Method b: The reaction was carried out under a nitrogen atmosphere. KH (19 mg, 0.48 mmol, 1.2 equiv) was added to a suspension of **28b** (214 mg, 0.402 mmol) and $Cu(OAc)_2$ (80 mg, 0.44 mmol, 1.1 equiv) in dry 1,2-dichloroethane (11 mL). After that, the reaction mixture was stirred at 70 °C for 4 d. The crude product was purified by column chromatography on silica gel (CH_2Cl_2/CH_3OH, 99:1→9:1) to yield a pale-yellow solid (123 mg, 0.230 mmol, 57%). The analytical data were identical to those reported above.

2-(2-Guanidinoethyl)-5-aminomethyl-1*H*-indole (10): In a 25 mL round-bottomed flask, **30a** (55 mg, 0.10 mmol) was dissolved in CH_3CN/2 M HCl (1:1; 8 mL). After the reaction mixture had been stirred at room temperature for 28 h, the solvent was removed under reduced pressure. The crude product was purified by column chromatography on RP silica gel with H_2O as the eluent. Freeze drying of the product fractions yielded a pale-rose solid of **10** as the hydrochloride salt (22 mg, 73 μmol, 73%). ^1H NMR (400 MHz, $CDCl_3$): $\delta = 11.37$ (s, 1H; NH1), 8.33 (s, 3H; Bn NH_2, guanidine NH), 7.86 (t, $J = 5.8$ Hz, 1H; guanidine NH), 7.54 (s, 1H; H4), 7.32 (d, $J = 8.7$ Hz, 1H; H7), 7.13 (dd, $J = 8.3$, 1.7 Hz, 1H; H6), 6.27 (s, 1H; H3), 4.01 (dt, $J = 5.8$, 5.8 Hz, 2H; Bn CH_2), 3.52 (dt, $J = 6.9$, 6.3 Hz, 2H; H9), 2.94 ppm (t, $J = 7.3$ Hz, 2H; H8); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 156.9$ (C10), 136.9 (C2), 135.4 (C7a), 127.9 (C3a), 123.8 (C5), 120.8 (C6), 120.4 (C4), 110.7 (C7), 99.1 (C3), 58.7 (C9), 42.6 (Bn CH_2), 28.8 ppm (C8); IR (neat): $\tilde{\nu} = 3326$, 3232, 3052, 2923, 1683, 1486, 1309, 1164, 967, 804, 641 cm^{-1}; HRMS (FAB): *m/z* calcd: 232.1557 $[M+H]^+$; found: 232.1552.

Biochemistry

DHS activity assay: The deoxyhypusine synthase reaction was conducted, in principle, as described previously.[57] Briefly, the reaction mixture contained eIF5A precursor protein (5 μg), DHS (3 μg), and [^{3}H]spermidine (2 μCi, 32.4 Ci mmol^{-1}) in a total volume of 200 μL of reaction buffer (300 mM glycine–NaOH (pH 9.0) containing 1 mM NAD^+, 1 mM 1,4-dithiothreitol (DTT), and 50 μg mL^{-1} bovine serum albumin (BSA)). To test for potential influence of the new inhibitors on DHS activity, the enzymatic reaction was performed in the presence of the indicated concentrations of the indicated compounds **1**–**10**, or with GC7 as a positive control, or without any compounds as a solvent or negative control, respectively. After 1 h at 37 °C, the reaction was stopped by adding 20 mM spermidine (200 μL) in PBS and was transferred onto a Millipore GSWPO2500 nitrocellulose membrane, which was previously blocked with 20 mM spermidine/PBS for 1 h. The reaction mixture was vacuum filtered and then the membrane was washed with PBS (5 mL). Finally, the membranes were air dried and the tritium signal was measured in a liquid scintillation counter.

Overexpression and purification of DHS and eIF-5A: *Escherichia coli* strain BL21 (DE3) (Novagen) was employed for protein expression.

His-DHS purification: The pTricHis-DHS clone was used for the preparation for recombinant DHS.[55] A 1 L culture of Luria–Bertani medium containing 50 μg mL^{-1} ampicillin was inoculated with 50 mL volume of an overnight culture of *E. coli* BL21 (DE3) transformed with pTricHis-DHS (preinoculum) and grown at 37 °C until the optical density value at 600 nm reached 0.6–0.8 (~3 h). The Tre recombinase gene expression was induced by addition of 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and the culture was grown for a further period of 4 h at 37 °C. After incubation, the bacteria were harvested by centrifugation (10 min, 5000 *g*, 4 °C) and stored at −80 °C until further use.

