Chapter 2
Preparation and Biological Evaluation of Synthetic and Polymer-Encapsulated Congeners of the Antitumor Agent Pactamycin: Insight into Functional Group Effects and Biological Activity

2.1 Introduction

An expeditious total synthesis of the aminocyclitol antibiotic pactamycin was described in Chap. 1, enabling fifteen-step preparation of the natural product from commercially available materials. Pactamycin, while bearing a remarkable array of valuable biological traits, is hindered in medicinal development by its high cytotoxicity. For this molecule to achieve its full potential as a therapeutic, chemical modifications to the parent structure must be enabled for structure-activity relationship (SAR) investigations to be possible. Accordingly, our synthesis of pactamycin was designed with the goal of late-stage structural modification toward the preparation of heretofore inaccessible synthetic analogs of pactamycin. This chapter will describe our successful efforts in the synthesis and biological analysis of twenty-five unique structural analogs of pactamycin. Additionally, as part of a collaboration with the DeSimone group (UNC Chapel Hill), the encapsulation of pactamycin and select derivatives into the PRINT® nanoparticle technology is demonstrated as proof-of-concept.

2.2 Background

2.2.1 Importance of Natural Products in Pharmaceutical Development

Pharmaceutical development through organic synthesis remains a critical feature of the drug discovery process [1]. Upon identification of an initial hit via...
high-throughput screening, a significant amount of structural modification is often required before a lead candidate can be advanced to clinical trials. Natural molecules are frequently identified as initial hits in these screenings; a recent survey study showed that natural products and their derivatives comprise over one-third of all FDA-approved new molecular entities (NMEs) [2]. Furthermore, Reynisson and co-workers reported that of the 39 % of known drug space (KDS) that is comprised of natural products and their derivatives, 74 % of this subset is made up of natural product derivatives [3]. Figure 2.1 illustrates a selection of natural product-derived structures (and their corresponding parent structures) approved for use by the FDA in the past decade [4]. The morphine-derived methylnaltrexone is currently in use for the treatment of opioid-induced constipation. Fingolimod, derived from myriocin, is being employed as a treatment for multiple sclerosis. Zucapsaicin and dapagliflazin (derived from the natural products capsaicin and phlorizin, respectively) are also in use as analgesic and diabetic medicines. These examples speak to the power of natural product scaffolds in the continued development of the pharmaceutical industry.

2.2.1.1 Medicinal Development of Pactamycin to Date

Despite these demonstrated successes, efficient modification of complex natural product structures toward the preparation of useful drug molecules can often be hindered by the deficiency of a practical and flexible chemical synthesis [5]. As a result, the continued advancement of synthetic organic methodology is critical for facile and flexible drug discovery and development. Pactamycin (2.1, Fig. 2.2) is an example of a valuable natural target that has yet to reach its full medicinal potential due to both its inherent cytotoxicity and challenges associated with preparation of structurally-distinct analogs.

Its impressive biology has attracted the attention of a multidisciplinary field in hopes of transforming pactamycin into a suitable therapeutic. In addition to 2.1, a number of naturally-occurring structural congeners have been isolated from related Streptomyces bacteria, displaying varied bioactivities (Fig. 2.3). Among these, 7-deoxypactamycin (2.2) and jogyamycin (2.3) have shown increased antiprotozoal activity relative to 2.1, albeit with increased cytotoxicity [6]. Additionally, natural derivatives including pactamycate (2.4) (bearing an oxazolidinone ring in place of the dimethylurea) and the 8″-hydroxypactamycin series have also been recently reported [7, 8].

Alternatively, biosynthetic engineering studies pioneered by Mahmud and co-workers have provided researchers with the first series of unnatural structural analogs (Fig. 2.4). An initial report disclosed the preparation of TM-025 (2.7) and TM-026 (2.8). In contrast to 2.2, these compounds demonstrated an increase in activity against Plasmodium falciparum relative to pactamycin in combination with a decrease in cytotoxic effects [8]. In 2013, Mahmud described the preparation of biosynthetically-generated fluorinated analogs (2.9, 2.10) displaying comparable antimalarial activity to 2.1 [9]. These findings have renewed promise for pactamycin analogs in drug development.
Moreover, encapsulation of natural cytotoxic agents into nanoparticles (NPs) has also shown improved clinical benefits, the most germane of these being reduction of undesired toxic side effects and increased therapeutic delivery to the target of...
interest. This approach has been successfully implemented in the case of doxorubicin (Doxil®) [10], paclitaxel (Abraxane®) [11] and others [12–14]. More recently, Bind Therapeutics [15] and Cerulean [16, 17] have ongoing clinical trials in NP formulations of cancer therapeutics (docetaxel, irinotecan, and camptothecin). DeSimone and co-workers have demonstrated the use of the Particle Replication in Non-Wetting Templates (PRINT®) technology to modulate the

Fig. 2.2 Pactamycin

Fig. 2.3 Naturally-occurring pactamycin congeners

Fig. 2.4 Biosynthetically-engineered derivatives produced by Mahmud
activity of cytotoxic agents such as docetaxel, reducing unwanted side-effects and increasing therapeutic activity in vivo [18–21]. To the best of our knowledge, however, the incorporation of pactamycin or its congeners into NPs of any type with the goal of bioactivity attenuation has not yet been explored.

While an efficient chemical synthesis of 2.1 might provide the most flexibility in structural derivatization, the inherent complexity of the molecule has rendered this a difficult undertaking. Indeed, one researcher went so far as to argue that a chemical approach to derivatives of 2.1 was “inaccessible by synthetic organic chemistry [8].” The heavily-compacted and heteroatom-rich functionality in pactamycin presents a number of challenges toward selective structural modification. Additionally, while the unique functional groups present in the molecule (salicylate, dimethylurea, aniline) offer novel branch points for structural diversification, methods with which to install these moieties are underexplored in the literature.1 As discussed in Chap. 1, a number of groups have undertaken this endeavor [30–34], most notably the landmark Hanessian total synthesis in 2011 [35, 36]. Since this initial publication, Hanessian has demonstrated the efficacy of his route in producing pactamycin derivatives (Fig. 2.5) [37, 38]. Entry into derivatives at the C1 position was accomplished via amine addition to late stage isocyanate 2.11. These analogs were carried through the remaining sequence to provide C1 derivatives 2.13–2.16. In addition, C3-aniline derivatives were accessed via a Lewis acid catalyzed epoxide opening strategy (2.17 → 2.18), which ultimately provided aniline

---

1Summary methods for synthesis of unsymmetrical dialkylureas: [22–29].
derivatives 2.19–2.22. These compounds have provided the medicinal community with the first library of synthetically-generated pactamycin analogs to date.

2.2.1.2 Application of the Johnson Synthesis to Structural Analog Preparation

Our work on the synthesis of pactamycin culminated in 2013 with a fifteen-step, asymmetric synthesis from commercially available 2,4-pentanedione [39, 40]. Critical to our approach was to assemble the molecule in a fashion such that key functional groups were installed both in their native form and in a late-stage fashion; we surmised that this approach would provide our synthesis platform with the greatest possible flexibility, facilitating investigations of structure-activity relationships at all critical branch points (Fig. 2.6). To this end, we envisaged a

**Fig. 2.6** Synthon analysis of pactamycin with branch points for structural derivatization

(readily available starting materials)
Our synthesis endgame was described in detail in Chap. 1 and is summarized in Scheme 2.1. Ketone intermediate 2.24 (synthesized in ten steps in gram quantities) would serve as our first point of derivatization. Nucleophilic methylation of 2.24 provided carbinol 2.25 in 75 % yield of a single diastereomer at C5. Sc(OTf)₃-promoted addition of m-acetylaniline installed the substituted C3-aniline necessary for elaboration to 2.1, upon which silyl deprotection afforded tetraol 2.26. Introduction of the remaining salicylate moiety to the C6-hydroxymethylene of 2.26 was accomplished via reaction with the reported acyl electrophile 2.27, which upon hydrogenative removal of the Cbz protecting group, delivered pactamycin in fifteen steps and 1.9 % overall yield. These late stage introductions of core functionality (and in their final forms for elaboration to pactamycin) would enable direct access to synthetic diversity at the indicated positions.

2.3 Results and Discussion

2.3.1 C3 Aniline Derivative Preparation

We first pursued the preparation of pactamycin congeners at the C3-aniline position, inspired by the related epoxide-opening strategy by Hanessian and co-workers [35, 36]. At this juncture, it is valuable to recall from Chap. 1 that the union of
epoxide 2.25 with m-acetylaniline requisite for pactamycin synthesis suffered from incomplete starting material conversion, poor solubility of the aniline, and the requirement of superstoichiometric Lewis acid promoter (Scheme 2.2). We initially surmised that this poor reactivity was a function of the electron-poor aniline employed and that the use of varied anilines in this reaction would proceed more readily. Indeed, we were pleased to find that when p-anisidine was substituted for m-acetylaniline in the epoxide opening, the reaction proceeded in 100% conversion and 95% yield. Additionally, the stoichiometry of Sc(OTf)3 could be lowered to catalytic amounts with no decrease in reaction efficiency.

With the aniline tolerance established, we turned our attention towards incorporation of a variety of anilines and nitrogen nucleophiles (Table 2.1). In the event, epoxide 2.25 reacted readily with a variety of electron-rich and electron-poor aniline nucleophiles. Even sterically-demanding fluorenyl (entry 6) and 4-bromo-1-naphthyl anilines (entry 7) proceeded in 59 and 87% yields, respectively. We surprised to find, however, that extension of the epoxide opening reaction to aliphatic amines (entries 10–11) resulted in no reaction. Additionally, NaN3 failed to react with 2.25, giving only cleavage of the TBS protecting group.

Although we were disappointed by the lack of reactivity observed in the use of non-aromatic amines, we moved forward with aniline addition products 2.28a–i towards preparation of their corresponding pactamycin analogs (Fig. 2.7). In general, this operation proceeded uneventfully to afford pactamycin derivatives 2.29a–h over the three-step sequence. However, thioether substrate 2.28i failed to undergo

Scheme 2.2 Reactivity difference of m-acetylaniline and p-anisidine in epoxide opening
Cbz deprotection in the final step (presumably due to poisoning of the catalyst by the substrate), rendering this derivative inaccessible via this route. In the case of bromonaphthyl addition product 2.28g, reduction of the aryl bromide was observed during Cbz deprotection, providing naphthylaniline 2.29g in decreased yield as the final derivative.

Table 2.1 Scope of addition of nitrogen nucleophiles in epoxide opening reaction

<table>
<thead>
<tr>
<th>Entry</th>
<th>Nucleophile</th>
<th>Product</th>
<th>% Yield (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F(_3)CO(\text{Ph})NH(_2)</td>
<td>2.28a</td>
<td>83</td>
</tr>
<tr>
<td>2</td>
<td>F(\text{Ph})NH(_2)</td>
<td>2.28b</td>
<td>43</td>
</tr>
<tr>
<td>3</td>
<td>MeO(\text{Ph})NH(_2)</td>
<td>2.28c</td>
<td>95</td>
</tr>
<tr>
<td>4</td>
<td>Br(\text{Ph})NH(_2)</td>
<td>2.28d</td>
<td>71</td>
</tr>
<tr>
<td>5</td>
<td>tert-Bu(\text{Ph})NH(_2)</td>
<td>2.28e</td>
<td>86</td>
</tr>
<tr>
<td>6</td>
<td>2-aminofluorene</td>
<td>2.28f</td>
<td>59</td>
</tr>
<tr>
<td>7</td>
<td>Br\text{Ph}NH(_2)</td>
<td>2.28g</td>
<td>87</td>
</tr>
<tr>
<td>8</td>
<td>F(_3)C\text{Ph}NH(_2)</td>
<td>2.28h</td>
<td>47</td>
</tr>
<tr>
<td>9</td>
<td>MeS\text{Ph}NH(_2)</td>
<td>2.28i</td>
<td>77</td>
</tr>
<tr>
<td>10</td>
<td>O\text{Ph}NH</td>
<td>–</td>
<td>TBS deprotection</td>
</tr>
<tr>
<td>11</td>
<td>BnNH(_2)</td>
<td>–</td>
<td>No reaction</td>
</tr>
<tr>
<td>12(^b)</td>
<td>NaN(_3)</td>
<td>–</td>
<td>TBS deprotection</td>
</tr>
</tbody>
</table>

\(^a\)Isolated yields
\(^b\)Conditions: NaN\(_3\) (1.1 equiv), Oxone (0.5 equiv), CH\(_3\)CN:H\(_2\)O (9:1), rt
2.3.2 Derivatization at the C1 Dimethyl Urea: A Surprising
Ring Closure

Hanessian’s approach toward preparation of pactamycin analogs at the C1 dimethylurea position substituent relied on the trapping of an in situ generated isocyanate electrophile late in the synthesis (2.11) [35, 36]. This tactic proved effective in the preparation of a series of functionalized ureas in good yields. By contrast, our synthesis of 2.1 utilized an early-stage N–H insertion reaction to install the urea [39, 40]. Synthetic diversification from this early intermediate would be a significant challenge. Consequently, we envisaged a similar isocyanate formation/trapping strategy from carbinol intermediate 2.25 via the acid-catalyzed elimination of dimethylamine (Scheme 2.3). The literature showed that isocyanate generation from ureas could be readily accomplished via treatment with mildly acidic conditions [41]; in a first pass, epoxide 2.25 was treated with NH₄Cl in refluxing MeOH. Upon observing clean conversion to a single product, we were surprised to isolate imidazolidinone 2.31 and not the desired isocyanate 2.30. Imidazolidinone 2.31 arises from intramolecular trapping of the in situ generated isocyanate with the C2 amine carbamate functionality.

Considering that the facility of this intramolecular process might preclude all attempts at intermolecular isocyanate addition, focus shifted to other avenues of
trapping isocyanate 2.30 (Scheme 2.4). We surmised that if the isocyanate were generated in the presence of an unprotected C7-hydroxyl (2.32), then intramolecular trapping would result preferentially in formation of the corresponding oxazolidinone 2.33 (and not the undesired imidazolidinone). This reactivity pattern would enable access to analogs of the naturally-occurring congener pactamycate (2.4), for which no synthetic derivatives had been previously reported. In practice, we selected the aniline addition product 2.28 for investigation. A screen of deprotection conditions revealed that treatment of 2.28 with Oxone® in aqueous CH₃CN furnished alcohol 2.32 in 84 % yield. Submission of this compound to the conditions developed for isocyanate generation resulted in the formation of a single product 2.33 in 73 % yield, although we were unsure of whether 2.32 had undergone oxazolidinone or imidazolidinone formation. To ascertain this structural confirmation, we subjected 2.33 to silyl deprotection and Cbz hydrogenation, whereupon we isolated de-6-MSA pactamycate 2.34, whose spectral data had been previously reported [36]. This result confirmed the identity of 2.33 as the product illustrated.

2.3.3 Preparation of Pactamycate Derivatives

With the feasibility of this sequence established, we set out to prepare a series of C3-aniline pactamycate derivatives (Scheme 2.5). Initiating with anilines 2.28b, 2.28c, and 2.28e already in our possession, TBS deprotection followed by oxazolidinone formation proceeded smoothly to give the corresponding oxazolidinones,
Scheme 2.4  Investigating access to pactamycate derivatives.  

(a) Hypothesis.

(b) Application

Scheme 2.5  Preparation of pactamycate derivatives
which upon desilylation, acylation, and hydrogenolysis, provided pactamycate derivatives 2.37a–c in 35, 23, and 25 % yields, respectively, over the five-step sequence.

### 2.3.4 C5 Derivatization

Access to pactamycin derivatives at C5 was envisioned via addition of alternative carbon nucleophiles to ketone 2.24. We were concerned, however, that the steric encumbrance of ketone 2.24 might preclude the application of nucleophiles with elevated complexity relative to MeMgBr (Table 2.2). Fortunately, we found that ketone 2.24 reacted efficiently with nucleophiles EtMgBr, n-hexylMgBr, and H₂C = CHMgBr to give the corresponding carbinols in 75, 73, and 43 % yields, respectively. Unfortunately, larger aliphatic nucleophiles (iPr, isopropenyl) failed to react with 2.24, returning only recovered starting material. As an additional example, the ketone was also reduced with NaBH₄ to give the seco-alcohol 2.38d in 88 % yield.

When a sterically-demanding aryl nucleophile (PhMgBr) was employed, complete conversion to a single product 2.39 matching the desired mass spectrum was observed (Scheme 2.6), although the ¹H NMR spectrum was significantly different from that expected. Additionally, this product was unreactive in the subsequent epoxide opening stage. On the basis of these facts, we speculate that the most probable identity of 2.39 is the result of an in situ Payne rearrangement in order to relieve the additional strain associated with the encumbering nucleophile [42]. Unfortunately, this unexpected side reaction was observed in the addition of all larger nucleophiles, precluding the further exploration of C5 ketone diversity.

<table>
<thead>
<tr>
<th>Table 2.2 Addition of nucleophiles to advanced ketone intermediate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entry</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6ᵇ</td>
</tr>
</tbody>
</table>

³Isolated yields
ᵇConditions: NaBH₄, MeOH, −45 °C
2.3.5 **Elaboration of C5 Addition Products to Pactamycin Derivatives**

With addition products 2.38a–d in hand, we proceeded in our studies to complete C5 analog preparation (Scheme 2.7). However, upon subjection of ethyl derivative 2.38a to the previously optimized conditions for *m*-acetylaniline addition, no reaction was observed. Increasing the loading of Sc(OTf)₃ or the reaction time/temperature had seemingly no effect. We reasoned at this impasse that the added steric encumbrance about C5 hinders addition of the electron-poor *m*-acetylaniline, either due to poor coordination of the Lewis acid or by an unfavorable substrate conformation for addition relative to the parent C5-methyl compound.

In order to circumvent this issue, we looked to the strategy of Hanessian and co-workers wherein the required *m*-acetylaniline was first incorporated as an *m*-isopropenyl derivative [35, 36]. The ketone was then revealed via Johnson-Lemieux oxidation of the olefin. Fortunately, epoxide opening of 2.38a with *m*-isopropenyl aniline afforded aniline 2.40a in 71% yield. Alkene 2.40a was then subjected to Johnson-Lemieux conditions, revealing acetophenone 2.41a in 53% yield over two steps. With this reactivity established, C5 addition products 2.38a, 2.38b, and 2.38d

**Scheme 2.6** Rearrangement observed in the case of Aryl Nucleophiles

**Scheme 2.7** Native aniline installation to pactamycin C5 derivatives
were carried through the revised aniline addition sequence (Scheme 2.8). Elaboration of vinyl addition product 2.38c to the corresponding pactamycin derivative was not attempted as reduction of the alkene was expected to occur during the final hydrogenation stage.

Finally, in order to probe the activity profile of a C5 derivative bearing an alternate functional group at the aniline position, a second C5-hydrido analog was prepared via addition of \( p \)-methoxyaniline to 2.38d in the epoxide opening stage (Scheme 2.9).

As anticipated based on the above studies, addition of this aniline proceeded uneventfully under the optimized conditions to give anisidine 2.43 in 83\% yield. Elaboration of this material through the remaining sequence provided derivative 2.44 in 30\% yield over the three-step sequence.

### 2.3.6 Derivative Preparation at the C6 Hydroxymethylene Position

The next point of synthetic diversification centered on manipulation of the salicylate-bearing C6 ester in 2.1. The esters we hoped to prepare included both simple esters as well as “salicylate-like” esters to examine the importance of this functional group in the key binding event of 2.1 (Scheme 2.10). Accordingly, we employed a modified procedure for that reported in the synthesis of 2.27 [43] to prepare electrophiles 2.48 and 2.50. Phenyl-substituted salicylate 2.48 was synthesized via Suzuki reaction of triflate 2.46 followed by hydrolysis and
esterification. Differentiated methoxyphenol was prepared from the known phenol 2.45 via etherification and hydrolysis/esterification.

With these electrophiles in hand, we began screening esters in the acylation of tetraol 2.26 (Table 2.3). Gratifyingly, efficient monoacylation was accomplished with a variety of the electrophiles examined in good yields. Modified salicylates 2.50 (entry 1) and 2.48 (entry 2) both underwent esterification under the conditions optimized for esterification with 2.27. An o-tolyl ester was also tolerated (entry 3). Esterification of 2.26 with aliphatic electrophiles performed well (entries 4–5), and the corresponding mesylate ester could also be prepared (entry 6). The resulting monoesters were then submitted to the optimized conditions for Cbz hydrogenolysis, affording C6 derivatives 2.51b–e. Unfortunately, in the case of the methoxyphenol 2.51a and mesylate 2.51f, only starting material decomposition was observed at this stage.
2.3.7 Attempts to Prepare C7-Deoxypactamycin Derivatives

The next series of derivatives we sought to prepare were those in which the C7 hydroxyl was removed. Cognizant of the known bioactivity differences between 2.1 and its 7-deoxy congener (2.2) [7, 8], we began probing selective reduction of the C7 hydroxyl to its corresponding methylene. Scheme 2.11 summarizes the approaches we elected to pursue. Because it was easily accessible and obviated potential issues associated with the reactive C3 acetophenone functionality, we selected aniline addition product 2.52 as a model substrate for examination (prepared via Oxone® deprotection of 2.28e). We first pursued radical reduction of a suitable C7 ester such as 2.54. To this end, we prepared the corresponding xanthate, oxalate, and diphenylsilyl ether. Unfortunately, all conditions examined towards radical reduction of these compounds (SnBu₃H, AIBN/Et₃B/(TMS)₃SiH) failed to provide 2.53. In most cases, only deacylation was obtained to return 2.52. A second

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Product</th>
<th>% Yield 1ᵃ</th>
<th>% Yield 2ᵃ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OMe</td>
<td>2.51aᵇ</td>
<td>57</td>
<td>Decomposition</td>
</tr>
<tr>
<td>2</td>
<td>Ph</td>
<td>2.51bᵇ</td>
<td>43</td>
<td>62</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>2.51 cᶜ</td>
<td>60</td>
<td>73</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>2.51dᶜ</td>
<td>83</td>
<td>76</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>2.51eᶜ</td>
<td>87</td>
<td>61</td>
</tr>
<tr>
<td>6</td>
<td>MsCl</td>
<td>2.51fᶜ</td>
<td>54</td>
<td>Decomposition</td>
</tr>
</tbody>
</table>

ᵃIsolated yields
ᵇConditions: K₂CO₃, DMA, rt
ᶜConditions: 2,4,6-collidine, CH₂Cl₂, −78 °C to rt

2.3.7 Attempts to Prepare C7-Deoxypactamycin Derivatives

The next series of derivatives we sought to prepare were those in which the C7 hydroxyl was removed. Cognizant of the known bioactivity differences between 2.1 and its 7-deoxy congener (2.2) [7, 8], we began probing selective reduction of the C7 hydroxyl to its corresponding methylene. Scheme 2.11 summarizes the approaches we elected to pursue. Because it was easily accessible and obviated potential issues associated with the reactive C3 acetophenone functionality, we selected aniline addition product 2.52 as a model substrate for examination (prepared via Oxone® deprotection of 2.28e). We first pursued radical reduction of a suitable C7 ester such as 2.54. To this end, we prepared the corresponding xanthate, oxalate, and diphenylsilyl ether. Unfortunately, all conditions examined towards radical reduction of these compounds (SnBu₃H, AIBN/Et₃B/(TMS)₃SiH) failed to provide 2.53. In most cases, only deacylation was obtained to return 2.52. A second
approach envisioned dehydration of the C7 hydroxyl followed by hydrogenation to arrive at 2.53. This approach was also unsuccessful, as even the Burgess reagent and the Martin sulfurane showed no reactivity towards alcohol 2.52. In a final case, oxidation of the C7 hydroxyl (TPAP, NMO) furnished the resulting ketone 2.56. From this compound, we investigated deoxygenation of the ketone via its derived enol triflate or dithiolane. However, this route also gave no promise for yielding access to 2.53.

As an alternative strategy, we envisaged masking of the C7 hydroxyl as its ester might serve the same purpose as deoxygenation (i.e. removal of the H-bonding interaction at C7) [44, 45]. From tetraol 2.26, this would take the form of bis-acylation of the C6 and C7 hydroxyl groups (Table 2.4). In practice, we were pleased to find that tetraol 2.26 underwent clean bis-acylation with Ac₂O, PivCl,
and cyclohexoyl chloride to give the corresponding diesters in 86, 92, and 76% yields, respectively. The ester identities were selected on the basis of varying levels of steric encumbrance about C7. Cbz hydrogenolysis of these compounds provided the diesters \[2.57a-c\].

### Table 2.4 Synthesis of C6,C7 Bis-Acylated pactamycin derivatives

<table>
<thead>
<tr>
<th>Entry</th>
<th>R Product</th>
<th>% Yield 1\textsuperscript{a, b}</th>
<th>% Yield 2\textsuperscript{a, b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.57a</td>
<td>86</td>
<td>38</td>
</tr>
<tr>
<td>2</td>
<td>2.57b</td>
<td>92</td>
<td>53</td>
</tr>
<tr>
<td>3</td>
<td>2.57c</td>
<td>76</td>
<td>72</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Isolated yields  
\textsuperscript{b}Conditions: electrophile (2.2 equiv), NE\textsubscript{3}, DMAP (10 mol%), CH\textsubscript{2}Cl\textsubscript{2}, 0 °C to rt

The ester identities were selected on the basis of varying levels of steric encumbrance about C7. Cbz hydrogenolysis of these compounds provided the diesters \[2.57a-c\].

### 2.3.8 Synthesis of Ent-Pactamycin

In order to better understand the effects of chirality on the parent pactamycin structure, we investigated the preparation of \textit{ent-2.1} (Scheme 2.12). As described in Chap. 1, the enantiomer identity in our total synthesis was established via an early-stage asymmetric Mannich addition (\[2.58 \rightarrow 2.60\]) \[39, 40\]. To translate this chemistry to the synthesis of \textit{ent-2.1}, cinchonine, the pseudoenantiomer of cinchonidine, would need to be employed. To this end, we were pleased to find that the asymmetric Mannich addition proceeded smoothly when cinchonine was used (\[2.58 \rightarrow \textit{ent-2.60}\]) with a yield and selectivity comparable to that of the parent reaction (68 %, 3:97 er).

This material was advanced through the remaining steps of the synthesis to provide \textit{ent-2.1}, the optical activity of which was confirmed via comparison of the specific rotation with natural pactamycin.
Carcinoma in Vitro Biological Evaluation

Having prepared a library of novel compounds, we set out to examine their varied biological profiles. Specifically, compounds were tested against human breast (MDA-MB-231), ovarian (SK-OV-3), and lung (A549) carcinoma cell lines. Additionally, the human embryonic cell line for which pactamycin’s toxicity has been established (MRC-5) was assayed for comparison [46]. The results for all derivatives are summarized in Table 2.5. As anticipated, pactamycin (2.1, entry 1) displayed exceptional potency, showing nanomolar inhibition against all three carcinoma cell lines. For comparison, the penultimate intermediate in our synthesis of pactamycin (2.61, entry 2) bearing Cbz protection at the C2-aminomethine (entry 2) showed a dramatic decrease in activity relative to 2.1. ent-Pactamycin (ent-2.1, entry 3) displayed a threefold order of magnitude decrease in bioactivity, illustrating the impact of the natural enantiomer of pactamycin to effective cell-growth inhibition.

Scheme 2.12 Synthesis of ent-Pactamycin. a Pactamycin mannich addition. b ent-Pactamycin synthesis

2.3.9 Carcinoma in Vitro Biological Evaluation

Having prepared a library of novel compounds, we set out to examine their varied biological profiles. Specifically, compounds were tested against human breast (MDA-MB-231), ovarian (SK-OV-3), and lung (A549) carcinoma cell lines. Additionally, the human embryonic cell line for which pactamycin’s toxicity has been established (MRC-5) was assayed for comparison [46]. The results for all derivatives are summarized in Table 2.5. As anticipated, pactamycin (2.1, entry 1) displayed exceptional potency, showing nanomolar inhibition against all three carcinoma cell lines. For comparison, the penultimate intermediate in our synthesis of pactamycin (2.61, entry 2) bearing Cbz protection at the C2-aminomethine (entry 2) showed a dramatic decrease in activity relative to 2.1. ent-Pactamycin (ent-2.1, entry 3) displayed a threefold order of magnitude decrease in bioactivity, illustrating the impact of the natural enantiomer of pactamycin to effective cell-growth inhibition.
### Table 2.5 Carcinoma biological evaluation of pactamycin derivatives

<table>
<thead>
<tr>
<th>Entry</th>
<th>Structure</th>
<th>Code Number</th>
<th>A549 EC50</th>
<th>MDA-MB-231 EC50</th>
<th>SK-OV-3 EC50</th>
<th>MRC-5 EC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1" alt="Structure" /></td>
<td>2.1</td>
<td>160 nM</td>
<td>124 nM</td>
<td>129 nM</td>
<td>53 nM</td>
</tr>
<tr>
<td>2</td>
<td><img src="image2" alt="Structure" /></td>
<td>2.61</td>
<td>11.8 μM</td>
<td>10.4 μM</td>
<td>12 μM</td>
<td>n.d.b</td>
</tr>
<tr>
<td>3</td>
<td><img src="image3" alt="Structure" /></td>
<td>ent-2.1</td>
<td>2.1 μM</td>
<td>1.2 μM</td>
<td>1.6 μM</td>
<td>933 nM</td>
</tr>
<tr>
<td>4</td>
<td><img src="image4" alt="Structure" /></td>
<td>2.29a</td>
<td>800 nM</td>
<td>659 nM</td>
<td>1.4 μM</td>
<td>380 nM</td>
</tr>
<tr>
<td>5</td>
<td><img src="image5" alt="Structure" /></td>
<td>2.29b</td>
<td>141 nM</td>
<td>556 nM</td>
<td>434 nM</td>
<td>314 nM</td>
</tr>
<tr>
<td>6</td>
<td><img src="image6" alt="Structure" /></td>
<td>2.29c</td>
<td>1.0 μM</td>
<td>n.d.</td>
<td>600 nM</td>
<td>582 nM</td>
</tr>
<tr>
<td>7</td>
<td><img src="image7" alt="Structure" /></td>
<td>2.29d</td>
<td>777 nM</td>
<td>4.0 μM</td>
<td>4.0 μM</td>
<td>682 nM</td>
</tr>
<tr>
<td>8</td>
<td><img src="image8" alt="Structure" /></td>
<td>2.29e</td>
<td>884 nM</td>
<td>3.3 μM</td>
<td>1.6 μM</td>
<td>2.3 μM</td>
</tr>
<tr>
<td>9</td>
<td><img src="image9" alt="Structure" /></td>
<td>2.29f</td>
<td>324 nM</td>
<td>376 nM</td>
<td>145 nM</td>
<td>431 nM</td>
</tr>
<tr>
<td>10</td>
<td><img src="image10" alt="Structure" /></td>
<td>2.29g</td>
<td>2.21 μM</td>
<td>1.84 μM</td>
<td>2.44 μM</td>
<td>860 nM</td>
</tr>
<tr>
<td>11</td>
<td><img src="image11" alt="Structure" /></td>
<td>2.29 h</td>
<td>760 nM</td>
<td>800 nM</td>
<td>436 nM</td>
<td>366 nM</td>
</tr>
</tbody>
</table>

(continued)
<table>
<thead>
<tr>
<th>Entry</th>
<th>Structure</th>
<th>Code Number</th>
<th>A549 EC$_{50}$</th>
<th>MDA-MB-231 EC$_{50}$</th>
<th>SK-OV-3 EC$_{50}$</th>
<th>MRC-5 EC$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td><img src="image1" alt="Structure" /></td>
<td>2.34</td>
<td>6.0 μM</td>
<td>n.d.</td>
<td>3.8 μM</td>
<td>2.9 μM</td>
</tr>
<tr>
<td>13</td>
<td><img src="image2" alt="Structure" /></td>
<td>2.37a</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>14</td>
<td><img src="image3" alt="Structure" /></td>
<td>2.37b</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>15</td>
<td><img src="image4" alt="Structure" /></td>
<td>2.37c</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>16</td>
<td>R = C$_2$H$_5$, Ar = m-acetyl</td>
<td>2.42a</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>2.1 μM</td>
</tr>
<tr>
<td>17</td>
<td>R = C$<em>6$H$</em>{14}$, Ar = m-acetyl</td>
<td>2.42b</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>11 μM</td>
</tr>
<tr>
<td>18</td>
<td>R = H, Ar = m-acetyl</td>
<td>2.42d</td>
<td>32 nM</td>
<td>50 nM</td>
<td>7 nM</td>
<td>6.5 nM</td>
</tr>
<tr>
<td>19</td>
<td>R = H, Ar = p-methoxy</td>
<td>2.44</td>
<td>83 nM</td>
<td>356 nM</td>
<td>91 nM</td>
<td>49 nM</td>
</tr>
<tr>
<td>20</td>
<td><img src="image5" alt="Structure" /></td>
<td>2.51b</td>
<td>88 nM</td>
<td>203 nM</td>
<td>103 nM</td>
<td>129 nM</td>
</tr>
<tr>
<td>21</td>
<td><img src="image6" alt="Structure" /></td>
<td>2.51c</td>
<td>114 nM</td>
<td>79 nM</td>
<td>80 nM</td>
<td>105 nM</td>
</tr>
</tbody>
</table>

(continued)
Generally, all C3-aniline derivatives (entries 4–11) showed a marginal to significant decrease in activity relative to 2.1 across all cell lines, although 2.29b (entry 5) showed comparable activity against A549 (EC50 = 141 nM) with a marginal decrease in MRC5 activity. With regard to the pactamycate series of analogs, De-6-MSA pactamycate 2.34 (entry 12) showed only minor cell-growth inhibition. This was not an unexpected result, however, as biological assays of 2.34 conducted by Hanessian and co-workers also showed little promising activity [37, 38]. Altering the C3 aniline position of the pactamycate parent structure (entries 13–15) resulted in complete loss of biological activity. These results, in combination with those of the pactamycin C3 analogs, speak to the importance of the m-acetyl functionality in pactamycin to its bioactivity [47, 48].

The results of compounds bearing diversity at C5 are shown in entries 16–19. Extending the length of the carbon chain at C5 (entries 16–17) had significantly deleterious effects to bioactivity as a complete loss of carcinoma activity was observed, leaving only low inhibition of MRC-5. However, removing alkyl functionality altogether at C5 (entries 18–19) had the opposite effect, as these C5 hydrido analogs (2.42d, 2.44) displayed the greatest activity across all cell lines of any compound tested in our study (including pactamycin). We speculate that these results are primarily a function of adjusting the lipophilicity of the structure relative to 2.1 [49]. As further evidence to the importance of the m-acetyl functionality, 2.44 (entry 19) showed less potency across all cell lines in comparison to 2.42d (entry 18).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Structure</th>
<th>Code Number</th>
<th>A549 EC50</th>
<th>MDA-MB-231 EC50</th>
<th>SK-OV-3 EC50</th>
<th>MRC-5 EC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td></td>
<td>2.51d</td>
<td>118 nM</td>
<td>300 nM</td>
<td>75 nM</td>
<td>100 nM</td>
</tr>
<tr>
<td>23</td>
<td></td>
<td>2.51e</td>
<td>194 nM</td>
<td>352 nM</td>
<td>436 nM</td>
<td>366 nM</td>
</tr>
<tr>
<td>24</td>
<td>R = Ac</td>
<td>2.57a</td>
<td>137 nM</td>
<td>458 nM</td>
<td>123 nM</td>
<td>132 nM</td>
</tr>
<tr>
<td>25</td>
<td>R = Piv</td>
<td>2.57b</td>
<td>175 nM</td>
<td>1.93 μM</td>
<td>86 nM</td>
<td>396 nM</td>
</tr>
<tr>
<td>26</td>
<td>R = CO(C6H11)</td>
<td>2.57c</td>
<td>588 nM</td>
<td>2.44 μM</td>
<td>593 nM</td>
<td>778 Nm</td>
</tr>
</tbody>
</table>

*aAssays were carried out as triplicates
bNot determined
The results of our diversification of the C6 hydroxymethylene (entries 20–23) are in agreement with Hanessian’s earlier findings. Namely, no significant gain (or loss) of biological activity was observed when the salicylate ester was altered relative to the parent pactamycin structure. These results further support the hypothesis that the C6 ester side chain has a limited role in the key binding event of 2.1 in the 30S ribosome [37, 38]. The three prepared C6,C7 bis-acylated derivatives (entries 24–26) showed a linear decrease in activity with steric encumbrance of the ester group. These results suggest that the C7 hydroxyl in 2.1 plays a larger role in the bioactivity of the structure than the C6 hydroxymethylene.

2.3.10 Analysis of Pactamycin Derivatives via NCI 60-Cell Line Screen

Upon collection of these initial data, derivatives ent-(2.1), 2.42d, 2.51c, 2.29f, and 2.57a were identified as the most promising lead compounds and assayed via the NCI-60 human tumor cell line screen. Upon initial one-dose screening, all five compounds were found to have sufficient activity to merit the subsequent five-dose assay. These derivatives were evaluated to determine GI50 (50 % growth inhibition) values. The results of these assays are described in detail in the Sect. 2.5 and summarized in Table 2.6. Additionally, the previously documented cell data for 2.1 is shown for comparison.

As expected based on our initial screen, ent-(2.1) showed multiple orders of magnitude loss in activity across the entire assay. By contrast, compound 2.42d bearing a secondary hydroxyl at C5 demonstrated exceptional activity, showing nM inhibition throughout the screen and outperforming pactamycin in multiple cell lines. Derivatives 2.51c (modified salicylate ester) and 2.57a (C6,C7 diacetoxy-pactamycin) also demonstrated general nM activity in the assay. The final derivative 2.29f bearing a fluorenyl aniline at C3 showed a general decrease in biological activity relative to 2.1 by factors of 10–100.

2.3.11 Pactamycin Nanoparticle Fabrication and Biological Evaluation

With these studies completed, we set out to examine the efficacy of pactamycin and select analogs to activity modulation via nanoparticle encapsulation. Polymeric PRINT® nanoparticles were fabricated by encapsulating compounds 2.1, 2.29e, and 2.42d, in poly(d,l-lactide) using previously described methods [19, 50]. Compounds 2.29e and 2.42d were selected on the basis of observing the effect of nanoformulation on derivatives both more and less active than 2.1. PRINT® NPs containing 2.1 and derivatives 2.29e and 2.42d all showed similar hydrodynamic
Table 2.6 Summary GI50 values from NCI-60 cell line screening

<table>
<thead>
<tr>
<th>GI50 (μM)</th>
<th>2.1b</th>
<th>2.42d</th>
<th>2.51c</th>
<th>2.29f</th>
<th>2.57a</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOLT-4</td>
<td>&lt;0.10</td>
<td>1.19</td>
<td>0.046</td>
<td>0.12</td>
<td>0.78</td>
</tr>
<tr>
<td>NCI-H322 M</td>
<td>0.12</td>
<td>3.72</td>
<td>0.016</td>
<td>0.33</td>
<td>1.07</td>
</tr>
<tr>
<td>HCT-15</td>
<td>0.03</td>
<td>20.0</td>
<td>0.16</td>
<td>0.65</td>
<td>1.46</td>
</tr>
<tr>
<td>SNB-19</td>
<td>&lt;0.10</td>
<td>3.07</td>
<td>0.52</td>
<td>0.19</td>
<td>1.40</td>
</tr>
<tr>
<td>M14</td>
<td>0.12</td>
<td>3.01</td>
<td>0.10</td>
<td>0.19</td>
<td>0.88</td>
</tr>
<tr>
<td>OVCAR-3</td>
<td>&lt;0.10</td>
<td>2.50</td>
<td>0.041</td>
<td>0.20</td>
<td>0.73</td>
</tr>
<tr>
<td>RXF 393</td>
<td>&lt;0.10</td>
<td>1.50</td>
<td>0.064</td>
<td>0.12</td>
<td>0.61</td>
</tr>
<tr>
<td>DU-145</td>
<td>&lt;0.01</td>
<td>7.26</td>
<td>0.15</td>
<td>0.26</td>
<td>1.37</td>
</tr>
<tr>
<td>MCF7</td>
<td>&lt;0.01</td>
<td>2.04</td>
<td>0.051</td>
<td>0.17</td>
<td>0.73</td>
</tr>
</tbody>
</table>

aData obtained from NCI-60 screening. See Sect. 2.5 for comprehensive results. MOLT-4, leukemia cell line; NCI-H322 M, nonsmall-cell lung cancer cell line; HCT-15, colon cancer cell line; SNB-19, CNS tumor cell lines; M14, melanoma; OVCAR-3, ovarian cancer cell line; RXF 393, renal cancer cell line; DU-145, prostate cancer cell line; MCF7, breast cancer cell line

Table 2.7 Cell-Based Assay Comparison for Pactamycin Derivatives and NP Counterparts

<table>
<thead>
<tr>
<th>Compound</th>
<th>A549 EC50</th>
<th>MDA-MB-231 EC50</th>
<th>MRC-5 EC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>160 nM</td>
<td>124 nM</td>
<td>53 nM</td>
</tr>
<tr>
<td>NP-2.1</td>
<td>52 nM</td>
<td>117 nM</td>
<td>52 nM</td>
</tr>
<tr>
<td>2.29e</td>
<td>884 nM</td>
<td>3.3 μM</td>
<td>2.3 μM</td>
</tr>
<tr>
<td>NP-2.29e</td>
<td>693 nM</td>
<td>5.5 μM</td>
<td>1.8 μM</td>
</tr>
<tr>
<td>2.42d</td>
<td>32 nM</td>
<td>50 nM</td>
<td>6.5 nM</td>
</tr>
<tr>
<td>NP-2.42d</td>
<td>6.5 nM</td>
<td>724 nM</td>
<td>18 nM</td>
</tr>
</tbody>
</table>

aAssays were carried out as triplicates
comparison to 2.1, showing a nominal reduction in EC$_{50}$ value for the A549 cell line. Of significant interest was the increase in selectivity observed for 2.42d, wherein the EC$_{50}$ for A549 decreased while the EC$_{50}$ for MDA-MB-231 and MRC5 increased.

2.4 Conclusion

In summary, we have demonstrated the efficacy of our synthesis of pactamycin to efficient and modular production of structural derivatives with a range of varied bioactivities. Enabled branch points for derivatization include the C3 aniline, C5 carbinol, and the C6 hydroxymethylene position. Additionally, this route has enabled unprecedented access to derivatives of the natural congener pactamycate and the enantiomeric series of pactamycin. These results have provided additional insight into the roles that each functional group plays in providing the observed activity of the parent structure. Additionally, we have established a heretofore undocumented proof-of-concept for modulation of pactamycin bioactivities via the use of the PRINT$^\text{®}$ nanoparticle delivery vehicle.

2.5 Experimental Details

Methods: General. Infrared (IR) spectra were obtained using a Jasco 460 Plus Fourier transform infrared spectrometer. Proton and carbon magnetic resonance spectra ($^1$H NMR and $^{13}$C NMR) were recorded on a Bruker Avance 400 ($^1$H NMR at 400 MHz and $^{13}$C NMR at 100 MHz), Bruker Avance III 500 ($^1$H NMR at 500 MHz and $^{13}$C NMR at 125 MHz) or a Bruker Avance III 600 ($^1$H NMR at 600 MHz and $^{13}$C NMR at 150 MHz) spectrometer with solvent resonance as the internal standard ($^1$H NMR: CDCl$_3$ at 7.26 ppm; $^{13}$C NMR: CDCl$_3$ at 77.0 ppm, $^1$H NMR: CD$_3$OD at 3.34 ppm; $^{13}$C NMR: CD$_3$OD at 49.8 ppm). $^1$H NMR data are reported as follows: chemical shift, multiplicity (s = singlet, br s = broad singlet, d = doublet, br d = broad doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet), coupling constants (Hz), and integration. Mass spectra were obtained using a Micromass Quattro-II triple quadrupole mass spectrometer in combination with an Advion NanoMate chip-based electrospray sample introduction system and nozzle or a Thermo LTqFT mass spectrometer with electrospray introduction and external calibration. All samples were prepared in methanol. Analytical thin layer chromatography (TLC) was performed on Sorbent Technologies 0.20 mm Silica Gel TLC plates. Visualization was accomplished with UV light, KMnO$_4$, and/or aqueous ceric ammonium nitrate solution followed by heating. Purification of the reaction products was carried out by flash chromatography using Siliaflash-P60 silica gel (40–63 μm) purchased from Silicycle. Supercritical fluid chromatography was performed on a Berger SFC system.
equipped with a Chiralcel OD column. Samples were eluted with SFC grade CO₂ at
the indicated percentage of MeOH. Unless otherwise noted, all reactions were
carried out under an atmosphere of dry nitrogen in oven-dried glassware with
magnetic stirring.

Materials: General. Tetrahydrofuran (THF), diethyl ether (Et₂O), dichlor-
omethane (CH₂Cl₂), and toluene (C₇H₈) were dried by passage through a column of
neutral alumina under nitrogen prior to use. Acetonitrile (CH₃CN), Triethylamine
(NEt₃) and diisopropylamine were freshly distilled from calcium hydride prior to
use. All other reagents were purchased from commercial sources and were used as
received unless otherwise noted. Poly(D,L-lactide) (lactide: 75,000–120,000; 0.55–
0.75 dL/g Inherent Viscosity) (PLA) was purchased from Sigma-Aldrich.
Chloroform and solvents (acetonitrile and water) for high performance liquid
chromatography (HPLC) were purchased from Fisher Scientific. Poly(ethylene
terephthalate) (PET) sheets (6” width) were purchased from KRS plastics.
Fluorocur⁰, diameter (d) = 80 nm; height (h) = 320 nm; (80 × 320 nm) prefabric-
cated molds were provided by Liquidia Technologies.

Experimental Procedures

General procedure A for the addition of m-acetylaniline to epoxide 2.25.

Benzyl ((1S,2R,3R,4S,5S)-5-((3-acetylphenyl)amino)-2-((S)-1-((tert-butyl-
dimethylsilyl) oxy) ethyl)-4-(((tert-butylidiphenylsilyl)oxy)methyl)-2-(3,3 dimethyl
ureido)-3,4-dihydroxy-3-methylcyclopentyl)carbamate (2.28): In a nitrogen-filled
glove box, a flame-dried 100-mL round-bottomed flask was charged with Sc(OTf)₃
(0.38 g, 0.77 mmol, 3.0 equiv). The flask was capped with a rubber septum and
removed from the glove box. Toluene (20 mL) was added and to the resulting sus-
pension were added m-acetylaniline (0.35 g, 2.6 mmol, 10.0 equiv) and a toluene
solution (1.5 mL) of epoxide 2.25 (0.20 g, 0.26 mmol, 1.0 equiv). The reaction was
heated to 60 °C with vigorous stirring and maintained for 14 h. (Note: increased
reaction times led to product decomposition). The reaction was cooled to rt, diluted
with H₂O (10 mL) and EtOAc (10 mL), and the resulting mixture was extracted with
EtOAc (3 × 10 mL). The combined organic extracts were washed with 0.5 M
HCl(aq.) (2 × 20 mL), saturated NaHCO₃(aq.) (15 mL), dried with magnesium sulfate,
and concentrated in vacuo. The crude product was purified via flash chromatography
(90:10 to 80:20 hexanes:EtOAc) to afford anilino-alcohol 2.28 as a yellow, viscous oil
(0.16 g, 66 %) with recovery of the unreacted epoxide 2.25 (0.04 g, 18 %).
Analytical data: [α]D° = −39.3 (c = 0.70, CHCl₃); ¹H NMR (600 MHz, CDCl₃): δ 8.21
(d, J = 6.6 Hz, 1H), 7.70 (d, J = 6.6 Hz, 2H), 7.51 (d, J = 7.2 Hz, 2H), 7.39 (t,
J = 7.2 Hz, 1H), 7.32 (t, J = 7.2 Hz, 2H), 7.28–7.22 (m, 8H), 7.16 (t, J = 7.2 Hz,
2H), 7.12 (t, \( J = 7.8 \text{ Hz}, 1 \text{H} \)), 6.79 (d, \( J = 7.8 \text{ Hz}, 1 \text{H} \)), 6.13 (s, 1H), 5.88 (s, 1H), 5.39–5.36 (m, 1H), 5.36 (s, 1H), 5.04 (d, \( J = 12.0 \text{ Hz}, 1 \text{H} \)), 5.01 (d, \( J = 12.0 \text{ Hz}, 1 \text{H} \)), 4.78 (dd, \( J = 4.6, 6.6 \text{ Hz}, 1 \text{H} \)), 4.37 (d, \( J = 10.2 \text{ Hz}, 1 \text{H} \)), 4.13 (s, 1H), 3.68 (dd, \( J = 4.6, 3.0 \text{ Hz}, 1 \text{H} \)), 3.48 (d, \( J = 10.8 \text{ Hz}, 1 \text{H} \)), 2.96 (s, 6H), 2.49 (s, 3H), 1.69 (s, 3H), 1.41 (d, \( J = 6.6 \text{ Hz}, 3 \text{H} \)), 0.98 (s, 9H), 0.92 (s, 9H), 0.12 (s, 3H), 0.02 (s, 3H); MS (ESI\(^+\)) Calcd. for C\(_{50}\)H\(_{70}\)N\(_8\)Si\(_2\) + H, 911.4812; Found, 911.4867.

General procedure B for the addition of varied anilines to epoxide 2.25.

(Benzyl \((1\text{S},2\text{R},3\text{R},4\text{S},5\text{S})\)-2-(((tert-butyl(dimethyl)silyl)oxy)ethyl)-4-(((tert-butyl(diphenyl)silyl)oxy)methyl)-2-(3,3-dimethylureido)-3,4-dihydroxy-5-((4-methoxyphenyl)amino)-3-methylcyclopentyl)carbamate) (2.28c): In a nitrogen-filled glove box, a flame-dried 100-mL round-bottomed flask was charged with Sc(OTf)\(_3\) (0.035 g, 0.065 mmol, 0.5 equiv). The flask was capped with a rubber septum and removed from the glove box. Toluene (9 mL) was added and to the resulting suspension were added \( p \)-anisidine (0.160 g, 1.29 mmol, 10 equiv) and a toluene solution (2 mL) of epoxide 2.25 (0.10 g, 0.129 mmol, 1.0 equiv). The reaction was heated to 60 °C with vigorous stirring and maintained for 14 h. (Note: increased reaction times led to product decomposition). The reaction was cooled to rt, diluted with H\(_2\)O (15 mL) and EtOAc (15 mL), and the resulting mixture was extracted with EtOAc (3 \( \times \) 10 mL). The combined organic extracts were washed with 0.5 M HCl (aq.) (2 \( \times \) 20 mL), saturated NaHCO\(_3\) (aq.) (15 mL), dried with magnesium sulfate, and concentrated in vacuo. The crude product was purified via flash chromatography (90:10 to 80:20 hexanes:EtOAc) to afford anilino-alcohol 2.28c as a yellow, viscous oil (0.110 g, 95%). Analytical data: \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta 8.10 \) (d, \( J = 6.4 \text{ Hz}, 1 \text{H} \)); 7.71 (d, \( J = 6.4 \text{ Hz}, 2 \text{H} \)); 7.56 (d, \( J = 6.8 \text{ Hz}, 2 \text{H} \)); 7.40–7.30 (m, 12H); 6.67 (d, \( J = 8.8 \text{ Hz}, 2 \text{H} \)); 6.56 (d, \( J = 8.8 \text{ Hz}, 2 \text{H} \)); 6.17 (s, 1H); 5.83 (s, 1H); 5.37 (q, \( J = 6.8 \text{ Hz}, 1 \text{H} \)); 5.02 (br s, 2H); 4.96 (s, 1H); 4.75–4.73 (m, 1H); 4.39 (d, \( J = 10.8 \text{ Hz}, 1 \text{H} \)); 3.74 (s, 3H); 3.61–3.54 (m, 2H); 2.95 (s, 2H); 1.67 (s, 3H); 1.40 (d, \( J = 6.4 \text{ Hz}, 3 \text{H} \)); 1.01 (s, 9H); 0.92 (s, 9H); 0.11 (s, 3H); 0.02 (s, 3H). MS (ESI\(^+\)) Calcd. For C\(_{49}\)H\(_{70}\)N\(_4\)O\(_8\)Si\(_2\) + H, 899.48; Found, 899.47.
Benzyl ((1S,2R,3R,4S,5S)-2-((S)-1-((tert-butyldimethylsilyl)oxy)ethyl)-4-(((tert-butyldiphenylsilyl)oxy)methyl)-2-(3,3-dimethylureido)-3,4-dihydroxy-3-methyl-5-((3-fluorophenyl)amino)cyclopentyl)carbamate (2.28a): Isolated from 2.25 via general procedure B using 3-fluoroaniline as the nucleophile in 43 % yield. Analytical data: \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.18 (d, \(J = 6.6\) Hz, 1H); 7.42 (d, \(J = 7.6\) Hz, 1H); 7.36 (d, \(J = 6.6\), 2H); 7.42 (d, \(J = 7.6\) Hz, 1H); 7.39–7.25 (m, 11H); 6.96 (d, \(J = 7.2\) Hz, 1H); 6.42–6.31 (m, 3H); 6.22 (s, 1H); 3.86 (s, 1H); 5.38 (q, \(J = 6.8\) Hz, 1H); 5.32 (br s, 1H); 4.75 (dd, \(J = 9.8, 6.8\) Hz, 1H); 4.35 (d, \(J = 10.4\) Hz, 1H); 4.13 (s, 1H); 3.6 (dd, \(J = 9.8, 3.6\) Hz, 1H); 3.52 (d, \(J = 10.8\) Hz, 1H) 2.97 (s, 6H); 1.68 (s, 3H); 1.42 (d, \(J = 6.8\) Hz, 1H), 3H); 1.01 (s, 9H); 0.93 (s, 9H); 0.12 (s, 3H); 0.02 (s, 3H); MS (ESI\(^+\)) Calcd. For C\(_{48}\)H\(_{77}\)F\(_3\)N\(_4\)O\(_7\)Si\(_2\) + H, 887.46; Found, 887.32.

Benzyl ((1S,2R,3R,4S,5S)-2-((S)-1-((tert-butyldimethylsilyl)oxy)ethyl)-4-(((tert-butyldiphenylsilyl)oxy)methyl)-2-(3,3-dimethylureido)-3,4-dihydroxy-3-methylcyclopentyl)carbamate (2.28b): Isolated from 2.25 via general procedure B using 3-fluoroaniline as the nucleophile in 43 % yield. Analytical data: \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.09 (d, \(J = 6.8\) Hz, 1H); 7.71 (d, \(J = 8.0\) Hz, 2H); 7.55 (d, \(J = 7.6\) Hz, 2H); 7.41–7.21 (m, 12H); 7.09–7.05 (m, 2H); 6.65–6.62 (m, 3H); 6.17 (s, 1H); 5.85 (s, 1H); 5.37 (q, \(J = 6.8\) Hz, 1H); 5.14 (br s, 1H); 5.02 (s, 2H); 4.76

Benzyl ((1S,2R,3R,4S,5S)-2-((S)-1-((tert-butyldimethylsilyl)oxy)ethyl)-4-(((tert-butyldiphenylsilyl)oxy)methyl)-2-(3,3-dimethylureido)-3,4-dihydroxy-3-methyl-5-(phenylamino)cyclopentyl)carbamate (2.28d): Isolated from 2.25 via general procedure B using aniline as the nucleophile in 71 % yield. Analytical data: \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.09 (d, \(J = 6.8\) Hz, 1H); 7.71 (d, \(J = 8.0\) Hz, 2H); 7.55 (d, \(J = 7.6\) Hz, 2H); 7.41–7.21 (m, 12H); 7.09–7.05 (m, 2H); 6.65–6.62 (m, 3H); 6.17 (s, 1H); 5.85 (s, 1H); 5.37 (q, \(J = 6.8\) Hz, 1H); 5.14 (br s, 1H); 5.02 (s, 2H); 4.76
Benzyl ((1S,2R,3R,4S,5S)-5-((4-(tert-butyl)phenyl)amino)-2-((S)-1-((tert-butyl-dimethylsilyl)oxy)ethyl)-4-(((tert-butylidiphenylsilyl)oxy)methyl)-2-(3,3-dimethylureido)-3,4-dihydroxy-3-methylcyclopentyl)carbamate (2.28e): Isolated from 2.25 via general procedure B using 4-tert-butylaniline as the nucleophile in 86 % yield. Analytical data: $^1$H NMR (600 MHz, CDCl$_3$); $\delta$ 8.11 (d, $J = 6.6$ Hz, 1H); 7.73 (d, $J = 7.2$ Hz, 2H); 7.56 (d, $J = 7.2$ Hz, 2H); 7.40 (d, $J = 6.6$ Hz, 1H); 7.36–7.31 (m, 5H); 7.27–7.23 (m, 5H); 7.20 (d, $J = 8.4$ Hz, 2H); 6.60 (d, $J = 8.4$ Hz, 2H); 6.20 (s, 1H); 5.83 (s, 1H); 5.38 (q, $J = 6.6$ Hz, 1H); 5.12 (s, 1H); 5.05 (d, $J = 12.6$ Hz, 1H); 5.00 (d, $J = 12.0$ Hz, 1H); 4.77 (m, 1H); 4.37 (d, $J = 10.8$ Hz, 1H); 4.16 (s, 1H); 4.14 (d, $J = 7.2$ Hz, 1H); 3.64–3.60 (m, 2H); 2.96 (s, 6H); 1.68 (s, 3H); 1.42 (d, $J = 6.6$ Hz, 3H); 1.29 (s, 9H); 1.01 (s, 9H); 0.93 (s, 9H); 0.12 (s, 3H); MS (ESI$^+$) Calcd. For C$_{48}$H$_{68}$N$_4$O$_7$Si$_2$ + H, 869.47; Found, 869.61.