The cell pellet was resuspended in lysis buffer (30 mL; 50 mM tris(hydroxymethyl)aminomethane (Tris; pH 8) containing 300 mM NaCl and 30 mM imidazole) and lysed by sonication. The soluble fraction was separated by centrifugation at 12000 rpm for 15 min. A bed volume of 1 mL of buffer-equilibrated nickel–nitriloacetate (Ni-NTA) beads was added to the supernatant and incubated with gentle rocking in an end-to-end rotor at 4 °C for 1 h. The beads were then packed in a column and washed with a wash buffer (50 mM Tris (pH 8) containing 300 mM NaCl and 30 mM imidazole), followed by a second wash buffer (50 mM Tris (pH 8) containing 300 mM NaCl and 50 mM imidazole). The protein was eluted with 50 mM Tris (pH 8) containing 300 mM NaCl and 300 mM imidazole. The purified His-DHS protein was analyzed by SDS-PAGE, dialyzed against 300 mM glycine–NaOH (pH 9) and 10% glycerol, and stored at −20 °C.

eIF-5A expression and purification: The pGEX-eIF5A clone was used to prepare eIF-5A protein, as described previously but with minor modifications.[58] GST-eIF5A overexpression was performed as described for DHS.

The IPTG-induced cell pellet was resuspended in lysis buffer (40 mL; 10 mM PBS (pH 7.4) containing 1 mM DTT, 5 mM ethylenediaminetetraacetate (EDTA), 2.6 mM $MnCl_2$, and 26 mM $MgCl_2$) with 0.5 μg mL^{-1} DNAse and protease inhibitors (0.1 mM phenylmethanesulfonyl fluoride (PMSF), 2 μg mL^{-1} leupeptin, and 2 μg mL^{-1} aprotinin) and lysed by sonication. 1% Triton X-100 was added to the total lysate (Triton X-100 was diluted in 1× PBS and then used) and the mixture was incubated at 4 °C for 1 h on an end-to-end rotor. The soluble fraction was separated by centrifugation at 12000 rpm for 15 min.

A bed volume of 1 mL of buffer-equilibrated glutathione (GSH) beads were added to the supernatant and the mixture was incubated with gentle rocking in an end-to-end rotor at 4 °C for 1–2 h. The lysate was passed through a column so that the beads were packed in a column. The beads were then washed with a wash buffer (100 mL; 50 mL PBS (pH 7.4) containing 1 mM DTT and 5 mM EDTA) and with a second buffer (150 mL; 50 mM Tris (pH 8) containing 200 mM NaCl and 5 mM EDTA). The protein was eluted with 50 mM Tris-HCl containing 200 mM NaCl and 20 mM reduced glutathione (pH 8.0). The purified protein fractions were dialyzed against the factor Xa cleavage buffer (50 mM Tris-HCl containing 100 mM NaCl and 1 mM $CaCl_2$). Factor Xa cleavage and final purification of eIF-5A was performed exactly as described previously.[58] eIF-5A was dialyzed in assay buffer (300 mM glycine/NaOH, pH 9) and stored at −20 °C.

HIV-1 infection experiments: PM1 cells were pre-incubated for 7 d in the presence of compound **10**, the DHS inhibitor GC7, or in medium alone. Subsequently, the cells were infected with the CCR5-tropic HIV-1 strain BaL.[59] For infection, roughly 3×10^6 cells were resuspended in culture medium (500 μL) without drugs and incubated at 37 °C for 3 h with HIV-1 viral stock (3 ng). After infection, cells were washed twice with PBS and further cultivated for another week in the presence of the compounds or in medium alone. At day 7 after infection, the p24 levels in the supernatant were determined with an enzyme-linked immunosorbent assay (Innogenetics NV). PM1 cells were maintained in RPMI medium (Invitrogen) containing 10% fetal calf serum (FCS; Biochrom AG), 100 U mL^{-1} penicillin, and 100 mg mL^{-1} streptomycin (Invitrogen).

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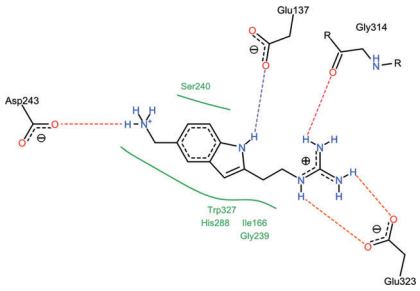
In silico Design, Synthesis, and Screening of Novel Deoxyhypusine Synthase Inhibitors Targeting HIV-1 Replication



Combating drug resistance: The in silico design, synthesis, and biological evaluation of deoxyhypusine synthase (DHS) inhibitors are described. DHS is involved in post-translational modification of the protein eIF-5A, which is needed for the translocation of HIV RNA from the nucleus into the cytosol. A newly designed inhibitor showed dose-dependent inhibition of DHS in vitro and suppression of HIV replication in cell cultures without cytotoxic effects.