Benzyl ((1S,2R,3R,4S,5S)-5-((9H-fluoren-2-yl)amino)-2-((S)-1-((tert-butyldimethylsilyl)oxy)ethyl)-4-(((tert-butyldiphenylsilyl)oxy)methyl)-2-(3,3-dimethylureido)-3,4-dihydroxy-3-methylcyclopentyl)carbamate (2.28f): Isolated from 2.25 via general procedure B using 2-fluorenyl aniline as the nucleophile in 59 % yield. Analytical data: $^1$H NMR (600 MHz, CDCl$_3$); $\delta$ 8.17 (d, $J = 6.7$ Hz, 1H); 7.72 (d, $J = 7.1$ Hz, 2H); 7.67 (t, $J = 7.0$ Hz, 1H); 7.56 (br s, 2H); 7.50 (br s, 2H); 7.39 (t, $J = 7.1$ Hz, 1H); 7.37–7.32 (m, 5H); 7.26–7.35 (m, 3H); 7.21 (t, $J = 8.1$ Hz, 1H); 7.15 (br s, 3H); 6.82 (s, 1H); 6.68 (d, $J = 5.6$ Hz, 1H); 6.22 (s, 1H); 5.92 (s, 1H); 5.43 (d, $J = 4.4$ Hz, 1H); 5.07 (s, 2H); 4.83–4.80 (m, 1H); 4.44 (d, $J = 7.2$ Hz, 1H); 4.21 (s, 1H); 3.84(s, 1H); 3.76–3.65 (m, 3H); 3.64 (d, $J = 7.2$ Hz, 1H); 3.01 (s, 6H); 1.73 (s, 3H); 1.45 (d, $J = 4.4$ Hz, 3H); 1.042 (s, 9H); 0.98 (s, 9H); 0.16 (s, 3H); 0.07 (s, 3H); MS (ESI$^+$) Calcd. For C$_{55}$H$_{72}$N$_4$O$_7$Si$_2$ + H, 957.50; Found, 957.42.
Benzyl ((1S,2R,3R,4S,5S)-5-((4-bromonaphthalen-1-yl)amino)-2-((S)-1-((tert-butyldimethylsilyl)oxy)ethyl)-4-(((tert-butylidiphenylsilyl)oxy)methyl)-2-(3,3-dimethylureido)-3,4-dihydroxy-3-methylcyclopentyl)carbamate (2.28 g): Isolated from 2.25 via general procedure B using 4-bromonaphthalen-1-amine as the nucleophile in 87 % yield. Analytical data: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.24 (d, $J$ = 6.4 Hz, 1H); 8.15 (d, $J$ = 8.4 Hz, 1H); 7.95 (d, $J$ = 8.4 Hz, 1H); 7.67 (d, $J$ = 6.9 Hz, 2H); 7.54 (t, $J$ = 7.1 Hz, 1H); 7.52–7.07 (m, 15H); 6.61 (d, $J$ = 8.4 Hz, 1H); 6.18 (d, $J$ = 12.4 Hz, 2H); 5.97 (s, 1H); 5.45 (d, $J$ = 6.8 Hz, 1H); 5.04–4.93 (m, 3H); 4.44 (d, $J$ = 10.4 Hz, 1H); 4.20 (s, 1H); 3.83 (d, $J$ = 10.0 Hz, 1H); 3.49 (d, $J$ = 10.8 Hz, 1H); 3.00 (s, 6H); 1.74 (s, 3H); 1.46 (d, $J$ = 6.8 Hz, 3H); 1.01 (s, 9H); 0.99 (s, 9H); 0.17 (s, 3H); 0.11 (s, 3H); MS (ESI$^+$) Calcd. For C$_{52}$H$_{69}$BrN$_4$O$_7$Si$_2$ + H, 997.40; Found, 997.48.

Benzyl ((1S,2R,3R,4S,5S)-2-((S)-1-((tert-butyldimethylsilyl)oxy)ethyl)-4-(((tert-butylidiphenylsilyl)oxy)methyl)-2-(3,3-dimethylureido)-3,4-dihydroxy-3-methyl-5-((4-(trifluoromethyl)phenyl)amino)cyclopentyl)carbamate (2.28 h): Isolated from 2.25 via general procedure B using 4-trifluoromethylaniline as the nucleophile in 47 % yield. Analytical data: $^1$H NMR (600 MHz, CDCl$_3$): $\delta$ 8.25 (d, $J$ = 6.6 Hz, 1H); 7.73 (d, $J$ = 7.2 Hz, 2H); 7.53 (d, $J$ = 7.8 Hz, 2H); 7.44–7.21 (m, 11H); 6.71 (d, $J$ = 7.8 Hz, 2H); 6.61 (d, $J$ = 8.4 Hz, 2H); 6.14 (s, 1H); 5.94 (s, 1H); 5.52 (s, 1H); 5.40 (q, $J$ = 6.6 Hz, 1H); 5.05 (s, 2H); 4.81–4.78 (m, 1H); 4.39 (d, $J$ = 10.8 Hz, 1H); 3.98 (s, 1H); 3.69–3.67 (m, 1H); 3.41 (d, $J$ = 10.8 Hz, 1H); 2.99 (s, 6H); 1.72 (s, 3H); 1.44 (d, $J$ = 6.6 Hz, 3H); 1.04 (s, 9H); 0.95 (s, 9H); 0.15 (s, 3H); 0.04 (s, 3H); MS (ESI$^+$) Calcd. For C$_{49}$H$_{67}$F$_3$N$_4$O$_7$Si$_2$ + H, 937.46; Found, 937.36.
Benzyl ((1S,2R,3R,4S,5S)-2-((S)-1-((tert-butyldimethylsilyl)oxy)ethyl)-4-(((tert-butyldiphenylsilyl)oxy)methyl)-2(3,3-dimethylureido)-3,4-dihydroxy-3-methyl-5-((4-(methylthio)phenyl)amino)cyclopentyl)carbamate (2.28i):
Isolated from 2.25 via general procedure B using 4-thiomethylaniline as the nucleophile in 77 % yield. Analytical data: $^1$H NMR (600 MHz, CDCl$_3$): $\delta$ 8.15 (d, $J = 6.6$ Hz, 1H); 7.71 (d, $J = 7.2$ Hz, 2H); 7.55 (d, $J = 7.2$ Hz, 2H); 7.41 (t, $J = 7.2$ Hz, 1H); 7.36–7.23 (m, 10H); 7.13 (d, $J = 9.0$ Hz, 2H); 6.57 (d, $J = 7.8$ Hz, 2H); 6.14 (s, 1H); 5.86 (s, 1H); 5.37 (d, $J = 6.6$ Hz, 1H); 5.23 (s, 1H); 5.02 (s, 2H); 4.75 (dd, $J = 9.6$, 6.6 Hz, 1H); 4.36 (d, $J = 10.8$ Hz, 1H); 4.13 (s, 1H); 3.61 (d, $J = 9.6$ Hz, 1H); 3.52 (d, $J = 10.8$ Hz, 1H); 2.96 (s, 6H); 2.39 (s, 3H); 1.67 (s, 3H); 1.14 (d, $J = 6.6$ Hz, 3H); 0.92 (s, 9H); 0.11 (s, 3H); 0.01 (s, 3H); MS (ESI$^+$) Calcd. For C$_{49}$H$_{70}$N$_4$O$_{7}$SSi$_2$ + Na, 937.46; Found, 937.36.

General procedure C for global silyl deprotection.

Benzyl ((1S,2R,3R,4S,5S)-5-((3-acetylphenyl)amino)-2-(3,3-dimethylureido)-3,4-dihydroxy-2-((S)-1-hydroxyethyl)-4-(hydroxymethyl)-3-methylcyclopentyl)carbamate (2.26):
A 20-mL scintillation vial was charged with silyl ether 2.28 (0.25 g, 0.28 mmol, 1.0 equiv) and THF (5.5 mL). The resulting solution was cooled to 0 °C, and TBAF (1 M solution in THF, 1.1 mL, 1.1 mmol, 4.0 equiv) was added. The reaction was allowed to stir at 0 °C until TLC analysis indicated consumption of the starting material, typically 30 min. The reaction was diluted with brine (3 mL) and EtOAc (3 mL) and extracted with EtOAc (3 $\times$ 7 mL). The combined organic extracts were dried with magnesium sulfate and concentrated in vacuo. The crude product was purified via flash chromatography (60:40 petroleum ether:acetone) to afford tetranol 2.26 as a pale yellow, viscous oil (0.14 g, 90 %). Analytical data: $[\alpha]_D^{19}$ +26.0 (c = 0.70, CHCl$_3$); $^1$H NMR (600 MHz, CDCl$_3$): $\delta$ 7.36 (s, 4H); 7.29 (br s, 1H); 7.23 (br s, 1H); 7.12 (br s, 1H); 6.99 (d, $J = 7.8$ Hz, 1H); 6.75 (d, $J = 6.6$ Hz, 1H); 6.02 (d, $J = 7.2$ Hz, 1H); 5.80 (br s, 1H); 5.48 (d, $J = 7.8$ Hz, 1H); 5.27 (br s, 1H); 5.13 (br s, 2H); 4.4–4.10 (m, 1H); 4.06 (br s, 2H); 3.80 (br s, 2H); 3.74–3.68 (m, 1H); 3.55 (m, 1H); 2.87 (s, 6H); 2.52 (s, 3H); 1.42 (s, 3H); 1.25 (br s, 3H); $^{13}$C NMR (150 MHz, CDCl$_3$): $\delta$ 198.7, 158.7, 155.8, 146.6, 138.3, 136.0, 129.7, 128.6, 128.4, 118.4, 112.0, 88.2, 83.9, 73.2, 71.7, 67.4, 66.9, 64.2, 61.8, 61.2, 36.7, 29.7, 26.7, 22.7, 21.2, 18.0, 14.1; MS (ESI$^+$) Calcd. For C$_{29}$H$_{38}$N$_4$O$_8$ + H, 559.2770; Found, 559.2800; IR (thin film, cm$^{-1}$) 3392, 2938, 1716, 1587, 155.8, 146.6, 138.3, 129.7, 128.6, 128.4, 118.4, 112.0, 88.2, 83.9, 73.2, 71.7, 67.4, 66.9, 64.2, 61.8, 61.2, 36.7, 29.7, 26.7, 22.7, 21.2, 18.0, 14.1; TLC (60:40 petroleum ether:acetone): R$_f$ = 0.30.
(Benzyl ((1S,2R,3R,4S,5S)-2-(3,3-dimethylureido)-3,4-dihydroxy-2-((S)-1-hydroxyethyl)-4-(hydroxymethyl)-5-((4-methoxyphenyl)amino)-3-methylcyclopentyl)carbamate) (S1c): Isolated from 2.28 via general procedure C in 94% yield. Analytical data: \(^1\text{H NMR}\) (400 MHz, CDCl\(_3\)): \(\delta\) 7.34–7.26 (m, 5H); 6.85 (br s, 1H); 6.73 (d, \(J = 8.4\) Hz, 2H); 6.5 (d, \(J = 8.0\) Hz, 2H); 6.06 (br s, 1H); 5.73 (br s, 1H); 5.28 (s, 1H); 5.13–5.10 (m, 2H); 4.95 (br s, 1H); 4.15 (br s, 1H); 4.04 (d, \(J = 11.2\) Hz, 2H); 3.72 (s, 3H); 3.66 (br s, 2H); 2.84 (s, 6H); 1.38 (s, 3H); 1.24 (br s, 3H). \(\text{MS (ESI}^+\text{)}\) Calcd. For C\(_{27}\)H\(_{38}\)N\(_4\)O\(_8\) + H + H, 547.28; Found, 547.28.

Benzyl ((1S,2R,3R,4S,5S)-2-(3,3-dimethylureido)-3,4-dihydroxy-2-((S)-1-hydroxyethyl)-4-(hydroxymethyl)-3-methyl-5-((4-(trifluoromethoxy)phenyl)amino)cyclopentyl) carbamate H(S1a): Isolated from 2.28a via general procedure C in 68% yield. Analytical data: \(^1\text{H NMR}\) (400 MHz, CDCl\(_3\)): \(\delta\) 7.34–7.26 (m, 5H); 7.00 (d, \(J = 8.0\) Hz, 2H); 6.71 (br s, 1H); 6.50 (d, \(J = 8.4\) Hz, 2H); 6.05 (br s, 1H); 5.72 (br s, 1H); 5.31 (br s, 1H); 5.26 (s, 1H); 5.12 (s, 2H); 4.17 (br s, 1H); 4.05 (br s, 1H); 4.01 (d, \(J = 12.0\) Hz, 1H); 3.82 (d, \(J = 11.6\) Hz, 1H); 3.69 (dd, \(J = 2.8, 9.0\) Hz, 1H); 3.52 (s, 1H); 2.87 (s, 6H); 1.41 (s, 3H); 1.25 (br s, 3H); \(\text{MS (ESI}^+\text{)}\) Calcd. For C\(_{27}\)H\(_{35}\)F\(_3\)N\(_4\)O\(_8\) + Na, 623.33; Found, 623.19.

Benzyl ((1S,2R,3R,4S,5S)-2-(3,3-dimethylureido)-5-((3-fluorophenyl)amino)-3,4-dihydroxy-2-((S)-1-hydroxyethyl)-4-(hydroxymethyl)-3-methylcyclopentyl)carbamate (S1b): Isolated from 2.28b via general procedure C in 85% yield. Analytical data: \(^1\text{H NMR}\) (400 MHz, CDCl\(_3\)): \(\delta\) 7.34–7.26 (m, 5H); 7.07 (d, \(J = 7.2\) Hz, 1H); 6.85 (br s, 1H); 6.42–6.38 (m, 1H); 6.32–6.26 (m, 2H); 6.07 (br s,
1H); 5.76 (br s, 1H); 5.39 (d, J = 8.0 Hz, 1H); 5.26 (s, 1H); 5.13 (d, J = 12.4 Hz, 1H); 5.10 (d, J = 12.4 Hz, 1H); 4.17 (br s, 1H); 4.06 (br s, 1H); 3.98 (d, J = 11.2 Hz, 1H); 3.80 (d, J = 11.2 Hz, 1H); 3.68 (dd, J = 2.4, 9.0 Hz, 1H); 3.6 (br s, 1H); 2.86 (s, 6H); 1.40 (s, 3H); 1.25 (d, J = 3.2 Hz, 3H); **MS (ESI⁺)** Calcd. For C₂₆H₃₅FN₄O₇ + H, 535.26; Found, 535.19.

**Benzyl ((1S,2R,3R,4S,5S)-2-(3,3-dimethylureido)-3,4-dihydroxy-2-((S)-1-hydroxyethyl)-4-(hydroxymethyl)-3-methyl-5-(phenylamino)cyclopentyl)carbamate (S1d):** Isolated from 2.28d via general procedure C in 91 % yield. Analytical data: ¹H NMR (400 MHz, CDCl₃): δ 7.34 (br s, 5H); 7.16–7.13 (m, 2H); 6.90 (br s, 1H); 6.72 (br s, 1H); 6.56 (br s, 2H); 6.05 (br s, 1H); 5.78 (s, 1H); 5.28 (br s, 1H); 5.10 (s, 2H); 4.18 (br s, 1H); 4.02 (br s, 2H); 3.78 (br s, 2H); 3.63 (s, 1H); 2.85 (s, 6H); 2.61 (br s, 1H); 1.85 (br s, 1H); 1.39 (s, 3H); 1.24 (br s, 3H); **MS (ESI⁺)** Calcd. For C₂₆H₃₆N₄O₇ + Na, 539.25; Found, 539.32.

**Benzyl ((1S,2R,3R,4S,5S)-5-((4-(tert-butyl)phenyl)amino)-2-(3,3-dimethylureido)-3,4-dihydroxy-2-((S)-1-hydroxyethyl)-4-(hydroxymethyl)-3-methylcyclopentyl)carbamate (S1e):** Isolated from 2.28e via general procedure C in 93 % yield. Analytical data: ¹H NMR (400 MHz, CDCl₃): δ 7.35 (br s, 5H); 7.18 (d, J = 8.4 Hz, 2H); 6.84 (br s, 1H); 6.51 (d, J = 8.0 Hz, 2H); 6.06 (br s, 1H); 5.78 (br s, 1H); 5.28 (br s, 1H); 5.15–5.08 (m, 3H); 4.21 (br s, 1H); 4.06–4.03 (m, 2H); 3.75 (d, J = 11.6 Hz, 1H); 3.70 (d, J = 8.8 Hz, 1H); 3.69 (br s, 1H); 2.86 (s, 6H); 1.39 (s, 3H); 1.27 (s, 9H); 1.25 (br s, 3H); **MS (ESI⁺)** Calcd. For C₃₀H₄₄N₄O₇ + H, 573.33; Found, 573.33.
Benzyl ((1S,2R,3R,4S,5S)-5-((9H-fluoren-2-yl)amino)-2-(3,3-dimethylureido)-3,4-dihydroxy-2-((S)-1-hydroxyethyl)-4-(hydroxymethyl)-3-methylcyclopentyl)carbamate (S1f): Isolated from 2.28f via general procedure C in 85 % yield. Analytical data: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.61 (d, $J$ = 7.3 Hz, 1H); 7.55 (br s, 1H); 7.45 (d, $J$ = 7.3 Hz, 1H); 7.42–7.26 (m, 6H); 7.20–7.17 (m, 1H); 6.97 (br s, 1H); 6.76 (s, 1H); 6.59 (br s, 1H); 6.06 (br s, 1H); 5.84 (br s, 1H); 5.35 (br s, 1H); 5.29 (br s, 1H); 5.12 (s, 2H); 4.18–4.06 (m, 3H); 3.82–3.75 (m, 4H); 3.64 (br s, 1H); 2.86 (s, 6H); 1.41 (s, 3H); 1.24 (s, 3H); MS (ESI$^+$) Calcd. For C$_{33}$H$_{40}$N$_4$O$_7$ + H, 605.30; Found, 605.23.

Benzyl ((1S,2R,3R,4S,5S)-5-((4-bromonaphthalen-1-yl)amino)-2-(3,3-dimethylureido)-3,4-dihydroxy-2-((S)-1-hydroxyethyl)-4-(hydroxymethyl)-3-methylcyclopentyl)carbamate (S1g): Isolated from 2.28 g via general procedure C in 65 % yield. Analytical data: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.19 (d, $J$ = 8.4 Hz, 1H); 7.70 (d, $J$ = 7.6 Hz, 1H); 7.59–7.55 (m, 2H); 7.48–7.45 (m, 1H); 7.27–7.21 (m, 5H); 7.20 (br s, 1H); 6.42 (br s, 1H); 6.32 (br s, 1H); 6.06 (br s, 2H); 5.25 (s, 1H); 5.14–5.12 (m, 2H); 4.15–4.12 (m, 3H); 3.89–3.82 (m, 2H); 3.64 (br s, 1H); 2.85 (s, 6H); 1.25 (br s, 3H); MS (ESI$^+$) Calcd. For C$_{30}$H$_{37}$BrN$_4$O$_7$ + H, 645.19; Found, 645.25.

Benzyl ((1S,2R,3R,4S,5S)-2-(3,3-dimethylureido)-3,4-dihydroxy-2-((S)-1-hydroxyethyl)-4-(hydroxymethyl)-3-methyl-5-((4-(trifluoromethyl)phenyl)amino)cyclopentyl)carbamate (S1h): Isolated from 2.28 h via general procedure C in 60 % yield. Analytical data: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.35–7.26 (m, 8H); 6.87 (br s, 2H); 6.10 (br s, 2H); 5.77 (br s, 1H); 5.62 (s, 1H); 5.12 (br s, 2H); 4.17 (s, 1H); 4.06 (s, 1H); 3.97 (d, $J$ = 10.4 Hz, 1H); 3.83 (d, $J$ = 10.4 Hz, 1H); 3.75 (d, $J$ = 6.8 Hz, 1H); 2.86 (s, 6H); 1.41 (s, 3H); 1.25 (d, $J$ = 4.0 Hz, 3H); MS (ESI$^+$) Calcd. For C$_{27}$H$_{35}$F$_3$N$_4$O$_7$ + H, 585.25; Found, 585.24.
Benzyl (1S,2R,3R,4S,5S)-2-(3,3-dimethylureido)-3,4-dihydroxy-2-((S)-1-hydroxyethyl)-4-(hydroxymethyl)-3-methyl-5-((4-(methylthio)phenyl)amino)cyclopentyl)carbamate (S1i): Isolated from 2.28i via general procedure C in 89 % yield. Analytical data: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.33 (bs, 5H); 7.16 (d, $J = 7.6$ Hz, 2H); 6.90 (bs, 1H); 6.49 (d, $J = 7.2$ Hz, 2H); 6.11 (bs, 1H); 5.74 (bs, 1H); 5.27 (bs, 2H); 5.11 (d, $J = 12.4$ Hz, 1H); 5.07 (s, $J = 12.4$ Hz, 1H); 4.15 (bs, 1H); 4.03 (bs, 1H); 3.97 (d, $J = 11.6$ Hz, 1H); 3.76 (d, $J = 12.0$ Hz, 1H); 3.69 (d, $J = 8.8$ Hz, 1H); 2.84 (s, 6H); 2.39 (s, 3H); 1.38 (s, 13H); 1.24 (d, $J = 5.6$ Hz, 13H); MS (ESI$^+$) Calcd. For C$_{27}$H$_{38}$N$_4$O$_7$S + H, 563.25; Found, 563.27.

General procedure D for salicylate ester formation.

((1S,2R,3R,4S,5S)-5-((3-acetylphenyl)amino)-4-(((benzyloxy)carbonyl)xamino)-3-(3,3-dimethylureido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-2-methylcyclopentyl)methyl 2-hydroxy-6-methylbenzoate (2.61): A flame-dried 20-mL scintillation vial was charged with cyanomethyl ester 2.27 (0.0075 g, 0.044 mmol, 1.1 equiv) and dimethylacetamide (DMA) (0.3 mL). K$_2$CO$_3$ (0.005 g, 0.04 mmol, 1.0 equiv) was added, and the resulting mixture was stirred for 1 h. The in situ generated ketene solution was transferred to a stirred solution of tetraol 2.26 (0.02 g, 0.04 mmol, 1.0 equiv) in DMA (0.7 mL). The reaction was stirred until TLC analysis indicated full consumption of the tetraol starting material, typically 3 h. The reaction was cooled to 0 °C and quenched by the dropwise addition of saturated NH$_4$Cl(aq.) (1.5 mL). The resulting mixture was extracted with EtOAc (3 × 5 mL), washed with H$_2$O (10 ml), brine (10 mL), dried with magnesium sulfate, and concentrated in vacuo. The crude product was purified via flash chromatography (50:50 hexanes:EtOAc) to afford an inseparable mixture of salicylate 2.61 (0.02 g, 80 %) and an unknown impurity (15 % by NMR analysis) as a pale yellow, viscous oil. Analytical data: $[\alpha]$_{D}^{19}$ +33.6 (c = 0.70, CHCl$_3$); $^1$H NMR (600 MHz, CDCl$_3$): $\delta$ 10.87 (s, 1H), 7.52 (br s, 1H), 7.36 (br s, 5H), 7.30–7.22 (m, 4H), 7.10 (s, 1H), 6.81 (d, $J = 8.4$ Hz, 1H), 6.63 (d, $J = 7.2$ Hz, 1H), 6.13 (s, 1H), 5.79 (d, $J = 9.0$ Hz, 1H), 5.72 (d, $J = 9.6$ Hz, 1H), 5.23–5.10 (m, 3H),
4.91–4.84 (m, 2H), 4.06 (br s, 2H), 3.80 (d, \( J = 9.6 \) Hz, 1H), 3.69 (s, 1H), 2.85 (s, 7H), 2.30 (s, 3H), 1.42 (s, 3H), 1.26 (s, 3H); \(^{13}\)C NMR (150 MHz, CDCl\(_3\)): 6 198.3, 173.4, 162.9, 158.5, 155.3, 146.0, 141.6, 138.3, 135.0, 129.7, 128.6, 128.5, 123.2, 119.4, 118.4, 115.8, 111.9, 111.6, 99.7, 88.6, 85.0, 73.9, 72.3, 67.5, 66.8, 66.6, 65.4, 62.7, 36.7, 23.9, 21.0, 18.0, 17.4; MS (ESI\(^+\)) Calcd. For C\(_{36}\)H\(_{44}\)N\(_4\)O\(_{10}\) + H, 693.3137; Found, 693.3172; IR (thin film, cm\(^{-1}\)) 3392, 2965, 1867, 1698, 1670, 1541, 1456, 1374, 1249, 874, 737; TLC (50:50 EtOAc:hexanes): \( R_f = 0.30 \).

((1S,2R,3R,4S,5S)-4-(((benzyloxy)carbonyl)amino)-3-(3,3-dimethylureido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-5-((4-methoxyphenyl)amino)-2-methylcyclopentyl)methyl 2-hydroxy-6-methylbenzoate) (S2c): Isolated from S1c via general procedure D in 80 % yield. Analytical data: \(^1\)H NMR (400 MHz, CDCl\(_3\)): 6 10.95 (s, 1H); 7.58 (d, \( J = 7.2 \) Hz, 1H); 7.36 (br s, 4H); 7.28 (d, \( J = 8.8 \) Hz, 1H); 6.82 (d, \( J = 8.4 \) Hz, 1H); 6.74 (d, \( J = 8.8 \) Hz, 2H); 6.66 (d, 7.6 Hz, 1H); 6.48 (d, \( J = 8.8 \) Hz, 2H); 6.04 (s, 1H); 5.90 (d, \( J = 8.0 \) Hz, 1H); 5.27 (d, \( J = 10.4 \) Hz, 1H); 5.23 (s, 1H); 5.17 (d, \( J = 12.0 \) Hz, 1H); 5.11 (d, \( J = 12.0 \) Hz, 1H); 4.88 (d, \( J = 12.8 \) Hz, 1H); 4.84 (d, \( J = 12.0 \) Hz, 1H); 4.07 (br s, 2H); 3.75 (s, 3H); 2.86 (s, 6H); 2.44 (s, 3H); 1.50 (s, 3H); 1.27 (br s, 3H). MS (ESI\(^+\)) Calcd. For C\(_{35}\)H\(_{44}\)N\(_4\)O\(_{10}\) + H, 681.31; Found, 681.26.

((1S,2R,3R,4S,5S)-4-(((benzyloxy)carbonyl)amino)-3-(3,3-dimethylureido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-2-methyl-5-((4-(trifluoromethoxy)phenyl)amino)cyclopentyl)methyl 2-hydroxy-6-methylbenzoate) (S2a): Isolated from S1a via general procedure D in 63 % yield. Analytical data: \(^1\)H NMR (400 MHz, CDCl\(_3\)): 6 10.90 (br s, 1H); 7.37–7.28 (m, 8H); 7.01 (d, \( J = 8.4 \) Hz, 2H); 6.84 (d, \( J = 8.4 \) Hz, 1H); 6.67 (d, \( J = 7.6 \) Hz, 1H); 6.48 (d, \( J = 8.8 \) Hz, 2H); 5.84 (d, \( J = 8.0 \) Hz, 1H); 5.64 (br s, 1H); 5.22–5.51 (m, 2H); 4.93 (d, \( J = 12.4 \) Hz, 1H); 4.05 (d, \( J = 7.6 \) Hz, 1H); 3.82 (br s, 1H); 3.72 (s, 1H); 3.00 (s, 1H); 2.88 (s, 6H); 2.31 (s, 3H); 1.52 (s, 3H); 1.29 (d, \( J = 6.8 \) Hz, 3H); MS (ESI\(^+\)) Calcd. For C\(_{35}\)H\(_{41}\)F\(_3\)N\(_4\)O\(_{10}\) + H, 735.29; Found, 735.20.
((1S,2R,3R,4S,5S)-4-(((benzyloxy)carbonyl)amino)-3-(3,3-dimethyleido)-5-((3-fluorophenyl)amino)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-2-methyl-cyclopentyl)methyl 2-hydroxy-6-methylbenzoate (S2b): Isolated from S1b via general procedure D in 80% yield. Analytical data: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 11.91 (s, 1H); 7.54 (br s, 1H); 7.34 (br s, 4H); 7.27 (d, $J = 8.0$ Hz, 1H); 7.25 (d, $J = 6.4$ Hz, 1H); 7.07 (q, $J = 7.2$ Hz, 1H); 6.81 (d, $J = 8.0$ Hz, 1H); 6.65 (d, $J = 7.6$ Hz, 1H); 6.39 (dd, $J = 2.0$, 8.0 Hz, 1H); 6.29 (dd, $J = 1.6$, 8.0 Hz, 1H); 6.21 (d, $J = 11.6$ Hz, 1H); 6.12 (s, 1H); 5.79 (d, $J = 8.8$ Hz, 1H); 5.69 (d, $J = 10.0$ Hz, 1H); 5.17–5.10 (m, 3H); 4.90 (d, $J = 12.4$ Hz, 1H); 4.80 (d, $J = 12.4$ Hz, 1H); 4.03 (d, $J = 8.4$ Hz, 1H); 3.74 (s, 1H); 3.70 (s, 1H); 1.48 (s, 3H); 1.24 (d, $J = 7.2$ Hz, 3H); MS (ESI$^+$) Calcd. For C$_{34}$H$_{41}$FN$_4$O$_9$ + H, 669.15; Found, 669.15.

((1S,2R,3R,4S,5S)-4-(((benzyloxy)carbonyl)amino)-3-(3,3-dimethyleido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-2-methyl-5-(phenylamino)cyclopentyl)methyl 2-hydroxy-6-methylbenzoate (S2d): Isolated from S1d via general procedure D in 69% yield. Analytical data: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 10.93 (s, 1H); 7.57 (br s, 1H); 7.35 (br s, 4H); 7.27–7.20 (m, 2H); 7.14 (t, $J = 7.6$ Hz, 2H); 6.80 (d, $J = 8.4$ Hz, 1H); 6.72 (t, $J = 7.2$ Hz, 1H); 6.65 (d, $J = 7.2$ Hz, 1H); 6.52 (d, $J = 7.6$ Hz, 2H); 6.05 (s, 1H); 5.84 (d, $J = 9.6$ Hz, 1H); 5.50 (d, $J = 10.0$ Hz, 1H); 5.21 (s, 1H); 5.17–5.07 (m, 2H); 4.91–4.81 (m, 2H); 4.28 (s, 1H); 4.05 (d, $J = 8.0$ Hz, 1H); 3.75 (br s, 2H); 2.83 (s, 6H); 2.34 (s, 3H); 1.48 (s, 3H); 1.13 (br s, 3H); MS (ESI$^+$) Calcd. For C$_{34}$H$_{42}$N$_4$O$_9$ + H, 651.30; Found, 651.39.

((1S,2R,3R,4S,5S)-4-(((benzyloxy)carbonyl)amino)-5-(4-(tert-butyl)phenyl)amino)-3-(3,3-dimethyleido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-2-methyl-
cyclopentyl)methyl 2-hydroxy-6-methylbenzoate (S2e): Isolated from S1e via general procedure D in 76 % yield. Analytical data: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 10.95 (br s, 1H); 7.49 (br s, 1H); 7.54 (br s, 5H); 7.27–7.24 (m, 2H); 7.15 (d, $J = 8.4$ Hz, 2H); 6.81 (d, $J = 8.4$ Hz, 1H) 6.65 (d, $J = 7.6$ Hz, 1H); 6.06 (s, 1H); 5.83 (d, $J = 8.4$ Hz, 1H); 5.37 (d, $J = 10.4$ Hz, 1H); 5.20–5.15 (m, 2H); 5.09 (d, $J = 12.0$ Hz, 1H); 4.85–4.80 (m, 2H); 4.31 (s, 1H); 4.05 (d, $J = 7.6$ Hz, 1H); 3.75 (d, $J = 9.2$ Hz, 1H); 3.66 (br s, 1H); 2.83 (s, 6H); 2.33 (s, 3H); 1.47 (s, 3H); 1.27–1.23 (m, 12H); MS (ESI$^+$) Calcd. For C$_{38}$H$_{50}$N$_4$O$_9$ + H, 707.37; Found, 707.24.

$\text{(1S,2R,3R,4S,5S)-5-((9H-fluoren-2-yl)amino)-4-((benzyloxy)carbonyl)amino)-3-(3,3-dimethylureido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-2-methycyclopentyl)methyl 2-hydroxy-6-methylbenzoate (S2f):}$ Isolated from S1f via general procedure D in 83 % yield. Analytical data: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 10.92 (s, 1H); 7.61 (d, $J = 7.2$ Hz, 1H); 7.54 (d, $J = 8.0$ Hz, 2H); 7.45 (d, $J = 7.2$ Hz, 1H); 7.42 (m, 4H); 7.31 (t, $J = 7.5$ Hz, 2H); 7.26–7.18 (m, 3H); 6.79 (d, $J = 8.4$ Hz, 1H); 6.71 (s, 1H); 6.61 (d, $J = 7.6$ Hz, 1H); 6.54 (d, $J = 8.0$ Hz, 1H); 6.16 (s, 1H); 5.82 (d, $J = 8.4$ Hz, 1H); 5.63 (d, $J = 10.0$ Hz, 1H); 5.21–5.14 (m, 3H); 4.91–4.88 (m, 2H); 3.82 (br s, 1H); 3.71 (br s, 2H); 2.85 (s, 6H); 2.32 (s, 3H); 1.50 (s, 3H); 1.24 (br s, 3H); LRMS (ESI$^+$) Calcd. For C$_{41}$H$_{46}$N$_4$O$_9$ + H, 739.33; Found, 739.21.

$\text{(1S,2R,3R,4S,5S)-4-((benzyloxy)carbonyl)amino)-5-((4-bromonaphthalen-1-yl)amino)-3-(3,3-dimethylureido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-2-methycyclopentyl)methyl 2-hydroxy-6-methylbenzoate (S2g):}$ Isolated from S1g via general procedure D in 97 % yield. Analytical data: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 10.88 (br s, 1H); 8.21 (d, $J = 8.4$ Hz, 1H); 7.73 (d, $J = 8.0$ Hz, 1H); 7.62–7.58 (m, 2H); 7.50 (t, $J = 7.2$ Hz, 2H); 7.36 (br s, 5H); 7.27–7.23 (m, 2H); 6.80 (d, $J = 8.0$ Hz, 1H); 6.64–6.59 (m, 2H); 6.36 (s, 1H); 6.20 (d, $J = 8.0$ Hz, 1H); 5.81 (br s, 1H); 5.20–5.18 (m, 3H); 5.00–4.98 (m, 2H); 4.05 (br s, 1H); 3.89
(d, J = 9.2 Hz, 1H); 3.81 (s, 1H); 2.86 (s, 6H); 2.21 (s, 3H); 1.65 (s, 3H) 1.22 (br s, 3H); MS (ESI⁺) Calcd. For C₃₈H₄₃BrN₄O₉ + H, 779.23; Found, 779.20.

((15S,2R,3S,4S,5S)-4-(((benzyloxy)carbonyl)amino)-3-(3,3-dimethylureido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-2-methyl-5-((4-(trifluoromethyl) phenyl)amino) cyclopentyl)methyl 2-hydroxy-6-methylbenzoate (S₂ h): Isolated from S₁ h via general procedure D in > 99 % yield. Analytical data: ¹H NMR (400 MHz, CDCl₃): δ 10.89 (br s, 1H); 7.52 (br s, 1H); 7.36–7.26 (m, 8H); 6.82 (d, J = 8.4 Hz, 1H); 6.65 (d, J = 7.6 Hz, 1H); 6.51 (d, J = 8.0 Hz, 2H); 6.16 (s, 1H); 5.92 (d, J = 9.6 Hz, 1H); 5.81 (br s, 1H); 5.17–5.11 (m, 3H); 4.93 (d, J = 12.4 Hz, 1H); 4.81 (d, J = 12.8 Hz, 1H); 2.27 (s, 3H); 1.50 (s, 3H); 1.26 (br s, 3H); MS (ESI⁺) Calcd. For C₃₅H₄₁F₃N₄O₉ + H, 719.29; Found, 719.23.

General procedure E for Carboxybenzyl group hydrogenolysis.

**Pactamycin (2.1):** A 4-mL vial was charged with salicylate 2.61 (0.0075 g, 0.01 mmol, 1.0 equiv), and Pd(OH)₂/C (20 wt%, 0.005 g). MeOH (1 mL) was added and the vial was sealed with a Teflon cap. The atmosphere was replaced by H₂ (balloon, ~1 atm) and stirred until TLC analysis indicated complete consumption of the starting material, typically 20 min. The resulting suspension was filtered through a pad of Celite and washed with MeOH. The homogeneous solution was concentrated in vacuo. The crude residue was purified by flash chromatography (98:2 CH₂Cl₂:MeOH) affording pactamycin (0.005 g, 82 %) as a pale yellow solid. Analytical data: [α]D²⁰ +27.4 (c = 0.40, CHCl₃); ¹H NMR (600 MHz, CDCl₃): δ 10.98 (br s, 1H), 7.91 (d, J = 10.8 Hz, 1H), 7.26–7.23 (m, 4H), 7.18–7.16 (m, 2H), 6.81–6.78 (m, 2H), 6.64 (d, J = 7.2 Hz, 1H), 5.78 (br s, 1H), 5.67 (d, J = 10.8 Hz, 1H), 4.84 and 4.79 (ABq, J = 12.6 Hz, 2H), 3.93 (m, 1H), 3.80 (d, J = 10.2 Hz, 1H), 2.99 (s, 6H), 2.95 (s, 1H), 2.55 (s, 3H), 2.38 (s, 3H), 1.55 (s, 3H), 1.04 (d, J = 6.0 Hz, 3H); ¹³C NMR (150 MHz, CDCl₃): δ 198.5, 172.6, 162.8, 159.2, 146.6, 141.2, 138.3, 134.6, 129.6, 123.0, 118.7, 118.4, 115.7, 112.0, 110.8, 88.8, 84.9, 74.3, 71.5, 68.7, 65.4, 63.2, 36.9, 29.7, 26.7, 24.1, 21.1, 18.1; MS (ESI⁺)
Calcd. for C_{28}H_{38}N_{4}O_{8} + H, 559.2762; Found, 559.2763; $\text{IR (thin film, cm}^{-1})$ 3393, 2938, 2359, 2341, 1698, 1652, 1520, 1473, 1418, 1338, 873, 668; $\text{TLC (95:5 CH}_2\text{Cl}_2$/MeOH): $R_f = 0.30$.

(((1S,2R,3R,4S,5S)-4-amino-3-(3,3-dimethylureido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-5-(4-methoxyphenyl)amino)-2-methylcyclopentyl)methyl 2-hydroxy-6-methylbenzoate) (2.29c): Isolated from S2c via general procedure E in 72 % yield. Analytical data: $[\alpha]_D^{19} +20.9$ ($c = 0.63$, CHCl$_3$); $^1\text{H NMR (400 MHz, CDCl}_3$): $\delta$ 10.80 (br s, 1H); 7.93 (br s, 1H); 7.28–7.24 (m, 2H); 7.21–7.18 (m, 2H); 6.83 (d, $J = 8.4$ Hz, 1H); 6.78 (d, $J = 8.8$ Hz, 2H); 6.68 (d, $J = 7.2$ Hz, 1H); 6.57 (d, $J = 8.4$ Hz, 2H); 5.68 (br s, 1H); 5.18 (br s, 1H); 4.85 (d, $J = 12.4$ Hz, 1H); 4.80 (d, $J = 12.0$ Hz, 1H); 3.97 (br s, 1H); 3.76 (s, 3H); 3.00 (s, 7H); 2.91 (s, 1H); 2.47 (s, 3H); 2.38 (s, 1H); 1.55 (s, 3H); 1.06 (d, $J = 6.4$ Hz, 3H); $^{13}\text{C NMR (100 MHz, CDCl}_3$): $\delta$ 172.3, 162.5, 159.2, 152.1, 141.3, 140.5, 134.3, 129.0, 128.2, 125.2, 122.9, 115.6, 115.1, 114.5, 112.40, 88.7, 84.7, 74.0, 71.3, 69.9, 65.4, 62.6, 55.7, 36.80, 23.9, 21.4, 18.1; $\text{MS (ESI}^+\text{)}$ Calcd. for C$_{27}$H$_{38}$N$_{4}$O$_{8}$ + H, 547.28; Found, 547.22; $\text{IR (thin film, cm}^{-1})$ 3774, 3406, 2935, 2359, 2069, 1610, 1511, 1377, 1251, 1105, 943; $\text{TLC (95:5 CH}_2\text{Cl}_2$/MeOH): $R_f = 0.30$.

(((1S,2R,3R,4S,5S)-4-amino-3-(3,3-dimethylureido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-5-(4-(trifluoromethoxy)phenyl)amino)cyclopentyl)methyl 2-hydroxy-6-methylbenzoate) (2.29a): Isolated from S2a via general procedure E in 96 % yield. Analytical data: $[\alpha]_D^{19} +34.4$ ($c = 0.70$, CHCl$_3$); $^1\text{H NMR (600 MHz, CDCl}_3$): $\delta$ 11.02 (br s, 1H); 7.94 (d, $J = 11.4$ Hz, 1H); 7.31–7.27 (m, 2H); 7.17 (br s, 1H); 7.05 (d, $J = 9.0$ Hz, 2H); 6.85 (d, $J = 7.8$ Hz, 1H); 6.69 (d, $J = 7.2$ Hz, 1H); 6.58 (d, $J = 8.4$ Hz, 1H); 5.84 (br s, 1H); 5.62 (d, $J = 10.2$ Hz, 1H); 4.87 (d, $J = 12.6$ Hz, 1H); 4.83 (d, $J = 12.6$ Hz, 1H); 3.99 (br s, 1H); 3.74 (d, $J = 10.2$ Hz, 1H); 3.02 (s, 6H); 2.40 (s, 3H); 1.58 (s, 3H); 1.10 (d, $J = 6.6$ Hz, 3H); $^{13}\text{C NMR (150 MHz, CDCl}_3$): $\delta$ 172.7, 162.8, 159.2, 145.2, 141.4, 140.6, 134.7, 123.1, 122.8, 115.7, 113.3, 112.1, 84.9, 65.4, 36.9, 24.0, 21.1, 18.1; $\text{MS (ESI}^+\text{)}$ Calcd. for C$_{27}$H$_{35}$F$_3$N$_{4}$O$_{8}$ + H, 601.25; Found, 601.28; $\text{IR$^{-1}$}$
((1S,2R,3R,4S,5S)-4-amino-3-(3,3-dimethylureido)-5-((3-fluorophenyl)amino)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-2-methylcyclopentyl)methyl 2-hydroxy-6-methyl benzoate (2.29b): Isolated from S2b via general procedure E in > 99 % yield. Analytical data: [α]D19 +34.8 (c = 0.90, CHCl3); 1H NMR (600 MHz, CDCl3): δ 11.04 (br s, 1H); 7.99 (d, J = 5.2 Hz, 1H); 7.30–7.28 (m, 2H); 7.27–7.18 (m, 2H); 7.12 (dd, J = 5.2, 10.0 Hz, 1H); 6.85 (d, J = 5.2 Hz, 1H); 6.70 (d, J = 5.2 Hz, 1H); 6.43 (dd, J = 1.2, 5.6 Hz, 1H); 6.39 (dd, J = 1.2, 11.6 Hz, 1H); 6.29 (d, J = 7.6 Hz, 1H); 5.83 (s, 1H); 5.69 (d, J = 6.8 Hz, H); 4.87 (d, J = 7.2 Hz, 1H); 4.82 (d, J = 8.4 Hz, 1H); 3.95 (br s, 1H); 3.72 (d, J = 7.2 Hz, 1H); 3.01 (s, 6H); 2.97 (s, 1H); 2.43 (s, 3H); 1.57 (s, 3H); 1.07 (d, J = 4.0 Hz, 3H); 13C NMR (150 MHz, CDCl3): δ 172.7, 162.8, 159.2, 146.3, 141.4, 134.5, 129.5, 123.0, 117.7, 115.7, 113.2, 112.1, 89.8, 88.8, 84.9, 74.2, 71.5, 68.9, 65.3, 63.0, 36.8, 29.7, 24.1, 21.2, 18.1; MS (ESI+) Calcd. For C26H37FN4O7 + H, 535.26; Found, 535.19; IR (thin film, cm−1) 3397, 2933, 2359, 1724, 1655, 1617, 1513, 1495, 1377, 1291, 1091, 943; TLC (95:5 CH2Cl2:MeOH): Rf = 0.29.

((1S,2R,3R,4S,5S)-4-amino-3-(3,3-dimethylureido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-2-methyl-5-(phenylamino)cyclopentyl)methyl 2-hydroxy-6-methyl benzoate (2.29d): Isolated from S2d via general procedure E in 83 % yield. Analytical data: [α]D19 −8.9 (c = 0.40, CHCl3); 1H NMR (600 MHz, CDCl3): δ 10.94 (br s, 1H); 7.90 (dd, J = 5.4 Hz, 1H); 7.26 (t, J = 7.8 Hz, 2H); 7.22–7.16 (m, 3H); 6.82 (d, J = 8.4 Hz, 1H); 6.71 (t, J = 7.8 Hz, 1H); 6.66 (d, J = 7.2 Hz, 1H); 6.59 (d, J = 7.8 Hz, 2H); 5.76 (s, 1H); 5.47 (d, J = 11.4 Hz, 1H); 4.84 (d, J = 12.6 Hz, 1H); 4.82 (d, J = 12.0 Hz, 1H); 3.94 (dd, J = 6.0, 10.2 Hz, 1H); 3.76 (d, J = 10.8 Hz, 1H); 2.98 (s, 6H); 2.94 (s, 1H); 2.42 (s, 3H); 1.54 (s, 3H); 1.03 (d, J = 6. Hz, 3H); 13C NMR (150 MHz, CDCl3): δ 172.7, 162.8, 159.2, 146.3, 141.4, 134.5, 129.5, 123.0, 117.7, 115.7, 113.2, 112.1, 89.8, 88.8, 84.9, 74.2, 71.5, 68.9, 65.3, 63.0, 36.8, 29.7, 24.1, 21.2, 18.1; MS (ESI+) Calcd. For C26H38N4O7 + H, 517.27; Found, 517.29; IR (thin film, cm−1)
3876, 3846, 3774, 3398, 2929, 2359, 1724, 1603, 1460, 1305, 1213, 977; TLC (95:5 CH₂Cl₂:MeOH): Rₖ = 0.26.

((1S,2R,3R,4S,5S)-4-amino-5-((4-((tert-butyl)phenyl)amino)-3-(3,3-dimethyl ureido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-2-methylcyclopentyl)methyl 2-hydroxy-6-methylbenzoate (2.29e): Isolated from S₂e via general procedure E in 88 % yield. Analytical data: [α]D₁⁹ +35.9 (c = 0.50, CHCl₃); ^1H NMR (400 MHz, CDCl₃): δ 11.03 (br s, 1H); 7.89 (d, J = 10.8 Hz, 1H); 7.28 (t, J = 6.0 Hz, 2H); 7.22–7.19 (m, 4H); 6.84 (d, J = 8.4 Hz, 1H); 6.69 (d, J = 7.6, 1H); 6.56 (d, J = 8.4 Hz, 2H); 5.70 (s, 1H); 5.33 (d, J = 11.2 Hz, 1H); 4.85 (d, J = 12.0 Hz, 1H); 4.81 (d, J = 12.4 Hz, 1H); 3.98 (dd, J = 6.4, 1.2 Hz, 1H); 3.76 (d, J = 10.8 Hz, 1H); 3.01 (s, 6H); 2.46 (s, 3H); 2.38 (s, 1H); 1.56 (s, 3H); 1.29 (s, 9H) 1.06 (d, J = 6.4 Hz, 3H); ^13C NMR (125 MHz, CDCl₃): δ 172.5, 167.7, 159.2, 143.8, 141.3, 140.4, 134.4, 126.2, 122.9, 115.7, 112.9, 112.2, 88.7, 84.8, 74.0, 71.4, 69.3, 65.3, 63.2, 36.8, 33.8, 31.5, 29.7, 24.1, 21.2, 18.1; MS (ESI⁺) Calcd. For C₃₀H₄₄N₄O₇ + H, 573.33; Found, 573.33; IR (thin film, cm⁻¹) 3379, 3204, 2961, 2360, 1723, 1607, 1518, 1364, 1255, 1082; TLC (90:10 CH₂Cl₂/MeOH): Rₖ = 0.92.

((1S,2R,3R,4S,5S)-5-((9H-fluoren-2-yl)amino)-4-amino-3-(3,3-dimethylureido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-2-methylcyclopentyl)methyl 2-hydroxy-6-methylbenzoate (2.29f): Isolated from S₂f via general procedure E in 40 % yield. Analytical data: [α]D₁⁹ +21.4 (c = 0.23, CHCl₃); ^1H NMR (600 MHz, CDCl₃): δ (400 MHz, CDCl₃): δ 11.02 (br s, 1H); 7.95 (d, J = 10.8 Hz, 1H); 7.64 (d, J = 7.6 Hz, 1H); 7.59 (d, J = 8.4 Hz, 1H); 7.48 (d, J = 7.6 Hz, 1H); 7.33 (t, J = 7.2 Hz, 1H); 7.29–7.19 (m, 3H); 6.81 (d, J = 8.0 Hz, 2H); 6.66–6.63 (m, 2H); 5.83 (s, 1H); 5.60 (d, J = 10.8 Hz, 1H); 4.90 (d, J = 12.4 Hz, 1H); 4.86 (d, J = 12.4 Hz, 1H); 4.01 (dd, J = 6.4, 10.4 Hz, 1H); 3.85 (d, J = 10.8 Hz, 2H); 3.81 (d, J = 7.6 Hz, 1H); 3.06
(s, 1H); 3.02 (s, 6H); 2.44 (s, 3H); 1.59 (s, 3H); 1.07 (d, J = 6.4 Hz, 3H); \textsuperscript{13}C NMR (150 MHz, CDCl\textsubscript{3}): δ 172.6, 162.7, 159.2, 145.8, 145.4, 142.1, 142.1, 141.3, 134.5, 132.3, 126.6, 125.0, 124.7, 123.0, 120.9, 118.4, 115.6, 122.1, 110.0, 88.8, 85.0, 74.2, 71.4, 69.2, 65.4, 65.1, 36.9, 36.8, 24.1, 21.2, 18.1; MS (ESI\textsuperscript{+}) Calcd. For C\textsubscript{33}H\textsubscript{40}N\textsubscript{4}O\textsubscript{7} + H, 605.30; Found, 605.29. IR (thin film, cm\textsuperscript{-1}) 3397, 2925, 1653, 1616, 1519, 1457, 1375, 1290, 1256, 1096, 804; TLC (95:5 CH\textsubscript{2}Cl\textsubscript{2}:MeOH): R\textsubscript{f} = 0.37.

((1S,2R,3S,4S,5S)-4-amino-3-(3,3-dimethylureido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-2-methyl-5-(naphthalen-1-ylamino)cyclopentyl)methyl 2-hydroxy-6-methylbenzoate (2.29 g) Isolated from S2 g via general procedure E in 48% yield. Analytical data: [\alpha]\textsubscript{D}\textsuperscript{19} +28.1 (c = 0.82, CHCl\textsubscript{3}) \textsuperscript{1}H NMR (600 MHz, CDCl\textsubscript{3}): δ 10.98 (s, 1H); 7.97 (br s, 1H); 7.79 (d, J = 6.0 Hz, 1H); 7.74 (d, J = 12.0 Hz, 1H); 7.46 (m, 2H); 7.31 (t, J = 6.0 Hz, 1H); 7.27-7.21 (m, 4H); 6.79 (d, J = 8.4 Hz, 1H); 6.61 (d, J = 7.2 Hz, 1H); 6.51 (m, 2H); 5.98 (s, 1H); 4.95 (dd, J = 12.0, 7.2 Hz, 2H); 3.97 (m, 2H); 3.06 (br s, 1H); 2.98 (s, 6H); 2.96 (m, 2H); 2.33 (s, 3H); 1.61 (s, 3H); 0.98 (d, J = 6.6 Hz, 3H); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}): δ 172.7, 162.7, 159.3, 141.7, 141.4, 134.6, 134.5, 128.5, 126.3, 126.1, 125.2, 123.7, 123.0, 120.1, 117.3, 115.6, 122.1, 103.3, 88.9, 85.1, 74.5, 71.6, 68.5, 65.4, 62.9, 36.8, 23.9, 21.2, 18.0; MS (ESI\textsuperscript{+}) Calcd. For C\textsubscript{30}H\textsubscript{38}N\textsubscript{4}O\textsubscript{7} + H, 567.28; Found, 567.28; IR (thin film, cm\textsuperscript{-1}) 3756, 3398, 3053, 2984, 2935, 2410, 2304, 1949, 1725, 1656, 1582, 1486, 1265, 1120, 943; TLC (95:5 CH\textsubscript{2}Cl\textsubscript{2}:MeOH): R\textsubscript{f} = 0.33.

((1S,2R,3R,4S,5S)-4-amino-3-(3,3-dimethylureido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-2-methyl-5-((4-(trifluoromethyl)phenyl)amino)cyclopentyl)methyl 2-hydroxy-6-methylbenzoate (2.29 h) Isolated from S2 h via general procedure E in 96% yield. Analytical data: [\alpha]\textsubscript{D}\textsuperscript{19} +30.0 (c = 0.15, CHCl\textsubscript{3}) \textsuperscript{1}H NMR (600 MHz, CDCl\textsubscript{3}): δ 10.98 (br s, 1H); 7.93 (d, J = 10.8 Hz, 1H); 7.39 (d, J = 8.4 Hz, 2H); 7.27 (m, 2H); 7.16 (s, 1H); 6.82 (d, J = 8.4 Hz, 1H); 6.66 (d, J = 7.2 Hz, 1H); 6.59 (d, J = 8.4 Hz, 2H); 5.91 (d, J = 9.6 Hz, 1H); 5.84 (s, 1H); 4.85 (d, J = 12.0 Hz, 1H); 4.79 (d, J = 12.0 Hz, 1H); 3.91 (m, 1H); 3.76
(d, J = 12.0 Hz, 1H); 2.99 (s, 6H); 2.36 (s, 3H); 2.17 (s, 3H); 2.07 (s, 3H); 1.55 (s, 3H); 1.05 (d, J = 6.0 Hz, 3H); 13C NMR (100 MHz, CDCl3): δ 172.5, 170.7, 162.7, 159.2, 148.5, 141.2, 134.6, 126.9, 126.9, 123.0, 115.7, 112.2, 112.1, 88.9, 84.7, 74.3, 71.5, 68.3, 65.3, 63.4, 38.0, 36.8, 35.2, 23.9, 21.5, 21.1, 18.1; MS (ESI+) Calcd. For C27H35F3N4O7 + H, 585.25; Found, 585.18; IR (thin film, cm⁻¹) 3413, 2359, 1617, 1524, 1457, 1375, 1321, 1255, 1213, 1160, 1106, 1064, 737; TLC (95:5 CH2Cl2:MeOH): Rf = 0.17.

Enantioselective Mannich reaction to provide enantioenriched material.

(R,E)-benzyl (4-acetyl-4-(3,3-dimethylureido)-5-oxo-1-phenylhex-1-en-3-yl)carbamate (2.60): A flame-dried 250-mL round-bottomed flask was charged with urea 2.58 (2.38 g, 12.28 mmol, 1.0 equiv), cinchonidine (0.72 g, 2.46 mmol, 0.2 equiv), and CH2Cl2 (65 mL). The resulting suspension was cooled to −78 °C and a cold solution of imine 2.59 (5.1 g, 19.24 mmol, 1.5 equiv) in CH2Cl2 (35 mL) was added via cannula transfer. The reaction was warmed to −65 °C and stirred until complete consumption of urea 2.58 was indicated by TLC analysis, typically 14–36 h (scale-dependent). The crude reaction was filtered through a short silica plug and rinsed with EtOAc (300 mL). The filtrate was concentrated in vacuo to give a pale yellow foam with a 84:16 enantiomeric ratio. Crystalline racemic product was isolated via trituration with 60:40 (v/v) hexanes:EtOAc (300 mL). The analytically-pure white solid was removed by filtration (1.33 g, 24 %) and the filtrate was concentrated in vacuo to give a yellow oil. The crude oil was purified by flash chromatography (60:40 to 50:50 hexanes:EtOAc) affording diketone 2.60 as a pale yellow foam (3.87 g, 70 %, 97:3 er). The enantiomeric ratio was determined by SFC analysis (Chiralcel, OD, 9.0 % MeOH, 1.5 mL/min, 150 bar, 210 nm; tR-minor 12.8 min, tR-major 14.7 min). Analytical data: [α]D²⁰ +16.5 (c = 1.00, CHCl3); mp (racemate) 130–134 °C; 1H NMR (600 MHz, CDCl3): δ 7.37–7.21 (m, 10H), 7.07 (br d, J = 6.0 Hz, 1H), 6.59 (d, J = 16.2 Hz, 1H), 6.50 (s, 1H), 5.96 (dd, J = 16.2 Hz, 7.2, 1H), 5.40 (t, J = 7.2 Hz, 1H), 5.14 (d, J = 12.0 Hz, 1H), 5.11 (d, J = 12.0 Hz, 1H), 2.97 (s, 6H), 2.28 (s, 3H), 2.14 (s, 3H); 13C NMR (150 MHz, CDCl3): δ 200.9, 200.4, 157.6, 156.7, 136.9, 136.5, 133.2, 128.7, 128.6, 128.2, 128.2, 128.1, 126.9, 124.6, 81.7, 67.0, 57.2, 36.8, 26.2, 25.4; MS
(ESI⁺) Calcd. For C$_{25}$H$_{29}$N$_3$O$_5$ + H, 452.2187; Found, 452.2212; IR (thin film, cm$^{-1}$) 3418, 2243, 1702, 1635, 1507, 1371, 1249, 1066, 912, 693; TLC (60:40 hexanes:EtOAc): R$_f$ = 0.20.

**ent-pactamycin (ent-2.1):** When cinchonidine is replaced by cinchonine in the above reaction, a crude enantiomeric ratio of 84:16 is obtained. Upon the analogous trituration protocol, **ent-2.60** is isolated in 68 % yield and 96.5:3.5 er (See SFC assay comparison below). When this material is carried forward in the synthesis, *ent*-pactamycin is obtained. The optical rotation was measured: [$\alpha$]$_D^{19}$ = -23.2 (c = 0.40, CHCl$_3$).

**SFC analysis of racemic Mannich product 2.60 (20 mol % NIPr$_2$Et)**

**Mannich product for natural pactamycin enantiomer synthesis**

Cinchonidine: Crude reaction mixture (16:84 e.r.) after purification/trituration (2:98 e.r.)
Mannich product for ent-pactamycin synthesis

Cinchonine: Crude reaction mixture (84:16 e.r.) after trituration/purification (96.5:3.5 e.r.)

General procedure F for isocyanate formation/trapping.

Benzyll (1aR,1bR,4aR,5R,5aR)-4a-((S)-1-((tert-butyldimethylsilyl)oxy)ethyl)-5a-((tert-butyldiphenylsilyl)oxy)methyl)-5-hydroxy-5-methyl-3-oxohexahydro oxireno [2′,3′:3,4]cyclopenta[1,2-d]imidazole-2(1aH)-carboxylate (2.31).

A 20-mL scintillation vial was charged with urea 2.25 (0.06 g, 0.077 mmol, 1.00 equiv) and an 8:1 mixture of MeOH:H2O (6.0 mL), and NH4Cl (0.123 g, 2.31 mmol, 30.0 equiv) was added. The vial was sealed with a screw-cap and the mixture was heated to 85 °C with vigorous stirring until TLC analysis indicated full conversion of the starting material, generally 16 h. The resulting mixture was concentrated and the remaining residue was dissolved in water (10 mL). The solution was extracted with EtOAc (3 × 8 mL) and the combined organics were washed with brine (10 mL), dried with magnesium sulfate, and concentrated in vacuo. The product was purified via flash chromatography (70:30 hexanes:EtOAc), obtaining 2.31 as a pale yellow solid (0.056 g, 73 %). Analytical Data: 1H NMR (400 MHz, CDCl3): δ 7.67–7.65 (m, 4H); 7.44–7.33 (m, 12H); 5.35 (s, 1H); 5.30 (s, 2H); 4.23 (br s, 2H); 3.99 (br s, 2H); 3.75 (s, 1H); 1.60 (s, 3H); 1.17 (d, J = 6.0 Hz, 3H); 0.87 (s, 9H); 0.10 (s, 9H); 0.08 (s, 9H); MS (ESI+) Calcd. For C40H54N2O7Si2 + H, 731.35; Found, 731.23.

General procedure G for selective TBS deprotection of C7 alcohol.
Benzyl ((1R,2R,3R,4R,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-3-(3,3-dimethylureido)-4-hydroxy-3-((S)-1-hydroxyethyl)-4-methyl-6-oxabicyclo[3.1.0]hexan-2-yl)carbamate (2.32) A 20-mL scintillation vial was charged with silyl ether 2.28 (0.067 g, 0.073 mmol, 1.00 equiv), and a 9:1 mixture of CH₃CN: H₂O (5 mL). To the resulting solution was added Oxone® (0.022 g, 0.15 mmol, 2.00 equiv), and the reaction was vigorously stirred until full conversion of 2.28 was observed by TLC analysis, typically 3 h. The mixture was diluted with H₂O (3 mL) and EtOAc (3 mL), and the layers were partitioned in a separatory funnel and extracted with EtOAc (3 × 10 mL). The combined organic extracts were washed with brine (10 mL), dried with magnesium sulfate, and concentrated in vacuo. The product was purified via flash chromatography (70:30 hexanes: EtOAc) to afford triol 2.32 (0.049 g, 84 %) as a clear, viscous oil. Analytical data: ¹H NMR (400 MHz, CDCl₃): δ 7.63 (d, J = 6.4 Hz, 2H); 7.50 (d, J = 8.0 Hz, 2H); 7.43–7.30 (m, 11H); 7.25 (d, J = 8.4 Hz, 2H); 7.19 (t, J = 7.2 Hz, 2H); 7.05 (s, 1H); 6.66 (d, J = 7.2 Hz, 1H); 5.92 (d, J = 9.6 Hz, 1H); 5.55 (br s, 1H); 5.22–5.13 (m, 3H); 4.02–3.99 (m, 3H); 3.74 (d, J = 9.6 Hz, 1H); 2.87 (s, 6H); 2.53 (s, 3H); 1.43 (s, 3H); 1.25 (d, J = 8.4 Hz, 3H); 1.02 (s, 9H); MS (ESI⁺) Calcd. For C₄₄H₅₆N₄O₈Si + H, 797.39; Found, 797.34.

Benzyl ((1S,2R,3R,4S,5S)-4-(((tert-butyldiphenylsilyl)oxy)methyl)-2-(3,3-dimethylureido)-5-((3-fluorophenyl)amino)-3,4-dihydroxy-2-((S)-1-hydroxyethyl)-3-methylcyclopentyl)carbamate (S3a): Isolated from 2.28b via general procedure G in 87 % yield. Analytical data: ¹H NMR (400 MHz, CDCl₃): δ 7.64 (d, J = 6.8 Hz, 2H); 7.51 (d, J = 7.2 Hz, 2H); 7.46–7.37 (m, 9H); 7.23–7.19 (m, 2H); 7.10 (br s, 1H); 6.44–6.41 (m, 1H); 6.25 (d, J = 8.0 Hz, 1H); 6.18 (d, J = 11.6 Hz, 1H); 5.95 (br s, 2H); 5.55 (d, J = 9.6 Hz, 1H); 5.20–5.17 (m, 3H); 4.12 (br s, 3H); 4.01 (d, J = 10.4 Hz, 1H); 3.69 (br s, 2H); 2.87 (s, 6H); 1.41 (s, 3H); 1.25 (br s, 3H); 1.03 (s, 9H); MS (ESI⁺) Calcd. For C₄₂H₅₃FN₄O₇Si + H, 773.37; Found, 773.46.
Benzyl ((1S,2R,3R,4S,5S)-4-(((tert-butyldiphenylsilyl)oxy)methyl)-2-(3,3-dimethylureido)-3,4-dihydroxy-2-((S)-1-hydroxyethyl)-5-((4-methoxyphenylamino)-3-methylcyclopentyl)carbamate (S3b): Isolated from 2.28c via general procedure G in 62 % yield. Analytical data: $^1$H NMR (600 MHz, CDCl$_3$): $\delta$ 7.65 (d, $J$ = 7.8 Hz, 2H); 7.58 (br s, 1H); 7.55 (d, $J$ = 7.8 Hz, 2H); 7.44–7.34 (m, 8H); 7.22 (t, $J$ = 7.8 Hz, 2H); 6.76 (d, $J$ = 8.4 Hz, 2H); 6.42 (d, $J$ = 7.8 Hz, 2H); 5.95 (s, 1H); 5.89 (br s, 1H); 5.20–5.18 (m, 2H); 5.14–5.11 (m, 2H); 4.00 (br s, 2H); 3.92 (d, $J$ = 9.6 Hz, 1H); 3.76 (s, 3H); 3.68 (s, 1H); 3.64 (d, $J$ = 6.6 Hz, 1H); 2.86 (s, 6H); 1.41 (s, 3H); 1.25 (br s, 3H); 1.03 (s, 9H); MS (ESI$^+$) Calcd. For C$_{43}$H$_{56}$N$_4$O$_8$Si + H, 785.39; Found, 785.49.

Benzyl ((1S,2R,3R,4S,5S)-5-((4-((tert-butyl)phenyl)amino)-4-(((tert-butyldiphenylsilyl)oxy)methyl)-2-(3,3-dimethylureido)-3,4-dihydroxy-2-((S)-1-hydroxyethyl)-3-methylcyclopentyl)carbamate (S3c): Isolated from 2.28e via general procedure G in 77 % yield. Analytical data: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.64 (d, $J$ = 6.8 Hz, 2H); 7.58 (d, $J$ = 9.8 Hz, 1H); 7.51 (d, $J$ = 6.8 Hz, 2H); 7.43–7.32 (m, 9H); 7.20–7.17 (m, 4H); 6.42 (d, $J$ = 8.4 Hz, 2H); 5.96–5.92 (m, 2H); 5.27 (dd, $J$ = 4.4, 14.4 Hz, 1H); 5.23–5.20 (m, 2H); 5.13 (d, $J$ = 12.0 Hz, 1H); 4.15–4.10 (m, 3H); 3.72 (s, 1H); 3.67 (d, $J$ = 8.8 Hz, 1H); 2.87 (s, 6H); 1.43 (s, 3H); 1.29 (s, 9H); 1.27 (d, $J$ = 7.2 Hz, 3H); 1.03 (s, 9H); MS (ESI$^+$) Calcd. For C$_{46}$H$_{62}$N$_4$O$_7$Si + H, 811.45; Found, 811.53.

Synthesis of De 6-MSA Pactamycate:

Benzyl ((4R,5R,6S,7S,8S,9R)-7-(((3-acetylphenyl)amino)-8-(((tert-butyldiphenylsilyl)oxy)methyl)-8,9-dihydroxy-4,9-dimethyl-2-oxo-3-oxa-1-azaspiro[4.4]nonan-6-yl)carbamate (2.33): Isolated from 2.32 via general procedure F in 73 % yield. Analytical data: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.53 (d, $J$ = 6.8 Hz, 2H); 7.45–7.44 (m, 3H); 7.37–7.34 (m, 4H); 7.28–7.26 (m, 6H); 7.14–7.10(m, 3H); 6.68 (d, $J$ = 6.4 Hz, 1H); 5.69 (s, 1H); 5.34 (d, $J$ = 6.4 Hz, 1H); 5.69 (s, 1H); 5.34 (d, $J$ = 10.4 Hz, 1H); 5.07 (d, $J$ = 12.4 Hz, 1H); 5.00 (d, $J$ = 12.4 Hz, 1H); 4.80 (q, $J$ = 6.4 Hz, 1H); 3.51–3.47 (m, 1H); 3.34 (s, 1H); 2.46 (s, 3H); 2.45(s, 1H);
2.04 (s, 1H); 1.45 (s, 3H); 143 (br s, 3H); 1.02 (s, 9H); **MS (ESI⁺)** Calcd. For C₄₂H₄₉N₃O₈Si + H, 752.34; Found, 752.48.

**Benzyl ((4R,5R,6S,7S,8S,9R)-7-((3-acetylphenyl)amino)-8,9-dihydroxy-8-(hydroxymethyl)-4,9-dimethyl-2-oxo-3-oxa-1-azaspiro[4.4]nonan-6-yl)carbamate (S4):** Isolated from 2.33 via general procedure C in > 95 % yield. Analytical data: **¹H NMR** (400 MHz, CDCl₃): δ 7.26–7.03 (m, 7H); 6.49 (br s, 2H); 6.30 (d, J = 8.0 Hz, 1H); 5.04 (d, J = 12.8 Hz, 1H); 4.95 (d, J = 12.4 Hz, 1H); 8.21 (br s, 1H); 4.53 (br s, 1H); 4.45 (s, 1H); 4.32 (br s, 1H); 4.24 (br s, 1H); 4.04 (d, J = 12.0 Hz, 1H); 3.59–3.56 (m, 3H); 2.35 (s, 1H); 2.27 (s, 3H); 1.39 (d, J = 6.0, 3H); 1.36 (s, 3H); **MS (ESI⁺)** Calcd. For C₂₆H₃₁N₃O₈ + H, 514.22; Found, 514.27.

**De-6-MSA Pactamycate (2.34):** Isolated from S₄ via general procedure E in 46 % yield. Analytical data: [α]D₁₉ +4.4 (c = 0.11, CHCl₃); **¹H NMR** (400 MHz, MeOD): δ 7.38 (br s, 1H); 7.21–7.19 (m, 2H); 7.01 (dt, J = 2.0, 6.8 Hz, 1H); 4.82 (q; J = 6.4 Hz, 1H); 3.91 (d, J = 11.6 Hz, 1H); 3.58–3.56 (m, 2H); 3.53 (d, J = 7.6 Hz; 1H); 2.55 (s, 3H); 1.54 (d, J = 6.8 Hz, 3H); 1.35 (s, 3H); **¹³C NMR** (150 MHz, MeOD): δ 201.5, 161.0, 150.9, 139.2, 130.3, 119.2, 118.0, 113.2, 84.1, 83.1, 78.4, 72.5, 70.7, 63.5, 60.7, 26.9, 17.5, 17.3; **MS (ESI⁺)** Calcd. For C₁₈H₂₅N₃O₆ + H, 380.18; Found, 380.23; **IR** (thin film, cm⁻¹) 2921, 2846, 2359, 1861, 1785, 1738, 1710, 1641, 1598, 1512, 1409, 1380, 1252, 1095; **TLC** (95:5 CH₂Cl₂:MeOH): Rf = 0.13.

**Synthesis of Pactamycate Derivatives:**
Benzyl \((4R,5R,6S,7S,8S,9R)-8-(((tert-butyldiphenylsilyl)oxy)methyl)-7-((3-fluorophenyl)amino)-8,9-dihydroxy-4,9-dimethyl-2-oxo-3-oxa-1-azaspiro[4.4]nonan-6-yl)carbamate (S5a): Isolated from S3a via general procedure F in 88 % yield. Analytical data: \(\text{\textit{H NMR}}\) (400 MHz, CDCl\(_3\)): \(\delta \) 7.55 (dd, \(J = 1.27, 8.31\) Hz, 2H); 7.50 (dd, \(J = 1.21, 8.29, 2H\)); 7.36–7.10 (m, 11H); 6.93 (dd, \(J = 7.6, 14.8\) Hz, 1H); 6.35–6.30 (m, 1H); 6.24 (d, \(J = 8.8\) Hz, 2H); 6.08 (s, 1H); 5.57 (d, \(J = 10.0\) Hz, 1H); 5.05 (d, \(J = 12.4\) Hz, 1H); 4.92 (d, \(J = 12.0\) Hz, 1H); 4.79 (q, \(J = 6.4\) Hz, 1H); 4.49–4.45 (m, 1H); 4.31 (d, \(J = 6.8\) Hz, 1H); 4.02 (d, \(J = 11.2\) Hz, 1H); 3.67 (d, \(J = 11.2\) Hz, 1H); 3.43 (s, 1H); 3.42 (s, 1H); 3.01 (s, 1H); 1.42 (s, 3H); 1.39 (d, \(J = 6.0\) Hz, 3H); 1.03 (s, 9H); \(\text{MS (ESI}^+\text{)}\) Calcd. For \(\text{C}_{40}\text{H}_{46}\text{FN}_{3}\text{O}_{7}\text{Si} + \text{H}, 728.32\); Found, 728.35.

Benzyl \((4R,5R,6S,7S,8S,9R)-8-(((tert-butyldiphenylsilyl)oxy)methyl)-8,9-dihydroxy-7-((4-methoxyphenyl)amino)-4,9-dimethyl-2-oxo-3-oxa-1-azaspiro[4.4]nonan-6-yl)carbamate (S5b): Isolated from S3b via general procedure F in 73 % yield. Analytical data: \(\text{\textit{H NMR}}\) (400 MHz, CDCl\(_3\)): \(\delta \) 7.55 (d, \(J = 7.2\) Hz, 2H); 7.51 (d, \(J = 7.2\) Hz, 2H); 7.45–7.22 (m, 12H); 6.46 (d, \(J = 8.4\) Hz, 1H); 5.83 (s, 1H); 5.34 (d, \(J = 10.4\) Hz, 1H); 5.05 (d, \(J = 12.0\) Hz, 1H); 4.97 (d, \(J = 12.0\) Hz, 1H); 4.78 (q, \(J = 6.4\) Hz, 1H); 4.45–4.40 (m, 1H); 4.06 (d, \(J = 11.6\) Hz, 1H); 3.81 (br s, 1H); 3.71 (s, 3H); 3.36 (d, \(J = 6.8\) Hz, 1H); 3.26 (s, 1H); 2.56 (s, 1H); 1.41 (br s, 6H); 0.87 (s, 9H); \(\text{MS (ESI}^+\text{)}\) Calcd. For \(\text{C}_{41}\text{H}_{49}\text{N}_{3}\text{O}_{8}\text{Si} + \text{H}, 740.34\); Found, 740.38.

Benzyl \((4R,5R,6S,7S,8S,9R)-7-((4-(tert-butyl)phenyl)amino)-8-(((tert-butyldiphenylsilyl)oxy)methyl)-8,9-dihydroxy-4,9-dimethyl-2-oxo-3-oxa-1-azaspiro[4.4]nonan-6-yl)carbamate (S5c): Isolated from S3c via general procedure F in 80 % yield. Analytical data: \(\text{\textit{H NMR}}\) (400 MHz, CDCl\(_3\)): \(\delta \) 7.55 (d, \(J = 6.8\) Hz, 2H); 7.49 (d, \(J = 6.8\) Hz, 2H); 7.42–7.22 (m, 12H); 7.06 (d, \(J = 8.4\) Hz, 2H); 6.46 (d, \(J = 8.4\) Hz, 2H); 5.93 (s, 1H); 5.39 (d, \(J = 10.0\) Hz, 1H); 5.02 (d, \(J = 12.0\) Hz, 1H); 4.98 (d, \(J = 12.4\) Hz, 1H); 4.80 (dd, \(J = 6.4, 13.2\) Hz, 1H); 4.50–4.77 (m, 1H); 4.05 (br s, 1H); 3.74 (d, \(J = 11.2\) Hz, 1H); 3.47 (br s, 1H); 3.42 (s, 1H); 2.75
(s, 1H); 1.42 (d, J = 9.2 Hz, 1H); 1.30 (s, 3H); 1.27 (s, 9H); 1.03 (s, 9H); **MS (ESI⁺)** Calcd. For C₄₄H₅₅N₃O₇Si + H, 766.39; Found, 766.42.

**Benzyl ((4R,5R,6S,7S,8S,9R)-7-((3-fluorophenyl)amino)-8,9-dihydroxy-8-(hydroxymethyl)-4,9-dimethyl-2-oxo-3-oxa-1-azaspiro[4.4]nonan-6-yl)carbamate (2.35a):** Isolated from **S5a** via general procedure C in 78 % yield. Analytical data: **¹H NMR** (400 MHz, d₆-Acetone): δ 7.26 (br s, 5H); 7.05 (d, J = 8.0 Hz, 1H); 6.65 (d, J = 10.4 Hz, 1H); 6.57 (d, J = 8.4 Hz, 2H); 6.52 (s, 1H); 6.33–6.29 (m, 1H); 6.08 (s, 1H); 5.15 (d, J = 8.8 Hz, 1H); 5.08 (d, J = 12.4 Hz, 1H); 5.00 (d, J = 12.4 Hz, 1H); 4.78 (q, J = 6.4 Hz, 1H); 4.74 (s, 1H); 4.57–4.52 (m, 1H); 4.45 (s, 1H); 4.05 (d, J = 11.2 Hz, 1H); 3.85 (t, J = 8.8 Hz, 1H); 3.67 (d, J = 11.2 Hz, 1H); 1.44–1.43 (m, 6H); **MS (ESI⁺)** Calcd. For C₂₄H₂₈FN₃O₇ + H, 490.20; Found, 490.26.

**Benzyl ((4R,5R,6S,7S,8S,9R)-8,9-dihydroxy-8-(hydroxymethyl)-7-((4-methoxyphenyl)amino)-4,9-dimethyl-2-oxo-3-oxa-1-azaspiro[4.4]nonan-6-yl)carbamate (2.35b):** Isolated from **S5b** via general procedure C in 76 % yield. Analytical data: **¹H NMR** (400 MHz, CDCl₃): δ 7.29 (br s, 2H); 7.26 (br s, 3H); 6.77 (d, J = 8.4 Hz, 2H); 6.70 (d, J = 8.4 Hz, 2H); 5.72 (s, 1H); 5.32 (d, J = 9.6 Hz, 1H); 5.09 (d, J = 12.4 Hz, 1H); 5.02 (d, J = 12.4 Hz, 1H); 4.86 (q, J = 6.4 Hz, 1H); 4.60–4.56 (m, 1H); 3.94 (d, J = 12.0 Hz, 1H); 3.88 (br s, 1H); 3.81 (d, J = 12.4 Hz, 1H); 3.75 (s, 3H); 3.44 (d, J = 8.4 Hz, 1H); 2.63 (s, 1H); 1.47 (d, J = 6.8 Hz, 3H); 1.38 (s, 3H); **MS (ESI⁺)** Calcd. For C₂₅H₃₁N₃O₈ + H, 502.22; Found, 502.17.
Benzyl \((4R,5R,6S,7S,8S,9R)-7-((4-(tert-butyl)phenyl)amino)-8,9-dihydroxy-8-(hydroxymethyl)-4,9-dimethyl-2-oxo-3-oxa-1-azaspiro[4.4]nonan-6-yl)carbamate (2.35c): Isolated from \(\text{SSc}\) via general procedure C in 80 % yield. Analytical data: \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.28–7.26 (m, 5H); 7.22 (d, \(J = 8.4\) Hz, 2H); 6.69 (d, \(J = 8.4\) Hz, 2H); 5.77 (br s, 1H); 5.33 (d, \(J = 9.2\) Hz, 1H); 5.10 (d, \(J = 12.4\) Hz, 1H); 5.04 (d, \(J = 12.4\) Hz, 1H); 4.86 (d, \(J = 6.4\) Hz, 1H); 4.35 (br s, 1H); 3.98 (d, \(J = 8.4\) Hz, 1H); 3.85 (br s, 1H); 3.78 (br s, 1H); 3.50 (d, \(J = 7.2\) Hz, 1H); 3.35 (s, 1H); 3.11 (br s, 1H); 1.46 (d, \(J = 6.8\) Hz, 3H); 1.38 (s, 3H); 1.28 (s, 9H); MS (ESI\(^+\)) Calcd. For C\(_{28}\)H\(_{37}\)N\(_3\)O\(_7\) + H, 528.27; Found, 528.34.

\(((4R,5R,6R,7S,8S,9S)-9-(((benzyloxy)carbonyl)amino)-8-((3-fluorophenyl)amino)-6,7-dihydroxy-4,6-dimethyl-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl) methyl 2-hydroxy-6-methylbenzoate (2.36a): Isolated from 2.35a via general procedure D in 89 % yield. Analytical data: \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 10.33 (br s, 1H); 7.26–7.17 (m, 5H); 7.05 (br s, 1H); 6.92 (q, \(J = 7.6\) Hz, 1H); 6.78 (d, \(J = 8.4\) Hz, 1H); 6.66 (d, \(J = 7.2\) Hz, 1H); 6.37–6.29 (m, 3H); 5.78 (d, \(J = 10.0\) Hz, 1H); 5.05 (d, \(J = 12.0\) Hz, 1H); 4.92 (d, \(J = 11.6\) Hz, 1H); 4.8 (d, \(J = 6.0\) Hz, 1H); 4.57 (t, \(J = 8.8\) Hz, 1H); 4.53 (d, \(J = 12.0\) Hz, 1H); 4.43 (d, \(J = 12.4\) Hz, 1H); 4.02 (s, 1H); 3.76 (s, 1H); 3.65 (br s, 1H); 2.57 (s, 3H); 1.43 (d, \(J = 6.0\) Hz, 3H); 1.24 (br s, 3H); MS (ESI\(^+\)) Calcd. For C\(_{32}\)H\(_{34}\)FN\(_3\)O\(_9\) + H, 624.24; Found, 624.30.

\(((4R,5R,6R,7S,8S,9S)-9-(((benzyloxy)carbonyl)amino)-6,7-dihydroxy-8-((4-methoxyphenyl)amino)-4,6-dimethyl-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl) methyl 2-hydroxy-6-methylbenzoate (2.36b): Isolated from 2.35b via general procedure D in 76 % yield. Analytical data: \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 10.46 (s, 1H); 7.31–7.23 (m, 6H); 6.83 (d, \(J = 8.0\) Hz, 1H); 6.70–6.68 (m, 3H); 6.62 (d, \(J = 8.4\) Hz, 2H); 5.96 (s, 1H); 5.48 (d, \(J = 10.8\) Hz, 1H); 5.08 (d, \(J = 12.4\) Hz, 1H); 5.00 (d, \(J = 12.4\) Hz, 1H); 4.80 (br s, 1H); 4.60–4.56 (m, 1H); 4.45 (d, \(J = 12.4\) Hz, 1H); 4.08 (br s, 1H); 3.76 (s, 1H); 3.69 (s, 3H); 3.55 (br s, 1H);
2.45 (s, 3H); 1.45 (d, $J = 6.4$ Hz, 3H); 1.26 (s, 3H); **MS (ESI$^+$)** Calcd. For $C_{33}H_{37}N_3O_{10} + H$, 636.26; Found, 636.27.

$((4R,5R,6R,7S,8S,9S)-9-(((benzyloxy)carbonyl)amino)-8-((4-(tert-butyl)phenyl)amino)-6,7-dihydroxy-4,6-dimethyl-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)methyl 2-hydroxy-6-methylbenzoate (2.36c):** Isolated from 2.35c via general procedure D in 95 % yield. Analytical data: $^1H$ NMR (400 MHz, CDCl$_3$): $\delta$ 10.42 (s, 1H); 7.23 (br s, 6H); 7.09 (d, $J = 8.0$ Hz, 2H); 6.81 (d, $J = 8.0$ Hz, 1H); 6.67 (d, $J = 7.2$ Hz, 1H); 6.60 (d, $J = 8.4$ Hz, 1H); 6.24 (s, 1H); 5.66 (d, $J = 9.6$ Hz, 1H); 5.07–4.97 (m, 2H); 4.82 (br s, 1H); 4.61 (m, 1H); 4.46 (d, $J = 12.8$ Hz, 1H); 4.28 (s, 1H); 4.10 (s, 1H); 3.67 (s, 1H); 3.36 (d, $J = 6.8$ Hz, 1H); 2.40 (s, 3H); 1.46 (d, $J = 6.4$ Hz, 3H); 1.28 (br s, 3H); 1.23 (s, 9H); **MS (ESI$^+$)** Calcd. For $C_{36}H_{43}N_3O_{9} + H$, 662.31; Found, 662.38.

$((4R,5R,6R,7S,8S,9S)-9-amino-8-((3-fluorophenyl)amino)-6,7-dihydroxy-4,6-dimethyl-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)methyl 2-hydroxy-6-methylbenzoate (2.37a):** Isolated from 2.36a via general procedure E in 66 % yield. Analytical data: $[\alpha]_D^{19} +37.3$ ($c = 0.8$, CHCl$_3$); $^1H$ NMR (600 MHz, MeOD): $\delta$ 7.16 (t, $J = 7.8$ Hz, 1H), 6.94 (q, $J = 7.2$ Hz, 1H), 6.69 (m, 2H), 6.51 (m, 2H), 6.17 (m, 1H), 4.81 (q, $J = 6.6$ Hz, 1H), 4.55 (d, $J = 11.4$ Hz, 1H), 4.50 (d, $J = 11.4$ Hz, 1H), 3.54 (s, 2H), 2.29 (s, 3H), 1.55 (d, $J = 6.6$ Hz, 3H), 1.35 (s, 3H); $^{13}C$ NMR (150 MHz, CDCl$_3$): $\delta$ 170.6, 164.7, 161.0, 158.4, 152.0, 140.3, 133.0, 131.1, 123.1, 119.3, 114.9, 109.9, 103.9, 103.7, 100.5, 100.4, 83.9, 82.7, 78.5, 72.7, 71.4, 67.1, 21.3, 17.2; IR (thin film, cm$^{-1}$) 3895, 3582, 3388, 3054, 2986, 2520, 2410, 2305, 1736, 1550, 1422, 1333, 1265, 1115; **MS (ESI$^+$)** Calcd. For $C_{24}H_{28}FN_3O_7 + H$, 490.20; Found, 490.26; **TLC** (95:5 CH$_2$Cl$_2$:MeOH): $R_f = 0.08$. 

![Image](2.36c)

![Image](2.37a)
((4R,5R,6R,7S,8S,9S)-9-amino-6,7-dihydroxy-8-((4-methoxyphenyl)amino)-4,6-dimethyl-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)methyl 2-hydroxy-6-methylbenzoate (2.37b): Isolated from 2.36b via general procedure E in 85% yield. Analytical data: $[\alpha]_D^{19} +18.8$ (c = 0.95, CHCl$_3$); $^1$H NMR (500 MHz, MeOD): δ 7.17 (t, $J = 8.0$ Hz, 1H); 6.69–6.68 (m, 4H); 6.62 (t, $J = 3.5$ Hz, 1H); 6.60 (t, $J = 2.0$ Hz, 1H); 4.82 (q, $J = 7.0$ Hz, 1H); 4.57 (d, $J = 12.0$ Hz, 1H); 4.51 (d, $J = 12.0$ Hz, 1H); 3.63 (s, 3H); 3.54 (s, 2H); 2.31 (s, 3H); 1.52 (d, $J = 6.5$ Hz, 3H); 1.41 (s, 3H); $^{13}$C NMR (150 MHz, CD$_3$OD): δ 170.7, 160.9, 158.9, 153.2, 143.8, 140.6, 133.2, 123.1, 118.8, 115.6, 115.5, 115.0, 84.2, 82.8, 78.5, 72.9, 72.3, 66.9, 61.3, 56.1, 21.6, 17.4, 17.0, 15.4; MS (ESI$^+$) Calcd. For C$_{25}$H$_{31}$N$_3$O$_8$ + H, 502.22; Found, 502.22; IR (thin film, cm$^{-1}$) 3828, 3740, 3389, 3054, 2986, 2521, 2359, 2305, 1735, 1550, 1441, 1265, 1114; TLC (95:5 CH$_2$Cl$_2$:MeOH): $R_f = 0.07$.

((4R,5R,6R,7S,8S,9S)-9-amino-8-((4-(tert-butyl)phenyl)amino)-6,7-dihydroxy-4,6-dimethyl-2-oxo-3-oxa-1-azaspiro[4.4]nonan-7-yl)methyl 2-hydroxy-6-methylbenzoate (2.37c): Isolated from 2.36c via general procedure E in 48% yield. Analytical data: $[\alpha]_D^{19} +27.0$ (c = 0.85, CHCl$_3$); $^1$H NMR (400 MHz, MeOD): δ 7.17 (t, $J = 7.6$ Hz, 1H); 7.05 (d, $J = 8.4$ Hz, 2H); 6.71–6.67 (m, 4H); 4.82 (q, $J = 6.8$ Hz, 1H); 4.54 (s, 2H); 3.57 (d, $J = 6.8$ Hz, 1H); 3.52 (d, $J = 6.8$ Hz, 1H); 2.29 (s, 3H); 1.53 (d, $J = 6.8$ Hz, 3H); 1.35 (s, 3H); 1.20 (s, 9H); $^{13}$C NMR (100 MHz, MeOD): δ 170.7, 161.0, 158.8, 147.3, 140.7, 140.6, 133.1, 126.7, 123.1, 119.0, 115.0, 84.1, 82.8, 78.6, 72.9, 72.00, 67.0, 61.4, 34.5, 32.0, 21.5, 17.4, 17.1; IR (thin film, cm$^{-1}$) 3390, 2960, 2359, 1707, 1649, 1552, 1482, 1385, 1303, 1198, 1071, 737; MS (ESI$^+$) Calcd. For C$_{28}$H$_{37}$N$_3$O$_7$ + H, 528.27; Found, 528.34; TLC (95:5 CH$_2$Cl$_2$:MeOH): $R_f = 0.13$.

General procedure H for addition of nucleophiles to ketone 2.24.
Benzyl ((1R,2R,3R,4R,5R)-3-((S)-1-((tert-butyl(dimethyl)silyl)oxy)ethyl)-5-(tert-butyldiphenylsilyl)oxy)methyl)-3-(3,3-dimethylureido)-4-hydroxy-4-methyl-6-oxabicyclo[3.1.0]hexan-2-yl)carbamate (2.25): A flame-dried 25-mL round-bottomed flask was charged with ketone 2.24 (1.7 g, 2.3 mmol, 1.0 equiv) and THF (23 mL). The solution was cooled to 0 °C and MeMgBr (3 M in THF, 7.6 mL, 22.9 mmol, 10.0 equiv) was added dropwise. The reaction was stirred at 0 °C until TLC analysis indicated complete ketone consumption, typically 2 h. Saturated NH₄Cl(aq.) (20 mL) was carefully added dropwise and the resulting mixture was extracted with EtOAc (3 × 15 mL). The combined organic extracts were washed with brine (20 mL), dried with magnesium sulfate, and concentrated in vacuo. The crude product was purified via flash chromatography (90:10 to 70:30 hexanes:EtOAc) to afford carbinol 2.25 as a clear, viscous oil with > 10:1 diastereoselection (1.3 g, 75 %). Analytical data: [α]D¹⁰ +7.2 (c = 0.70, CHCl₃); ¹H NMR (600 MHz, CDCl₃): δ 7.73 (d, J = 7.8 Hz, 2H), 7.70 (d, J = 7.2 Hz, 2H), 7.45–7.30 (m, 12H), 5.55 (br s, 1H), 5.21 (d, J = 12.6 Hz, 1H), 5.17 (br s, 1H), 5.07 (d, J = 12.0 Hz, 1H), 4.77 (br s, 1H), 4.64 (dd, J = 8.4, 3.6 Hz, 1H), 4.21 (d, J = 12.6 Hz, 1H), 4.12 (d, J = 12.6 Hz, 1H), 3.90 (s, 1H), 2.75 (s, 6H), 1.30 (s, 3H), 1.25 (d, J = 6.0 Hz, 3H), 1.07 (s, 9H), 0.97 (s, 9H), 0.11 (s, 3H), −0.01 (s, 3H); ¹³C NMR (150 MHz, CDCl₃): δ 158.8, 156.5, 136.3, 135.6, 135.5, 134.7, 133.3, 132.9, 129.6, 129.4, 128.4, 128.2, 128.1, 127.7, 127.6, 127.6, 67.1, 66.8, 62.1, 58.3, 36.1, 26.7, 26.5, 25.7, 23.8, 19.6, 19.2, 17.8, −4.2, −5.5; MS (ESI⁺) Calcd. for C₄₂H₆₁N₃O₇Si₂ + H, 776.4128; Found, 776.4179; IR (thin film, cm⁻¹) 3430, 2429, 2359, 1716, 1635, 1506, 1456, 1112, 831, 700; TLC (90:10 hexanes/ EtOAc): Rf = 0.35.

Benzyl ((1R,2R,3R,4R,5R)-3-(((S)-1-((tert-butyl(dimethyl)silyl)oxy)ethyl)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-3-(3,3-dimethylureido)-4-ethyl-4-hydroxy-6-oxabicyclo[3.1.0]hexan-2-yl)carbamate (2.38a): Isolated from 2.24 via general procedure H using EtMgBr as the nucleophile in 75 % yield. Analytical data: ¹H NMR (500 MHz, CDCl₃): δ 7.66–7.63 (m, 4H); 7.42–7.33 (m, 11H); 5.31 (br s, 1H); 5.24 (br s, 1H); 5.16 (d, J = 12.0 Hz, 1H); 5.04 (d, J = 12.5 Hz, 1H); 4.66 (s, 1H); 4.61–4.57 (m, 2H); 4.33 (d, J = 12.5 Hz, 1H); 3.98 (s, 1H); 3.95–3.94 (m, 1H); 2.68 (s, 6H); 1.44–1.41 (m, 1H); 1.36–1.32 (m, 1H); 1.27–1.25 (m, 3H); 1.01 (s, 9H); 0.95 (s, 9H); 0.81 (t, J = 7.5 Hz, 3H); 0.08 (s, 3H); 0.07 (s, 3H); MS (ESI⁺) Calcd. For C₄₃H₆₃N₃O₇Si₂ + H, 790.43; Found, 790.43.
Benzyl ((1R,2R,3R,4R,5R)-3-(((S)-1-((tert-butyldimethylsilyl)oxy)ethyl)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-3-(3,3-dimethylureido)-4-hydroxy-6-oxabicyclo[3.1.0]hexan-2-yl)carbamate (2.38b): Isolated from 2.24 via general procedure H using ("Hexyl)MgBr as the nucleophile in 73 % yield. Analytical data: $^1$HNMR (400 MHz, CDCl$_3$): $\delta$ 7.67–7.63 (m, 4H), 7.40–7.31 (m, 12H), 5.32 (d, J = 6.8 Hz, 1H), 5.23 (br s, 1H), 5.18 (d, J = 12.4 Hz, 1H), 5.07 (d, J = 12.4 Hz, 1H), 4.72 (s, 1H), 4.59 (m, 2H), 4.34 (d, J = 13.2 Hz, 1H), 3.97 (m, 2H), 2.70 (s, 6H), 1.26 (m, 4H), 1.18 (m, 4H), 1.02 (s, 9H), 0.96 (s, 9H), 0.09 (s, 3H), 0.06 (s, 3H); MS (ESI$^+$) Calcd. For C$_{47}$H$_{71}$N$_3$O$_7$Si$_2$ + H, 846.49; Found, 846.59.

Benzyl ((1R,2R,3R,4R,5R)-3-(((S)-1-((tert-butyldimethylsilyl)oxy)ethyl)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-3-(3,3-dimethylureido)-4-hydroxy-4-vinyl-6-oxabicyclo[3.1.0]hexan-2-yl)carbamate (2.38c): Isolated from 2.24 via general procedure H using (vinyl)MgBr as the nucleophile in 43 % yield. Analytical data: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.65–7.64 (m, 4H), 7.40–7.35 (m, 11H), 5.93 (dd, J = 10.8, 17.20 Hz, 1H), 5.70 (br s, 1H), 5.35 (d, J = 17.2 Hz, 1H), 5.19 (d, J = 12.0 Hz, 1H), 5.11–5.06 (m, 3H), 4.93 (br s, 1H), 4.19 (d, J = 12.8 Hz, 1H), 4.02 (s, 1H), 3.97 (d, J = 12.4 Hz, 1H), 2.72 (s, 6H), 1.23 (d, J = 6.4 Hz, 3H), 1.01 (s, 9H), 0.93 (s, 9H), 0.09 (s, 3H), 0.01 (s, 3H); MS (ESI$^+$) Calcd. For C$_{43}$H$_{61}$N$_3$O$_7$Si$_2$ + Na, 810.39; Found, 810.24.

Benzyl ((1R,2R,3S,4R,5S)-3-(((S)-1-((tert-butyldimethylsilyl)oxy)ethyl)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-3-(3,3-dimethylureido)-4-hydroxy-6-oxabicyclo[3.1.0]hexan-2-yl)carbamate (2.38d): A flame-dried 100 mL round bottomed flask was charged with ketone 2.24 (0.280 g, 0.369 mmol, 1.0 equiv) and MeOH (20 mL) under an atmosphere of nitrogen. The solution was cooled to $-45 \, ^\circ\text{C}$ and NaBH$_4$ (0.056 g, 1.47 mmol, 4.0 equiv) was added in one portion. The reaction was allowed to stir at this temperature until TLC analysis indicated full conversion of the starting material, generally 1 h. Saturated NH$_4$Cl$_{aq.}$ (10 mL) was added slowly followed by EtOAc (20 mL) and the mixture was allowed to warm to r.t. The mixture was partitioned and the aqueous layer was extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with H$_2$O (2 × 20 mL), brine (15 mL), dried with magnesium sulfate and concentrated in vacuo. The crude product was purified via flash chromatography (90:10
hexanes:EtOAc) to obtain the title compound as a white foam (249 mg, 88 %). Analytical Data: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.67–7.64 (m, 4H); 7.39–7.34 (m, 11H); 5.49 (br s, 1H); 5.28 (br s, 1H); 5.20 (d, $J = 12.0$ Hz, 1H); 5.02 (d, $J = 12.0$ Hz, 1H); 4.97 (s, 1H); 4.61(dd, $J = 2.8, 8.8$ Hz, 1H); 4.48 (bs, 1H); 4.16 (s, 1H); 4.15 (d, $J = 12.4$ Hz, 1H); 4.09 (d, $J = 12.4$ Hz, 1H); 3.92 (br s, 1H); 2.70 (s, 6H); 1.18 (d, $J = 6.4$ Hz, 3H); 1.02 (s, 9H); 0.92 (s, 9H); 0.06 (s, 3H); −0.07 (s, 3H); MS (ESI$^+$) Calcd. For C$_{41}$H$_{59}$N$_3$O$_7$Si$_2$ + Na, 784.38; Found, 784.44.

Benzyl (1S,2R,3R,4S,5S)-2-((S)-1-((tert-butyldimethylsilyl)oxy)ethyl)-4-(((tert-butyldiphenylsilyl)oxy)methyl)-2-(3,3-dimethylureido)-3-ethyl-3,4-dihydroxy-5-((3-(prop-1-en-2-yl)phenyl)amino)cyclopentyl)carbamate (2.40a): Isolated from 2.38a via general procedure B using 3-isopropenylaniline as the nucleophile in 71 % yield. Analytical data: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.07 (d, $J = 6.8$ Hz, 1H), 7.66 (d, $J = 6.8$ Hz, 2H), 7.47 (d, $J = 6.8$ Hz, 2H), 7.39 (d, $J = 7.6$ Hz, 1H), 7.34–7.19 (m, 10H), 7.05 (t, $J = 8.0$ Hz, 1H), 6.80 (m, 2H), 6.57 (d, $J = 7.6$ Hz, 1H), 6.15 (s, 1H), 5.47 (s, 1H), 5.36 (s, 1H), 5.27 (q, $J = 6.4$ Hz, 1H), 5.17 (d, $J = 3.6$ Hz, 1H), 5.02 (br s, 3H), 4.72 (dd, $J = 7.2, 2.8$ Hz, 1H), 4.36 (s, 1H), 4.13 (d, $J = 10.8$ Hz, 1H), 3.75 (d, $J = 10.8$ Hz, 1H), 3.66 (dd, $J = 4.0, 6.0$ Hz, 1H), 2.95 (s, 6H), 2.12 (s, 3H), 1.88 (m, 1H), 1.35 (d, $J = 6.8$ Hz, 3H), 0.96 (s, 9H), 0.898 (br s, 12H), 0.09 (s, 3H), 0.01 (s, 3H); MS (ESI$^+$) Calcd. For C$_{52}$H$_{74}$N$_4$O$_7$Si$_2$ + H, 923.52; Found, 923.54.

Benzyl (4S,5S)-2-((S)-1-((tert-butyldimethylsilyl)oxy)ethyl)-4-(((tert-butyldiphenylsilyl)oxy)methyl)-2-(3,3-dimethylureido)-3-hexyl-3,4-dihydroxy-5-((3-(prop-1-en-2-yl)phenyl)amino)cyclopentyl)carbamate (2.40b): Isolated from 2.38b via general procedure B using 3-isopropenylaniline as the nucleophile in 63 % yield. Analytical data: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.07 (d, $J = 6.8$ Hz, 1H), 7.66 (d, $J = 6.8$ Hz, 2H), 7.48 (d, $J = 6.8$ Hz, 2H), 7.40(m, 1H), 7.35–7.19 (m, 10H), 7.05 (t, $J = 7.6$ Hz, 1H), 6.79 (m, 2H), 6.57 (d, $J = 7.6$ Hz, 1H), 6.18 (s, 1H), 5.49 (s, 1H), 5.37 (s, 1H), 5.28 (q, $J = 6.4$ Hz, 1H), 5.16 (s, 1H), 5.01 (s, 3H), 4.73 (dd, $J = 7.2, 2.4$ Hz, 1H), 4.38 (s, 1H), 4.13 (d, $J = 10.8$ Hz, 1H), 3.76 (d, ...
2.5 Experimental Details

\[ J = 10.8 \text{ Hz, 1H}, 3.67 \text{ (dd, } J = 3.6, 2 \text{ Hz, 1H}), 2.96 \text{ (s, 6H), 2.12 \text{ (s, 3H), 1.75 (m, 1H), 1.45 (m, 1H), 1.34 (d, } J = 6.4 \text{ Hz 3H), 1.22 (br s, 4H), 0.97 (s, 9H), 0.91 (s, 11H), 0.10 (s, 3H), 0.01 (s, 3H); MS (ESI\textsuperscript{+}) \text{ Calcd. for } C_{50}H_{82}N_{4}O_{7}Si_{2} + H, 979.58; \text{ Found, 979.58.} \]

**Benzyl ((1S,2S,3R,4S,5S)-2-((S)-1-(((tert-butyl(dimethyl)silyl)oxy)ethyl)-4-(((tert-butyl(diphenyl)silyl)oxy)methyl)-2-(3,3-dimethylureido)-3,4-dihydroxy-5-((4-methoxyphenyl)amino)cyclopentyl)carbamate (2.43):** Isolated from 2.38d via general procedure B using \( p \)-anisidine as the nucleophile in 83 % yield. Analytical data: \( ^1H \text{ NMR (400 MHz, CDCl}_3 \)): \( \delta \ 8.27 \text{ (d, } J = 6.0 \text{ Hz, 1H}); 7.74 \text{ (d, } J = 6.8 \text{ Hz, 2H}); 7.66 \text{ (d, } J = 6.8 \text{ Hz, 2H}); 7.40–7.26 \text{ (m, 12H)); 6.69 (d, } J = 8.8 \text{ Hz, 2H}); 6.60 \text{ (d, } J = 8.40 \text{ Hz, 2H}); 6.02 \text{ (s, 1H}); 5.33 \text{ (s, 1H); 5.31 (d, } J = 6.8 \text{ Hz, 1H); 5.02 (s, 2H); 4.67 (dd, } J = 6.4, 9.6 \text{ Hz, 1H); 4.38 (d, } J = 10.8 \text{ Hz, 1H); 3.92 (s, 1H); 3.83 (s, 1H); 3.74 (s, 3H); 3.64 (d, } J = 10.0 \text{ Hz, 1H); 3.59 (d, } J = 10.8 \text{ Hz, 1H); 2.93 (s, 6H)); 1.39 (d, } J = 6.0 \text{ Hz, 3H); 1.04 (s, 9H); 0.91 (s, 9H); 0.12 (s, 3H); 0.01 (s, 3H)); MS (ESI\textsuperscript{+}) \text{ Calcd. for } C_{48}H_{68}N_{4}O_{8}Si_{2} + H, 885.47; \text{ Found, 885.53.} \)

**Benzyl ((1S,2S,3R,4S,5S)-2-((S)-1-(((tert-butyl(dimethyl)silyl)oxy)ethyl)-4-(((tert-butyl(diphenyl)silyl)oxy)methyl)-2-(3,3-dimethylureido)-3,4-dihydroxy-5-((3-(prop-1-en-2-yl)phenyl)amino)cyclopentyl)carbamate (2.40d):** Isolated from 2.38d via general procedure B using \( m \)-isopropenylaniline as the nucleophile in 63 % yield. Analytical data: \( ^1H \text{ NMR (400 MHz, CDCl}_3 \)): \( \delta \ 8.28 \text{ (d, } J = 6.4 \text{ Hz, 1H}); 7.75 \text{ (d, } J = 6.8 \text{ Hz, 2H}); 7.60 \text{ (d, } J = 6.8 \text{ Hz, 2H}); 7.38 \text{ (d, } J = 7.2 \text{ Hz, 2H}); 7.33–7.25 \text{ (m, 8H)); 7.12 (t, } J = 7.6 \text{ Hz, 1H); 7.05 (t, } J = 7.6 \text{ Hz, 1H); 6.88 (d, } J = 7.6 \text{ Hz, 1H}); 6.84 (s, 1H); 6.80 (br s, 1H); 6.61 \text{ (d, } J = 8.0 \text{ Hz, 1H)); 6.58 (d, } J = 8.0 \text{ Hz, 1H)); 6.03 (s, 1H); 5.37–5.29 (m, 3H); 5.04–5.01 (m, 2H); 4.69 (dd, } J = 6.4,10.0 \text{ Hz, 1H); 4.40 (d, } J = 10.8 \text{ Hz, 1H); 3.93 (s, 1H); 3.84 (s, 1H); 3.74(d, } J = 8.0 \text{ Hz, 1H); 3.58 (d, } J = 10.8 \text{ Hz, 1H); 2.94 (s, 6H); 2.12 (s, 3H); 1.40 (d, } J = 6.4 \text{ Hz, 3H)); 1.02 (s, 9H); 0.91 (s, 9H); 0.13 (s, 3H); 0.00 (S, 3H); MS (ESI\textsuperscript{+}) \text{ Calcd. for } C_{50}H_{70}N_{4}O_{7}Si_{2} + H, 895.49; \text{ Found, 895.59.} \)
General procedure I for isopropenyl group oxidative cleavage:

Benzyl ((4S)-5-((3-acetylphenyl)amino)-2-((S)-1-((tert-butyldimethylsilyl)oxy)ethyl)-4-(((tert-butyldiphenylsilyl)oxy)methyl)-2-(3,3-dimethylureido)-3-ethyl-3,4-dihydroxycyclopentyl)carbamate (2.41a). A 20 mL scintillation vial was charged with olefin 2.40a (0.154 g, 0.17 mmol, 1.00 equiv) and THF (2 mL), Acetone (2 mL), and H₂O (0.4 mL) were added. The solution was cooled to 0 °C and NMO (0.101 g, 0.860 mmol, 5.00 equiv) was added followed by two flaks of OsO₄. The resulting mixture and stirred for 1 h at r.t. Saturated NaHSO₃ (aq) (3 mL) was added, and the mixture was stirred 30 min. The mixture was then extracted with EtOAc (3 × 30 mL) and the combined organic layers were washed with water (10 mL), brine (10 mL), dried with magnesium sulfate and concentrated in vacuo. The crude diol was judged clean by ¹H NMR spectroscopy and was submitted to the next step without further purification.

The crude diol was added to a 20 mL scintillation vial and dissolved in THF (3 mL) and water (3 mL). NaIO₄ (0.065 g, 0.30 mmol, 2.40 equiv) was added at rt and the reaction was allowed to stir for 3 h. The reaction mixture was partitioned between EtOAc (5 mL) and H₂O (5 mL), and the mixture was extracted with EtOAc (3 × 30 mL) and the combined organic layers were washed with water (10 mL), brine (10 mL), dried with magnesium sulfate and concentrated in vacuo. The crude product was purified via flash chromatography (90:10 to 80:20 hexanes:EtOAc) to give acetophenone 2.41a as a pale yellow foam (98 mg, 65 % over two steps)

Analytical data: ¹H NMR (400 MHz, CDCl₃): δ 8.18 (d, J = 6.8 Hz, 1H), 7.63 (d, J = 8.0 Hz, 2H), 7.44 (d, J = 6.8 Hz, 2H), 7.38 (d, J = 7.2 Hz, 1H), 7.33–7.12 (m, 13H), 6.81 (d, J = 8.8 Hz, 1H), 6.11 (s, 1H), 5.45 (s, 1H), 5.33 (d, J = 4.0 Hz, 1H), 5.26 (q, J = 6.8 Hz, 1H), 5.02 (d, J = 1.2 Hz, 2H), 4.72 (dd, J = 6.8, 2.8 Hz, 1H), 4.32 (s, 1H), 4.06 (d, J = 10.8 Hz, 1H), 6.68 (d, J = 10.8 Hz, 1H), 3.64 (d, J = 4.0 Hz, 1H), 2.96 (s, 6H), 2.50 (s, 3H), 2.09 (m, 1H), 2.05 (s, 3H), 1.85 (m, 1H), 1.34 (d, J = 6.8 Hz, 3H), 0.92 (br s, 11H), 0.89 (br s, 10H), 0.09 (s, 3H), 0.00 (s, 3H); MS (ESI⁺) Calcd. for C₅₁H₇₄N₄O₈Si₂ + H, 925.50; Found, 925.52.
Benzyl ((4S,5S)-5-((3-acetylphe nyl)amino)-2-((S)-1-((ter t-butyldimethylsilyl)oxy)ethyl)-4-(((tert-butyldiphenylsilyl)oxy)methyl)-2-(3,3-dimethylureido)-3-hexyl-3,4-dihydroxycyclopentyl)carbamate (2.41b): Isolated from 2.40b via general procedure I in 63 % yield. Analytical data: $^1$H NMR (400 MHz, CDCl$_3$): δ 8.17 (d, $J$ = 6.4 Hz, 1H), 7.62 (d, $J$ = 6.8 Hz, 2H), 7.43 (d, $J$ = 6.8 Hz, 2H), 7.38 (d, $J$ = 7.2 Hz, 1H), 7.33–7.12 (m, 13H), 6.80 (d, $J$ = 8.0 Hz, 1H), 6.13 (s, 1H), 5.47 (s, 1H), 5.31 (d, $J$ = 3.6 Hz, 1H), 5.26 (q, $J$ = 6.4 Hz, 1H), 5.02 (s, 1H), 4.34 (s, 1H), 4.07 (d, $J$ = 10.8 Hz, 1H), 3.69 (d, $J$ = 10.8 Hz, 1H), 3.65 (dd, $J$ = 4.0, 6.0 Hz, 1H), 2.96 (s, 6H), 2.50 (s, 3H), 1.74 (m, 1H), 1.42 (br s, 3H), 1.33 (d, $J$ = 6.4 Hz, 3H), 1.20 (br s, 4H), 0.95–0.86 (m, 24H), 0.09 (s, 3H), 0.00 (s, 3H); MS (ESI$^+$) Calcd. for C$_{55}$H$_{80}$N$_{4}$O$_{8}$Si$_{2}$ + H, 981.56; Found, 981.55.

Benzyl ((1S,2S,3R,4S,5S)-5-((3-acetylphe nyl)amino)-2-((S)-1-((tert-butyldimethylsilyl)oxy)ethyl)-4-(((tert-butyldiphenylsilyl)oxy)methyl)-2-(3,3-dimethylureido)-3,4-dihydroxycyclopentyl)carbamate (2.41d): Isolated from 2.40d via general procedure I in 65 % yield. Analytical data: $^1$H NMR (400 MHz, CDCl$_3$): δ 8.36 (br s, 1H); 7.72 (d, $J$ = 7.2 Hz, 2H); 7.56 (d, $J$ = 6.8 Hz, 2H); 7.38 (d, $J$ = 7.6 Hz, 1H); 7.33–7.20 (m, 12H); 5.99 (s, 1H); 5.46 (s, 1H); 5.39 (s, 1H); 4.99 (d, $J$ = 12.0 Hz, 1H); 4.69 (dd, $J$ = 6.4, 10.0 Hz, 1H); 4.34 (d, $J$ = 10.4 Hz, 1H), 3.94 (s, 1H); 3.86 (s, 1H); 3.74 (d, $J$ = 10.4 Hz, 1H); 3.47 (d, $J$ = 10.8 Hz, 1H); 2.94 (s, 6H); 2.50 (s, 3H); 1.86 (m, 2H), 1.4 (d, $J$ = 6.4 Hz, 3H); 1.00 (s, 9H); 0.93 (s, 9H); 0.13 (s, 3H); 0.00 (s, 3H); MS (ESI$^+$) Calcd. For C$_{49}$H$_{68}$N$_{4}$O$_{8}$Si$_{2}$ + H, 897.47; Found, 897.56.

Benzyl ((4S)-5-((3-acetylphe nyl)amino)-2-((S)-1-hydroxyethyl)-3,4-dihydroxy-2-((S)-1-hydroxyethyl)-4-(hydroxymethyl)cyclopentyl)carbamate (S6a): Isolated from 2.41a via general procedure C in 88 % yield. Analytical data: $^1$H NMR (400 MHz, CDCl$_3$): δ 7.33–7.15 (m, 5H), 6.96 (br s, 1H), 6.78 (d, $J$ = 7.2 Hz, 2H), 6.56 (br s, 2H), 6.06 (br s, 1H), 5.58 (s, 1H), 5.47 (b s, 1H), 5.37–5.25 (m, 3H), 5.11 (br s, 2H), 4.33 (br s, 1H), 4.07 (b s, 1H), 3.85 (s, 2H), 3.71 (s, 1H), 2.87 (s, 6H), 2.50 (s, 3H), 1.86 (m, 2H), 1.25 (br s, 5H); MS (ESI$^+$) Calcd. for C$_{29}$H$_{40}$N$_{4}$O$_{8}$ + H, 573.29; Found, 573.27.
Benzyl ((4S,5S)-5-((3-acetylphenyl)amino)-2-(3,3-dimethylureido)-3-hexyl-3,4-dihydroxy-2-((S)-1-hydroxyethyl)-4-(hydroxymethyl)cyclopentyl)carbamate (S6b): Isolated from 2.41b via general procedure C in 78 % yield. Analytical data: \(^1\text{HNMR}\) (400 MHz, CDCl\(_3\)): \(\delta\) 7.32–7.05 (m, 10H), 6.94 (br s, 1H), 6.77 (br s, 2H), 6.14 (d, \(J = 7.6\) Hz, 1H), 5.43 (br s, 1H), 5.25 (s, 1H), 5.10 (br s, 2H), 4.23 (br s, 1H), 4.10 (br s, 1H), 3.86–3.73 (m, 3H), 2.87 (s, 6H), 2.49 (s, 3H), 1.93 (br s, 2H), 1.56 (t, \(J = 12.0\) Hz, 1H), 1.50 (br s, 1H), 1.26 (br s, 9H), 1.00 (br s, 1H); \textbf{MS (ESI\(^+\))} Calcd. for C\(_{32}\)H\(_{48}\)N\(_4\)O\(_8\) + H, 629.36; Found, 629.36.

Benzyl ((1S,2R,3R,4S,5S)-2-(3,3-dimethylureido)-3,4-dihydroxy-2-((S)-1-hydroxyethyl)-4-(hydroxymethyl)-5-((4-methoxyphenyl)amino)cyclopentyl)carbamate (S8): Isolated from 2.43 via general procedure C in 73 % yield. Analytical data: \(^1\text{HNMR}\) (400 MHz, CDCl\(_3\)): \(\delta\) 7.33–7.26 (m, 8H); 6.72 (d, \(J = 8.4\) Hz, 2H); 6.64 (br s, 2H); 6.00 (br s, 1H); 5.72 (br s, 1H); 5.61 (br s, 1H); 5.10 (d, \(J = 11.2\) Hz, 1H); 5.04 (d, \(J = 12.0\) Hz, 1H); 4.40 (s, 1H); 4.04–3.97 (m, 3H); 3.80 (d, \(J = 11.8\) Hz, 1H); 3.73 (s, 3H); 3.59 (br s, 1H); 2.90 (s, 6H); 1.19 (d, \(J = 4.4\) Hz, 3H); \textbf{MS (ESI\(^+\))} Calcd. For C\(_{26}\)H\(_{36}\)N\(_4\)O\(_8\) + H, 533.26; Found, 533.26.

Benzyl ((1S,2R,3R,4S,5S)-5-((3-acetylphenyl)amino)-2-(3,3-dimethylureido)-3,4-dihydroxy-2-((S)-1-hydroxyethyl)-4-(hydroxymethyl)cyclopentyl)carbamate (S6d): Isolated from 2.41d via general procedure C in 91 % yield. Analytical data: \(^1\text{HNMR}\) (400 MHz, CDCl\(_3\)): \(\delta\) 7.29–7.22 (m, 7H); 7.15 (t, \(J = 8.0\) Hz, 1H); 6.84 (d, \(J = 6.4\) Hz, 1H); 6.32 (d, \(J = 8.4\) Hz, 1H); 5.85 (s, 1H); 5.60 (br s, 1H); 5.04 (d, \(J = 12.0\) Hz, 1H); 4.97 (d, \(J = 12.4\) Hz, 1H); 4.78 (br s, 1H); 4.43 (s, 1H); 4.22–4.15 (m, 2H); 4.03–3.97 (m, 2H); 3.73 (d, \(J = 11.6\) Hz, 1H); 2.87 (s, 6H);
2.47 (s, 3H); 1.16 (d, \( J = 5.2 \) Hz, 3H); \textbf{MS (ESI\(^+\)} \) Calcd. For \( \text{C}_{27}\text{H}_{36}\text{N}_4\text{O}_8 + \text{H} \), 545.26; Found, 545.31.

\[
\begin{align*}
\text{((1S,5S)-5-((3-acetylphenyl)amino)-4-((benzyloxy)carbonyl)amino)-3-(3,3-dimethylureido)-2-ethyl-1,2-dihydroxy-3-((S)-1-hydroxyethyl)cyclopentyl) methyl 2-hydroxy-6-methylbenzoate (S7a)} \text{: Isolated from S6a via general procedure D in 80 \% yield. Analytical data: } & \text{\textbf{^{1}H-NMR} (400 MHz, CDCl}_3\text{)}: \delta 10.89 (s, 1H), 7.49 (d, \( J = 8.4 \) Hz, 1H), 7.35 (br s, 4H), 7.27–7.20 (m, 4H), 7.05 (s, 1H), 6.79 (d, \( J = 8.0 \) Hz, 1H), 6.68 (d, \( J = 6.8 \) Hz, 1H), 6.58 (d, \( J = 7.2 \) Hz, 1H), 5.88 (s, 1H), 5.83 (m, 1H), 5.16 (s, 2H), 5.107–5.063 (m, 2H), 4.88 (d, \( J = 12.8 \) Hz, 1H), 4.04 (br s, 2H), 3.71 (d, \( J = 9.2 \) Hz, 1H), 2.84 (s, 6H), 2.49 (s, 3H), 2.19 (s, 3H), 2.12 (m, 1H), 1.97 (m, 1H), 1.24 (d, \( J = 7.2 \) Hz, 3H), 1.04 (t, \( J = 7.6 \) Hz, 3H); \textbf{MS (ESI\(^+\))} \text{ Calcd. for } \text{C}_{37}\text{H}_{46}\text{N}_4\text{O}_{10} + \text{H}, 707.33; \text{ Found, 707.36.}
\end{align*}
\]

\[
\begin{align*}
\text{((1S,5S)-5-((3-acetylphenyl)amino)-4-((benzyloxy)carbonyl)amino)-3-(3,3-dimethylureido)-2-hexyl-1,2-dihydroxy-3-((S)-1-hydroxyethyl)cyclopentyl) methyl 2-hydroxy-6-methylbenzoate (S7b)} \text{: Isolated from S6b via general procedure D in 84 \% yield. Analytical data: } & \text{\textbf{^{1}H-NMR} (400 MHz, CDCl}_3\text{)}: \delta 10.90 (s, 1H), 7.44–7.18 (m, 9H), 7.06 (s, 1H), 6.80 (d, \( J = 8.4 \) Hz, 1H), 6.69, (d, \( J = 7.6 \) Hz, 1H), 6.59 (d, \( J = 7.2 \) Hz, 1H), 5.90 (s, 2H), 5.78 (d, \( J = 9.6 \) Hz, 1H), 5.16–5.04 (m, 4H), 4.87 (d, \( J = 12.8 \) Hz, 1H), 4.03 (s, 2H), 2.85 (s, 6H), 2.49 (s, 3H), 2.20 (s, 3H), 1.83 (m, 1H), 1.61 (m, 1H), 1.28 (br s, 12H); \textbf{MS (ESI\(^+\))} \text{ Calcd. for } \text{C}_{41}\text{H}_{54}\text{N}_4\text{O}_{10} + \text{H}, 763.39; \text{ Found, 763.46.}
\end{align*}
\]

\[
\begin{align*}
\text{((1S,2R,3R,4S,5S)-4-((benzyloxy)carbonyl)amino)-3-(3,3-dimethylureido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-5-((4-methoxyphenyl)amino)cyclopentyl) methyl 2-hydroxy-6-methylbenzoate (S9)} \text{: Isolated from S8 via general}
\end{align*}
\]
procedure D in 56 % yield. Analytical data: $^1$H NMR (400 MHz, CDCl$_3$): δ 10.78 (br s, 1H); 7.34–7.24 (m, 5H); 7.17 (d, $J$ = 7.6 Hz, 1H); 6.83 (d, $J$ = 8.4 Hz, 2H); 6.75–6.69 (m, 4H); 6.53 (br s, 2H); 5.88 (br s, 2H); 5.50 (s, 1H); 5.15–5.09 (m, 3H); 4.93 (d, $J$ = 12.0 Hz, 1H); 4.75 (d, $J$ = 12.4 Hz, 1H); 4.31 (s, 1H); 4.09 (br s, 1H); 3.88 (br s, 1H); 3.74 (s, 3H); 2.96 (s, 1H); 2.86 (s, 6H); 2.47 (s, 3H); 1.21 (br s, 1H); MS (ESI$^+$) Calcd. For C$_{34}$H$_{42}$N$_4$O$_{10}$ + H, 667.30; Found, 667.31.

|((1S,2R,3R,4S,5S)-5-((3-acetylphenyl)amino)-4-(((benzyloxy)carbonyl)amino)-3-(3,3-dimethylureido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)cyclopentyl)methyl 2-hydroxy-6-methylbenzoate (S7d): Isolated from S6d via general procedure D in 66 % yield. Analytical data: $^1$H NMR (400 MHz, CDCl$_3$): δ 10.61 (br s, 1H); 7.29 (m, 10H); 6.77 (d, $J$ = 8.4 Hz, 2H); 6.64 (d, $J$ = 7.2 Hz, 1H); 6.37 (br s, 1H); 6.21 (br s, 1H); 5.64 (br s, 2H); 5.19–4.09 (m, 3H); 4.86 (d, $J$ = 12.0 Hz, 1H); 4.67 (d, $J$ = 12.0 Hz, 1H); 4.41 (s, 1H); 4.13–4.08 (m, 1H); 3.86 (br s, 1H); 2.85 (s, 6H); 2.46 (s, 3H); 1.18 (br s, 3H); MS (ESI$^+$) Calcd. For C$_{35}$H$_{42}$N$_4$O$_{10}$ + H, 679.30; Found, 679.35.

|((1S,5S)-5-((3-acetylphenyl)amino)-4-amino-3-(3,3-dimethylureido)-2-ethyl-1,2-dihydroxy-3-((S)-1-hydroxyethyl)cyclopentyl)methyl 2-hydroxy-6-methyl benzoate (2.42a): Isolated from S7a via general procedure E in 48 % yield. Analytical data: [α]$_D^{19}$ +39.3 (c = 0.25, CHCl$_3$); $^1$HNMR (400 MHz, CDCl$_3$): δ 10.99 (s, 1H), 7.84 (d, $J$ = 10.8 Hz, 1H), 7.25–7.21 (m, 5H), 7.11 (s, 1H), 6.79 (d, $J$ = 8.0 Hz, 1H), 6.75 (m, 1H), 6.60 (d, $J$ = 7.6 Hz, 1H), 5.75 (d, $J$ = 10.0 Hz, 1H), 5.63 (s, 1H), 4.99 (d, $J$ = 12.8 Hz, 1H), 4.88 (d, $J$ = 12.4 Hz, 1H), 3.98 (m, 1H), 3.76 (d, $J$ = 10.4 Hz, 1H), 2.99 (s, 6H), 2.91 (s, 1H), 2.53(s, 3H), 2.29 (s, 3H), 2.16 (m, 1H), 2.05 (m, 1H), 1.05 (m, 6H). $^{13}$C NMR (150 MHz, CDCl$_3$): δ 198.5, 173.0, 162.8, 159.2, 146.5, 141.5, 138.2, 134.6, 129.6, 129.0, 128.2, 125.3, 123.0, 118.2, 115.6, 111.9, 90.4, 86.0, 73.8, 72.8, 68.4, 66.8, 62.2, 36.90, 26.7, 25.8, 23.8, 18.1, 8.6; IR (thin film, cm$^{-1}$) 3381, 2926, 1724, 1667, 1604, 1522, 1485, 1464, 1389, 1253, 1115, 1074, 735; MS (ESI$^+$) Calcd. for C$_{29}$H$_{40}$N$_4$O$_8$ + H, 573.29; Found, 573.33; TLC (95:5 CH$_2$Cl$_2$:MeOH): $R_f$ = 0.18.
((1S,5S)-5-((3-acetylphenyl)amino)-4-amino-3-(3,3-dimethylureido)-2-hexyl-1,2-dihydroxy-3-((S)-1-hydroxyethyl)cyclopentyl)methyl 2-hydroxy-6-methylbenzoate (2.42b): Isolated from S7b via general procedure E in 61 % yield. Analytical data: \([\alpha]_D^{19}+28.8 \ (c = 0.15, \text{CHCl}_3); \ \ ^1\text{HNMR} \ (400 \text{ MHz, CDCl}_3): \ \delta \ 10.98 \ (\text{br s, 1H}), 7.85 \ (\text{br s, 1H}), 7.25–7.11 \ (\text{m, 5H}), 6.79 \ (d, J = 8.4 \text{ Hz, 1H}), 6.75 \ (m, 1H), 6.60 \ (d, J = 7.2 \text{ Hz, 1H}), 5.73 \ (d, J = 10.0 \text{ Hz, 1H}), 5.67 \ (s, 1H), 4.94 \ (d, J = 12.4 \text{ Hz, 1H}), 4.86 \ (d, J = 12.4 \text{ Hz, 1H}), 3.98 \ (\text{br s, 1H}), 3.76 \ (d, J = 10.0 \text{ Hz, 1H}), 3.64 \ (\text{br s, 1H}), 2.99 \ (s, 6H), 2.95 \ (s, 1H), 2.53 \ (s, 3H), 2.29 \ (s, 3H), 2.07 \ (m, 1H), 1.93 \ (\text{m, 2H}), 1.62 \ (\text{m, 2H}), 1.31 \ (\text{m, 2H}), 1.04 \ (d, J = 6.0 \text{ Hz, 3H}), \ \ ^{13}\text{C NMR} \ (150 \text{ MHz, CDCl}_3): \ \delta \ 198.59, 172.88, 162.73, 159.17, 146.49, 141.47, 138.15, 134.52, 129.58, 122.98, 118.46, 118.20, 115.61, 111.96, 110.52, 90.22, 86.00, 73.67, 72.82, 68.23, 66.68, 62.31, 36.93, 33.40, 31.84, 30.51, 29.68, 26.68, 24.05, 23.84, 22.66, 18.13, 14.15; \ \text{IR} \ (\text{thin film, cm}^{-1}) \ 3413, 2928, 2359, 1653, 1509, 1438, 1378, 1252, 1213, 1095, 736; \ \text{MS (ESI$^+$)} \ \text{Calcd. for} \ C_{33}H_{48}N_4O_8 + \text{H, 629.36; Found, 629.42; TLC (95:5 CH}_2\text{Cl}_2:\text{MeOH):} \ R_f = 0.21.

((1S,2R,3R,4S,5S)-4-amino-3-(3,3-dimethylureido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-5-((4-methoxyphenyl)amino)cyclopentyl)methyl 2-hydroxy-6-methylbenzoate (2.44): Isolated from S9 via general procedure E in 67 % yield. Analytical data: \([\alpha]_D^{19}+4.4 \ (c = 0.25, \text{CHCl}_3); \ \ ^1\text{HNMR} \ (400 \text{ MHz, CDCl}_3): \ \delta \ 10.92 \ (\text{br s, 1H}), 7.46 \ (\text{br s, 1H}), 7.29–7.11 \ (\text{m, 5H}), 6.99 \ (\text{br s, 1H}), 6.82 \ (d, J = 8.0 \text{ Hz, 1H}), 6.79 \ (d, J = 8.8 \text{ Hz, 2H}), 6.70 \ (d, J = 7.2 \text{ Hz, 1H}), 6.60 \ (d, J = 8.8 \text{ Hz, 2H}), 5.52 \ (\text{br s, 1H}), 4.87 \ (d, J = 12.4 \text{ Hz, 1H}), 4.75 \ (d, J = 12.0 \text{ Hz, 1H}), 4.70 \ (\text{br s, 1H}), 4.32 \ (\text{s, 1H}), 3.84 \ (\text{br s, 1H}), 3.74 \ (\text{s, 3H}), 3.13 \ (\text{br s, 1H}), 2.96 \ (\text{m, 6H}), 2.51 \ (\text{s, 3H}), 1.06 \ (d, J = 6.4 \text{ Hz, 3H}), \ \ ^{13}\text{C NMR} \ (150 \text{ MHz, CDCl}_3): \ \delta \ 171.5, 158.6, 158.4, 141.1, 140.7, 134.2, 122.9, 115.6, 115.0, 114.5, 64.4, 55.7, 50.9, 36.7, 31.9, 29.7, 29.6, 24.8, 23.6, 22.7, 17.7, 14.1; \ \text{IR} \ (\text{thin film, cm}^{-1}) \ 3939, 3389, 3054, 2931, 2359, 2054, 1640, 1512, 1442, 1382, 1265, 1119, 736; \ \text{MS (ESI$^+$)} \ \text{Calcd. for} \ C_{26}H_{36}N_4O_8 + \text{H, 533.26; Found, 533.26; TLC (95:5 CH}_2\text{Cl}_2:\text{MeOH):} \ R_f = 0.05.
((1S,2R,3R,4S,5S)-5-((3-acetylphenyl)amino)-4-amino-3-(3,3-dimethylureido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)cyclopentyl)methyl 2-hydroxy-6-methylbenzoate (2.42d): Isolated from S7d via general procedure E in 72 % yield. Analytical data: [α]_D^19 +39.3 (c = 0.25, CHCl_3); ^1H NMR (400 MHz, CDCl_3): δ 10.86 (br s, 1H); 7.55 (br s, 1H); 7.52–7.54 (m, 4H); 7.20 (s, 1H); 7.10 (s, 1H); 6.83–6.79 (m, 2H); 6.67 (d, J = 7.6 Hz, 1H); 5.61 (br s, 1H); 5.11 (s, 1H); 4.84 (d, J = 12.4 Hz, 1H); 4.76 (d, J = 12.4 Hz, 1H); 4.81 (s, 1H); 3.85 (d, J = 10.4 Hz, 1H); 3.80 (br s, 1H); 3.48 (s, 1H); 3.07 (s, 6H); 2.55 (s, 3H); 2.47 (s, 3H); 1.04 (d, J = 6.4 Hz, 3H); ^13C NMR (150 MHz, CDCl_3): δ 198.6, 171.7, 162.3, 159.0, 146.6, 141.2, 138.2, 134.3, 129.7, 123.0, 118.7, 118.6, 115.6, 112.5, 111.0, 83.9, 82.9, 74.1, 69.7, 68.5, 65.9, 63.4, 36.6, 29.7, 26.7, 17.9; IR (thin film, cm⁻¹) 3381, 2926, 1724, 1667, 1604, 1522, 1485, 1464, 1389, 1253, 1115, 1074, 735; MS (ESI⁺) Calcd. For C_{27}H_{36}N_{4}O_{8} + H, 545.26; Found, 545.32; TLC (90:10 CH_2Cl_2:MeOH): R_f =0.90.

C6 Hydroxymethylene Derivatives

5-methoxy-2,2-dimethyl-4H-benzo[d][1,3]dioxin-4-one (2.49): A flame dried 250-mL round bottomed flask was charged with 2.45 (0.50 g, 2.57 mmol, 1.00 equiv), acetone (70 mL) and anhydrous K₂CO₃ (0.53 g, 3.85 mmol, 1.50 equiv). The mixture was cooled to 0 °C and MeI (0.239 mL, 3.85 mmol, 1.50 equiv) was added slowly, and then warmed slowly to rt. After 1 h the reaction was poured into H₂O (20 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (15 mL), dried with magnesium sulfate, and concentrated in vacuo. The crude product was purified via flash chromatography (60:40 hexanes:EtOAc), affording ether 2.49 (400 mg, 74 % yield) as a pale yellow solid. Analytical Data: ^1H NMR (400 MHz, CDCl_3): δ 7.45 (t, J = 8.4 Hz, 1H); 6.63 (d, J = 8.4 Hz, 1H); 6.56 (d, J = 9.6 Hz, 1H); 3.96 (s, 3H); 3.56 (s, 3H); 1.70 (s, 6H).

cyanomethyl 2-hydroxy-6-methoxybenzoate (2.50): Methyl ether 2.49 (0.40 g, 1.90 mmol, 1.00 equiv) was dissolved in THF (2 mL) and a solution of KOH (0.53 g, 9.50 mmol, 5.00 equiv) in 2 mL of H₂O was added. The mixture was
refluxed overnight, subsequently cooled to rt, acidified to pH 1 with 6 M HCl (aq.) (10 mL), and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (10 mL), dried with magnesium sulfate, and concentrated in vacuo, to afford the crude acid, which was carried on to the next step without further purification.

The crude product was dissolved distilled acetone (7 mL). Et₃N (379 µL, 2.85 mmol, 1.50 equiv) and chloroacetonitrile (181 µL, 2.85 mmol, 1.50 equiv) were added to the solution and the mixture was refluxed for 3 h. The solvent was removed via rotary evaporator and pH 4 buffer (10 mL) was added. The aqueous layer was extracted with EtOAc (3 × 15 mL). The organic layers were combined, washed with brine (10 mL), dried with magnesium sulfate, and concentrated in vacuo. The crude product was purified with flash chromatography (80:20 Hexane: EtOAc) to afford ester **2.50** as a pale yellow solid (113 mg, 29 % yield). Analytical Data: **1H NMR** (400 MHz, CDCl₃): δ 10.86 (s, 1H); 3.40 (t, J = 8.4 Hz, 1H); 6.62 (d, J = 9.2 Hz, 1H); 6.44 (d, J = 8.4 Hz, 1H); 4.96 (s, 2H); 3.87 (s, 3H).

**2,2-Dimethyl-5-phenyl-4H-benzo[d][1,3]dioxin-4-one (2.47):** A 100-mL round-bottomed flask was charged with **2.46** (0.948 g, 2.91 mmol, 1.00 equiv), phenyl boronic acid (0.531 g, 4.36 mmol, 1.5 equiv), KBr (0.346 g, 2.91 mmol, 1equiv), K₃PO₄ (0.928 g, 4.36 mmol, 1.50 equiv) and dioxane (12 mL). Pd(PPh₃)₄ (0.169 g, 0.15 mmol, 0.05 equiv) was added as a suspension in dioxane (3 mL) and the mixture was stirred at 100 °C for 12 h. The mixture was cooled to room temperature, diluted with H₂O (10 mL), and the aqueous layer was extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with water (20 mL), brine (20 mL), dried with magnesium sulfate, and concentrated in vacuo. The product was purified via flash chromatography (60:40 hexanes:EtOAc), affording the desired product **2.47** (413 mg, 55 %) as a pale yellow solid. Analytical Data: **1H NMR** (400 MHz, CDCl₃): δ 7.52 (t, J = 8.0 Hz, 1H); 7.42–7.37 (m, 3H); 7.34–7.32 (m, 2H); 7.01 (d, J = 6.4 Hz, 1H); 6.98 (d, J = 7.2, 1H); 1.79 (s, 6H). **MS (ESI⁺)** Calcd. For C₁₆H₁₄O₃ + Na, 277.08; Found, 277.11.

**Cyanomethyl 3-hydroxybiphenyl-2-carboxylate (2.48):** **2.47** (0.40 g, 1.90 mmol, 1.00 equiv) was dissolved in THF (2 mL), and a solution of KOH (0.53 g, 9.50 mmol, 5.00 equiv) in 2 mL H₂O was added. The mixture was heated at reflux for 12 h. The resulting mixture was cooled to room temperature and acidified with 6 M HCl (aq.) (10 mL) and extracted with EtOAc (3 × 10 mL). The
combined organic layers were washed with brine, dried with magnesium sulfate and concentrated in vacuo to afford crude the crude acid, which was used in the next step without further purification.

The crude acid was dissolved in acetone (7 mL), and triethylamine (379 µL, 2.85 mmol, 1.50 equiv) and chloroacetonitrile (181 µL, 2.85 mmol, 1.50 equiv) were added to the solution. The mixture was refluxed for 3 h, upon which the solvent was removed in vacuo. A pH = 4 buffer solution (10 mL) was then added to the residue, and the mixture was extracted with EtOAc (3 × 15 mL). The organic layers were combined, washed with brine, dried with magnesium sulfate, and concentrated in vacuo. The product was purified via flash chromatography (80:20 hexanes:EtOAc) to afford ester 2.48 as a pale yellow solid (113 mg, 29 %).

1H NMR (400 MHz, CDCl3): δ 10.11 (br s, 1H); 7.50–7.40 (m, 1H); 7.39–7.37 (m, 3H); 7.36–7.23 (m, 2H); 7.05 (d, J = 9.6 Hz, 1H); 6.86 (d, J = 8.4 Hz, 1H); 4.52 (s, 2H). MS (ESI+). Calcd. For C15H11NO3 + Na, 276.06; Found, 276.49.

((1S,2R,3R,4S,5S)-5-((3-acetylphenyl)amino)-4-(((benzyloxy)carbonyl)amino)-3-(3,3-dimethylureido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-2-methylcyclopentyl)methyl 2-hydroxy-6-methoxybenzoate (S10a): Isolated from 2.26 via general procedure D using cyanomethyl ester 2.50 as the electrophile in 57 % yield. Analytical data: 1H NMR (400 MHz, CDCl3): δ 11.08 (s, 1H); 7.37–7.20 (m, 8H); 7.12 (s, 1H); 6.73 (d, J = 6.4 Hz, 1H); 6.59 (d, J = 6.0 Hz, 1H); 6.39 (d, J = 8.4 Hz, 1H); 6.04 (s, 1H); 5.89 (br s, 1H); 5.59 (d, J = 7.9 Hz, 1H); 5.27 (s, 1H); 5.20 (d, J = 13.5 Hz, 1H); 5.14 (d, J = 11.8 Hz, 1H); 4.72 (d, J = 12.0 Hz, 1H); 4.66 (d, J = 11.6 Hz, 1H); 4.07 (br s, 1H); 3.84 (d, J = 9.6 Hz, 1H); 3.79 (s, 1H); 3.75 (s, 3H); 2.88 (s, 6H); 2.50 (s, 3H); 1.48 (s, 3H); 1.23 (br s, 3H); MS (ESI+) Calcd. For C36H44N4O11 + H, 709.31; Found, 709.15.

((1S,2R,3R,4S,5S)-5-((3-acetylphenyl)amino)-4-(((benzyloxy)carbonyl)amino)-3-(3,3-dimethylureido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-2-methylcyclopentyl)methyl 3-hydroxy-[1,1′-biphenyl]-2-carboxylate (S10b): Isolated from 2.26 via general procedure D using S17 as the electrophile in 43 % yield. Analytical data: 1H NMR (400 MHz, CDCl3): δ 11.04 (s, 1H); 7.51–7.46 (m, 6H); 7.40–7.32 (m, 3H); 7.26–7.23 (m, 4H); 7.19–7.17 (m, 1H); 7.04 (s, 1H); 6.98 (d, J = 8.0 Hz, 1H); 6.61 (t, J = 8.4 Hz, 2H); 5.86 (s, 1H); 5.38–5.34 (m, 1H); 5.30 (s, 1H); 5.20 (d, J = 11.2 Hz,
1H); 5.05 (d, J = 8.0 Hz, 1H); 5.00 (s, 1H); 4.56 (d, J = 11.6 Hz, 1H); 4.08 (d, J = 12.0 Hz, 1H); 3.91 (br s, 1H); 3.82 (d, J = 8.4 Hz, 1H); 2.91 (d, J = 10.4 Hz, 1H); 2.85 (s, 6H); 2.25 (s, 3H); 1.261.22 (m, 6H); MS (ESI⁺) Calcd. For C₄₁H₄₆N₄O₁₀ + H, 755.33; Found, 755.19.

((1S,2R,3R,4S,5S)-5-(3-acetylphenylamino)-4-(benzyloxycarbamylamino)-3-(3,3-dimethylureido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-2-methylcyclopentyl)methyl 2-methylbenzoate (S10c): A flame-dried 20-mL scintillation vial was charged with tetraol 2.26 (0.041 g, 0.073 mmol, 1.00 equiv) and CH₂Cl₂ (3.3 mL) was added under an atmosphere of N₂. The solution was cooled to −78 °C and 2,4,6-collidine (0.02 mL, 0.47 mmol, 2.00 equiv) and DMAP (0.001 g, 0.01 mmol, 0.10 equiv) were added sequentially. The mixture was stirred for 30 min, and 2-methylbenzoyl chloride was added. The reaction was allowed to stir for 1 h, warmed to room temperature and stirred until TLC analysis indicated full conversion of the starting material, generally 8 h. A 1:1 mixture of saturated NH₄Cl(aq.):1M HCl (aq.) (3 mL) was added, followed by EtOAc (4 mL). The mixture was partitioned in a separatory funnel, and the aqueous layer was extracted with EtOAc (3 × 6 mL), and the combined organic layers were washed with saturated NaHCO₃(aq.) (5 mL), water (5 mL), brine (5 mL) and dried with magnesium sulfate. The crude product was concentrated in vacuo and purified via flash chromatography (60:40 to 50:50 hexanes:EtOAc) to give the ester S10c (0.03 g, 60 %) as a pale yellow foam. Analytical Data: ¹H NMR (400 MHz, CDCl₃): δ 7.31 (d, J = 7.6 Hz, 1H); 7.51 (br s, 1H); 7.38–7.12 (m, 11H); 6.74 (d, J = 7.6 Hz, 1H); 6.07 (s, 1H); 5.87 (d, J = 9.2 Hz, 1H); 5.74 (d, J = 10.0 Hz, 1H); 5.20 (s, 1H); 5.16 (d, J = 12.4 Hz, 1H); 5.10 (d, J = 12.0 Hz, 1H); 4.8 (d, J = 12.8 Hz, 1H); 4.73 (d, J = 12.4 Hz, 1H); 4.12–4.05 (m, 2H); 3.97 (s, 1H); 3.81 (d, J = 9.6 Hz, 1H); 2.85 (s, 6H); 2.50 (s, 3H); 2.49 (s, 3H); 1.51 (s, 3H); 1.25 (br s, 3H); MS (ESI⁺) Calcd. For C₃₆H₄₄N₄O₉ + H, 677.32; Found, 677.25.

**General Procedure J for primary alcohol esterification using aliphatic electrophiles.**
((1S,2R,3R,4S,5S)-5-((3-acetylphenyl)amino)-4-(((benzyloxy)carbonyl)amino)-3-(3,3-dimethylureido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-2-methylcyclopentyl)methyl 2-phenylacetate (S10e): A flame-dried scintillation vial was charged with tetraol 2.26 (0.032 g, 0.056 mmol, 1.00 eq.) and CH₂Cl₂ (2.5 mL) under an atmosphere of N₂. The solution was cooled to −78 °C and 2,4,6-collidine (15 µL, 0.112 mmol, 2.00 eq.) was added. The resulting mixture was stirred for 30 min, and phenylacetyl chloride was added. The reaction was allowed to stir at this temperature until TLC analysis indicated full conversion of the starting material, typically 1 h. A mixture of saturated NH₄Cl(aq.):1 M HCl (aq.) (1:1) (3 mL) was added followed by EtOAc (4 mL) and the reaction was allowed to warm to rt. The mixture was partitioned in a separatory funnel and the aqueous layer was extracted with EtOAc (3 × 6 mL). The combined organic layers were washed with saturated NaHCO₃(aq.) (5 mL), water (5 mL), brine (5 mL), dried with magnesium sulfate, and concentrated in vacuo. The crude product was purified via flash chromatography (60:40 to 50:50 hexanes:EtOAc) to give the title compound S10e as a pale yellow foam (0.033 g mg, 87 %). Analytical data: ¹H NMR (400 MHz, CDCl₃): δ 7.39–7.34 (m, 5H); 7.32 (d, J = 7.6 Hz, 1H); 7.26–7.23 (m, 5H); 7.17 (d, J = 6.4 Hz, 2H); 6.67 (d, J = 7.6 Hz, 1H); 5.98 (s, 1H); 5.65 (d, J = 9.2 Hz, 1H); 5.59 (d, J = 10.4 Hz, 1H); 5.15–5.13 (m, 1H); 4.53 (s, 1H); 4.01 (br s, 1H); 3.96 (d, J = 8.4 Hz, 1H); 3.59 (s, 2H); 3.55 (d, J = 9.6 Hz, 1H); 3.21 (s, 1H); 2.84 (s, 6H); 2.54 (s, 3H); 1.37 (s, 3H); 1.20 (d, J = 7.2 Hz, 3H); MS (ESI⁺) Calcd. For C₃₆H₄₄N₄O₉ + H, 677.32; Found, 677.31.

((1S,2R,3R,4S,5S)-5-((3-acetylphenyl)amino)-4-(((benzyloxy)carbonyl)amino)-3-(3,3-dimethylureido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-2-methylcyclohexyl)methyl cyclohexanecarboxylate (S10d): Isolated from 2.26 via general procedure J using cyclohexoyl chloride as the electrophile in 83 % yield. Analytical data: ¹H NMR (400 MHz, CDCl₃): δ 7.37 (br s, 1H); 7.31–7.23 (m, 6H); 7.10 (s, 1H); 6.71 (d, J = 6.4 Hz, 1H); 6.02 (d, J = 8.0 Hz, 1H); 5.64 (d, J = 10.0 Hz, 1H); 5.16–5.10 (m, 3H); 4.52 (d, J = 12.0 Hz, 1H); 4.51 (d, J = 12.4 Hz, 1H); 4.02 (br s, 1H); 3.72–3.68 (m, 2H); 2.85 (s, 6H); 2.54 (s, 3H); 2.29–2.35 (m, 1H); 1.83–1.74 (m, 2H); 1.67–1.63 (m, 4H); 1.44 (s, 3H); 1.36–1.27 (m, 2H); 1.25–1.18 (m, 5H); MS (ESI⁺) Calcd. For C₃₅H₄₈N₄O₉ + H, 669.35; Found, 669.35.
((1S,2R,3R,4S,5S)-5-((3-acetylphenyl)amino)-4-(((benzyloxy)carbonyl)amino)-3-(3,3-dimethylureido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-2-methylcyclopentyl)methyl methanesulfonate (S10f): Isolated from 2.26 via general procedure J using cyclohexoyl chloride as the electrophile in 54 % yield. Analytical data: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.34–7.24 (m, 7H); 7.14 (s, 1H); 6.75 (d, $J = 7.6$ Hz; 1H); 5.96 (d, $J = 9.6$ Hz, 1H); 5.55 (bs, 1H); 5.25 (s, 1H); 5.12 (bs, 2H); 4.65 (d, $J = 11.2$ Hz, 1H); 4.53 (d, $J = 11.2$ Hz, 1H); 4.09 (bs, 1H); 3.85 (bs, 1H); 2.90 (s, 3H); 2.85 (s, 6H); 2.53 (s, 3H); 1.46 (s, 3H); 1.25 (bs, 3H); MS (ESI$^+$) Calcd. For C$_{29}$H$_{40}$N$_4$O$_{10}$S + H, 637.25; Found, 669.14.

((1S,2R,3R,4S,5S)-5-((3-acetylphenyl)amino)-4-amino-3-(3,3-dimethylureido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-2-methylcyclopentyl)methyl 3-hydroxy-[1,1′-biphenyl]-2-carboxylate (2.51b): Isolated from S10b via general procedure E in 62 % yield. Analytical data: $[\alpha]_D^{19}$ +8.4 (c = 0.3, CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 11.05 (br s, 1H); 7.85 (d, $J = 10.8$ Hz, 1H); 7.57 (t, $J = 7.4$ Hz, 2H); 7.44–7.33 (m, 5H); 7.18–7.15 (m, 2H); 7.10 (s, 1H); 6.97 (d, $J = 8.4$ Hz, 1H); 6.67 (d, $J = 7.2$ Hz, 1H); 6.62 (d, $J = 7.6$ Hz, 1H); 5.48 (s, 1H); 5.32 (d, $J = 10.4$ Hz, 1H); 5.11 (br s, 1H); 4.09 (d, $J = 12.0$ Hz, 1H); 3.80 (br s, 1H); 2.98 (s, 6H); 2.95 (s, 1H); 2.91–2.83 (m, 2H); 2.75 (s, 1H); 2.54 (s, 3H); 1.30 (s, 3H); IR (thin film, cm$^{-1}$) 3376, 2925, 2853, 2359, 1727, 1672, 1602, 1520, 1438, 1267, 1216; MS (ESI$^+$) Calcd. For C$_{33}$H$_{40}$N$_4$O$_8$ + H, 621.29; Found, 621.22; TLC (95:5 CH$_2$Cl$_2$:MeOH): R$_f$ = 0.24.

((1S,2R,3R,4S,5S)-5-((3-acetylphenyl)amino)-4-amino-3-(3,3-dimethylureido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-2-methylcyclopentyl)methyl 2-methyl benzoate (2.51c): Isolated from S10c via general procedure E in 73 % yield. Analytical data: $[\alpha]_D^{19}$ +9.9 (c = 0.28, CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.75 (d, $J = 8.0$ Hz, 1H); 7.39 (t, $J = 7.6$ Hz, 1H); 7.26–7.25 (m, 6H); 6.83 (d, $J = 6.8$ Hz, 1H); 5.8 (br s, 1H); 5.68 (d, $J = 10.4$ Hz, 1H); 4.74 (s, 2H); 4.01 (br s, 1H); 3.86 (d, $J = 10.4$ Hz, 1H); 3.01 (s, 6H); 2.55 (s, 3H); 2.52 (s, 3H); 1.58 (s, 3H); 1.08 (d, $J = 6.4$ Hz, 3H); $^{13}$C NMR (150 MHz, CDCl$_3$): $\delta$ 198.6, 168.7, 159.2, 146.8, 140.4,
Preparation and Biological Evaluation of Synthetic...

((1S,2R,3R,4S,5S)-5-(3-acetylphenyl)amino)-4-amino-3-(3,3-dimethylureido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-2-methylcyclopentyl)methyl 2-phenyl acetate (2.51e): Isolated from S10e via general procedure E in 61% yield. Analytical data: [α]D +48.9 (c = 0.45, CHCl3); 1H NMR (400 MHz, CDCl3): δ 7.88 (d, J = 11.2 Hz, 1H); 7.29–7.24 (m, 6H); 7.18–7.24 (m, 4H); 6.74 (d, J = 7.6 Hz, 1H); 5.55 (d, J = 10.4 Hz, 1H); 4.53 (d, J = 12.4 Hz, 1H); 4.44 (d, J = 12.4 Hz, 1H); 3.87 (br s, 1H); 3.58 (s, 2H); 3.55 (s, 1H); 2.96 (s, 6H); 2.85 (s, 1H); 1.41 (s, 3H); 1.01 (d, J = 6.4 Hz, 3H). 13C NMR (150 MHz, CDCl3): δ 198.7, 172.4, 159.2, 146.6, 138.3, 133.7, 129.6, 129.2, 129.1, 128.6, 127.2, 118.2, 118.9, 118.2, 110.5, 88.5, 84.6, 74.2, 71.4, 68.3, 64.6, 63.1, 41.3, 36.8, 29.7, 26.8, 21.0, 18.1; IR (thin film, cm⁻¹) 3398, 2928, 2359, 1733, 1671, 1602, 1519, 1514, 1373, 1327, 1266, 1094, 780; MS (ESI⁺) Calcd. For C28H38N4O7 + H, 543.28; Found, 543.21; TLC (95:5 CH₂Cl₂:MeOH): Rf = 0.32.

Preparation and Biological Evaluation of Synthetic...

((1S,2R,3R,4S,5S)-5-(3-acetylphenyl)amino)-4-amino-3-(3,3-dimethylureido)-1,2-dihydroxy-3-((S)-1-hydroxyethyl)-2-methylcyclopentyl)methyl cyclohexancarboxylate (2.51d): Isolated from S10d via general procedure E in 76% yield. Analytical data: [α]D +22.3 (c = 0.3, CHCl3); 1H NMR (400 MHz, CDCl3): δ 7.86 (br s, 1H); 7.24–7.21 (m, 3H); 7.16–7.13 (m, 2H); 6.77 (d, J = 6.8 Hz, 1H); 5.69 (br s, 1H); 5.57 (d, J = 10.4 Hz, 1H); 4.48 (d, J = 12.0 Hz, 1H); 4.43 (d, J = 12.0 Hz, 1H); 3.95 (br s, 1H); 3.72 (d, J = 10.4 Hz, 1H); 2.98 (s, 6H); 2.57 (br s, 1H); 1.83–1.60 (m, 5H); 1.49 (s, 3H); 1.36–1.15 (m, 5H); 1.03 (d, J = 6.0 Hz, 3H); 13C NMR (150 MHz, CDCl3): δ 198.7, 177.3, 159.2, 146.8, 138.2, 129.6, 118.8, 118.2, 110.5, 84.6, 65.9, 64.2, 43.0, 36.8, 29.0, 28.8, 26.7, 25.6, 25.3, 25.2, 21.1, 18.1, 15.3; IR (thin film, cm⁻¹) 3380, 3054, 2986, 2935, 2410, 1727, 1679, 1603, 1514, 1440, 1265, 738; MS (ESI⁺) Calcd. For C27H42N4O7 + H, 535.31; Found, 535.37; TLC (95:5 CH₂Cl₂:MeOH): Rf = 0.36.
General Procedure K for C6,C7 bis acylation:

((1S,2R,3R,4S,5S)-3-((S)-1-acetoxyethyl)-5-((3-acetylphenyl)amino)-4-(((benzylloxy)carbonyl)amino)-3-(3,3-dimethylureido)-1,2-dihydroxy-2-methylcyclopentyl)methyl acetate (S11a): A flame-dried 20-mL scintillation vial was charged with tetraol 2.26 (0.020 g, 0.036 mmol, 1.00 equiv) and CH₂Cl₂ (1 mL) under an atmosphere of N₂. The solution was cooled to 0 °C and NEt₃ (0.01 mL, 0.07 mmol, 2.00 equiv) and DMAP (0.001 g, 0.01 mmol, 0.27 equiv) were added followed lastly by Ac₂O (0.01 mL, 0.11 mmol, 3.00 equiv). The mixture was allowed to warm to rt and stirred until full conversion of the starting material was observed by TLC analysis, typically 12 h. The reaction was quenched via addition of saturated NaHCO₃(aq.) (5 mL), and the layers were partitioned in a separatory funnel. The aqueous layer was extracted with CH₂Cl₂ (3 × 5 mL), and the combined organics were washed with brine (5 mL), dried with magnesium sulfate, and concentrated in vacuo. The product was purified via flash chromatography (70:30 to 60:40 petroleum ether:acetone) to afford diester S11a (0.02 g, 86 %) as a colorless foam. Analytical Data: ¹H NMR (400 MHz, CDCl₃): δ 7.67 (d, J = 8.0 Hz, 1H), 7.25–7.23 (m, 8H), 6.20 (q, J = 6.4 Hz, 1H), 5.57 (s, 1H), 5.07 (d, J = 12.4 Hz, 1H), 5.00 (d, J = 12.8 Hz, 1H), 4.82 (d, J = 5.2 Hz, 1H), 4.59 (m, 3H), 4.21 (d, J = 12.4 Hz, 1H), 3.85 (m, 1H), 3.62 (s, 1H), 2.96 (s, 6H), 2.55 (s, 3H), 2.06 (s, 3H), 1.91 (s, 3H), 1.48 (s, 3H), 1.37 (d, J = 6.4 Hz, 3H); MS (ESI⁺) Calcd. For C₃₂H₄₂N₄O₁₀ +Na, 665.28; Found, 665.34.

((1S,2R,3R,4S,5S)-5-((3-acetylphenyl)amino)-4-(((benzylloxy)carbonyl)amino)-3-(3,3-dimethylureido)-1,2-dihydroxy-2-methyl-3-((S)-1-(pivaloyloxy)ethyl)cyclopentyl)methyl pivalate (S11b): Isolated from 2.26 via general procedure K using pivaloyl chloride as the electrophile in 92 % yield. Analytical data: ¹H NMR (400 MHz, CDCl₃): δ 7.86 (d, J = 8.0 Hz, 1H), 7.25–7.19 (m, 8H), 6.88 (d, J = 8.0 Hz, 1H), 6.16 (q, J = 8.0 Hz, 1H), 5.61 (s, 1H), 5.03 (dd, J = 4.8, 12.0 Hz, 2H), 4.95 (d, J = 5.2 Hz, 1H), 4.66 (m, 2H), 4.57 (t, J = 12.5 Hz, 1H), 4.17 (d, J = 12.4 Hz, 1H), 3.86 (m, 1H), 3.64 (s, 1H), 2.96 (s, 6H), 2.55 (s, 3H), 2.17 (s, 3H), 1.46 (s, 3H), 1.38 (d, J = 6.8 Hz, 3H), 1.19 (s, 9H), 1.14 (s, 9H); MS (ESI⁺) Calcd. For C₃₈H₅₄N₄O₁₀ +Na, 749.37; Found, 749.45.
\(((1S,2R,3R,4S,5S)-5-((3-acetylphenyl)amino)-4-((benzyloxy)carbonyl)amino)-3-((S)-1-((cyclohexanecarbonyloxy)ethyl)-3-(3,3-dimethylureido)-1,2-dihydroxy-2-methylcyclopentyl)methyl cyclohexanecarboxylate (S11c)\): Isolated from 2.26 via general procedure K using cyclohexylacetyl chloride as the electrophile in 76 % yield. Analytical data: $^1$HNMR (400 MHz, CDCl$_3$): $\delta$ 7.85 (d, $J$ = 8.0 Hz, 1H), 7.28–7.20 (m, 8H), 6.88 (d, $J$ = 8.4 Hz, 1H), 6.23 (q, $J$ = 6.8 Hz, 1H), 5.64 (s, 1H), 5.04 (dd, $J$ = 5.6, 12.4 Hz, 2H), 4.96 (d, $J$ = 5.2 Hz, 1H), 4.64 (m, 3H), 4.22 (d, $J$ = 12.4 Hz, 1H), 3.79 (s, 1H), 2.97 (s, 6H), 2.56 (s, 3H), 2.27 (m, 1H), 2.16 (t, $J$ = 10.8 Hz, 1H), 1.96–1.62 (m, 11H), 1.38 (d, $J$ = 6.4 Hz, 3H), 1.32–1.13 (m, 9H); MS (ESI$^+$) Calcd. For C$_{42}$H$_{58}$N$_4$O$_{10}$ + H, 801.41; Found, 801.54.

\(((1S,2R,3R,4S,5S)-3-((S)-1-acetoxyethyl)-5-((3-acetylphenyl)amino)-4-amino-3-(3,3-dimethylureido)-1,2-dihydroxy-2-methylcyclopentyl)methyl acetate (2.57a)\): Isolated from S11a via general procedure E in 38 % yield. Analytical data: $[\alpha]_D^{19}$ +5.6 (c = 0.17, CHCl$_3$); $^1$HNMR (600 MHz, CDCl$_3$): $\delta$ 7.27–7.21 (m, 4H), 6.95 (d, $J$ = 6.6 Hz, 1H), 6.10 (q, $J$ = 6.6 Hz, 1H), 5.54 (s, 1H), 4.51 (d, $J$ = 12.0 Hz, 2H), 4.21 (d, $J$ = 12.0 Hz, 1H), 4.06 (br s, 1H), 3.44 (br s, 1H), 2.99 (s, 7H), 2.55 (s, 3H), 2.11 (s, 1H), 1.82 (s, 1H), 1.44 (m, 6H); $^{13}$CNMR (150 MHz, CDCl$_3$): $\delta$ 198.9, 172.2, 169.7, 158.7, 148.2, 138.0, 129.3, 117.8, 112.1, 85.7, 81.7, 72.1, 69.3, 65.6, 36.7, 29.7, 26.8, 21.5, 20.8, 20.2, 16.6, 1.0; IR (thin film, cm$^{-1}$) 3421, 2926, 2846, 1637, 1541, 1246, 1066; MS (ESI$^+$) Calcd. For C$_{24}$H$_{36}$N$_4$O$_{8}$ + H; Found, 509.33; TLC (98:2 CH$_2$Cl$_2$:MeOH): $R_f$ = 0.10.
\[(\text{1S,2R,3R,4S,5S})-5-((\text{3-acetylphenyl})\text{amino})-4\text{-amino}-3-(\text{3,3-dimethylureido})-1,2\text{-dihydroxy}-2\text{-methyl}-3-(\text{5}-(\text{1-((cyclohexane carbonyl) oxy})ethyl)-3-(\text{3,3-dimethylureido})-1,2\text{-dihydroxy}-2\text{-methylcyclopentyl})methyl pivalate}\]

(2.57b): Isolated from S11b via general procedure E in 53 \% yield. Analytical data: \([\alpha]_{D}^{25} +22.8 (c = 0.45, \text{CHCl}_3)\); \(^1\text{H} NMR\) (600 MHz, CDCl\(_3\)): \(\delta 7.29–7.22\) (m, 4H), 6.95 (d, \(J = 6.6\) Hz, 1H), 6.08 (q, \(J = 6.6\) Hz, 1H), 5.55 (s, 1H), 4.60 (d, \(J = 12.0\) Hz, 1H), 4.33 (br s, 1H), 4.17 (d, \(J = 12.0\) Hz, 1H), 4.04 (t, \(J = 7.8\) Hz, 1H), 3.34 (d, \(J = 6.8\) Hz, 1H), 2.98 (s, 6H), 2.55 (s, 3H), 1.43 (d, \(J = 6.6\) Hz, 3H), 1.38 (s, 3H), 1.23 (s, 9H), 1.09 (s, 9H); \(^{13}\text{C} NMR\) (150 MHz, CDCl\(_3\)): \(\delta 198.7, 179.9, 177.0, 158.6, 148.2, 138.1, 129.5, 117.7, 117.4, 112.2, 85.5, 81.5, 69.6, 66.0, 38.9, 38.8, 36.7, 27.1, 26.8, 20.2, 16.6; \text{IR}(\text{thin film, cm}^{-1})\) 3895, 3399, 2972, 2359, 1710, 1642, 1530, 1461, 1367, 1284, 1165; \text{MS (ESI+)}\) Calcd. For C\(_{30}\)H\(_{48}\)N\(_4\)O\(_8\) + H, 593.36; Found, 593.38; \text{TLC} (90:10 CH\(_2\)Cl\(_2\):MeOH): \(R_f = 0.34\).

\[(\text{1S,2R,3R,4S,5S})-5-((\text{3-acetylphenyl})\text{amino})-4\text{-amino}-3-((\text{S})-1-((\text{cyclohexane carbonyl}) oxy)ethyl)-3-(\text{3,3-dimethylureido})-1,2\text{-dihydroxy}-2\text{-methylcyclopentyl})methyl cyclohexanecarboxylate}\]

(2.57c): Isolated from S11c via general procedure E in 72 \% yield. Analytical data: \([\alpha]_{D}^{25} +22.6 (c = 0.85, \text{CHCl}_3)\); \(^1\text{H} NMR\) (600 MHz, CDCl\(_3\)): \(\delta 7.28–7.20\) (m, 4H), 6.93 (d, \(J = 7.2\) Hz, 1H), 6.14 (q, \(J = 6.6\) Hz, 1H), 5.55 (s, 1H), 4.53 (d, \(J = 12.6\) Hz, 1H), 4.34 (s, 1H), 4.18 (d, \(J = 16.2\) Hz, 1H), 3.98 (t, \(J = 7.8\) Hz, 1H), 3.30 (d, \(J = 7.2\) Hz, 1H), 2.98 (s, 6H), 2.55 (s, 3H) 2.30 (m, 1H), 2.05 (m, 1H), 1.91 (m, 2H), 1.76 (m, 3H), 1.66–1.57 (m, 5H), 1.42 (d, \(J = 6.6\) Hz, 3H), 1.38 (s, 3H), 1.28–1.10 (m, 10H); \(^{13}\text{C} NMR\) (150 MHz, CDCl\(_3\)): \(\delta 198.7, 177.4, 174.5, 158.5, 148.4, 138.0, 129.3, 117.6, 117.5, 112.2, 85.6, 81.6, 69.5, 65.5, 43.4, 43.1, 36.7, 28.7, 26.8, 25.8, 25.6, 25.5, 25.4, 25.3, 20.3, 16.6; \text{IR}(\text{thin film, cm}^{-1})\) 3390, 3055, 2935, 2857, 2305, 2054, 1723, 1642, 1538, 1450, 1332, 1247, 1132, 737; \text{MS (ESI+) Calcd. For C\(_{34}\)H\(_{52}\)N\(_4\)O\(_8\) + H, 645.39; Found, 645.47; TLC} (90:10 CH\(_2\)Cl\(_2\):MeOH): \(R_f = 0.36\).
correcting for the supernatant (TA Instruments). Excitation, emission, and absorbance wavelength scans of PLGA Quantum Dot NPs were performed on a 96 well plate reader (Molecular Devices SpectraMax M5). QD concentrations were determined by inductively coupled plasma mass spectroscopy (ICP-MS).

**High Pressure Liquid Chromatography Analysis and Drug Loading**

HPLC analysis of PRINT-therapeutics was performed with an analytical Agilent 1200 HPLC system equipped with a variable wavelength absorbance detector using a reverse phase C18 column (Agilent, Zorbax Eclipse XDB-C18, 5 Å, 4.6 × 150 mm). A binary gradient of water (10 % isopropyl alcohol), acetonitrile (10 % isopropyl alcohol), 1 mL min⁻¹ was used and the eluent was monitored by UV absorbance at 205 and 210 nm.

**In vitro cytotoxicity assays**

A549 (ATCC® CCL-185™), MDAMB231 (ATCC® HTB-26™), SK-OV-3 (ATCC® HTB-77™), MRC-5 (ATCC® CCL-171™), were purchased directly from and authenticated by ATCC immediately prior to initiation of these studies. All cell-based assays were performed utilizing passage number for each cell line ranging from 6–16. Each cell line was seeded in 200 μL of media [RPMI1640 (A549), Leibovitz’s L-15 medium (MDAMB231), McCoy’s 5A (SK-OV-3), and EMEM (MRC-5) with 10 % fetal bovine serum] at a density of 5000 cells per cm² into a 96-well microtiter plate. Cells were allowed to adhere for 24 h and subsequently incubated with PRINT particles at drug concentrations ranging from 4 μM to 0.05 nM for 72 h at 37 °C in a humidified 5 % CO₂ atmosphere. After the incubation period, all medium/particles were aspirated off cells. 100 μL fresh medium was added back to cells followed by the addition of 100 μL CellTiter-Glo® Luminescent Cell Viability Assay reagent. Plates were placed on a microplate shaker for 2 min, then incubated at room temperature for 10 min to stabilize luminescent signal. The luminescent signal was recorded on a SpectraMax M5 plate reader (Molecular Dynamics). The viability of the cells exposed to PRINT particles was expressed as a percentage of the viability of cells grown in the absence of particles (Figs. 2.8, 2.9, 2.10, 2.11, 2.12 and 2.13, Table 2.8).

![Fig. 2.8](image-url) Scanning electron microscope (SEM) images of 80 × 320 nm PRINT-therapeutic NPs containing derivative (a) 2.1, (b) 2.29e, and (c) 2.42d, show that the particles are monodisperse and uniform in size and shape despite containing different therapeutics.
National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

Report Date: October 06, 2014
Test Date: May 01, 2014

NCCI: 140/5RS75
Com: JH3/JS75/58 (150758)
Stain Reagent: SRB Dual-Pass Related
SPL: DYO

Log10 Concentration

<table>
<thead>
<tr>
<th>Panel/Cell Line</th>
<th>Time</th>
<th>Mean Optical Densities</th>
<th>Percent Growth</th>
<th>G50</th>
<th>T1</th>
<th>LC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>LNCaP</td>
<td>20.0</td>
<td>1.356 0.257</td>
<td>0.749 0.685 0.046</td>
<td>15</td>
<td>2.376 0.045</td>
<td>0.046</td>
</tr>
<tr>
<td>LNCaP</td>
<td>10.0</td>
<td>1.356 0.257</td>
<td>0.749 0.685 0.046</td>
<td>15</td>
<td>2.376 0.045</td>
<td>0.046</td>
</tr>
<tr>
<td>LNCaP</td>
<td>10.0</td>
<td>1.356 0.257</td>
<td>0.749 0.685 0.046</td>
<td>15</td>
<td>2.376 0.045</td>
<td>0.046</td>
</tr>
<tr>
<td>LNCaP</td>
<td>10.0</td>
<td>1.356 0.257</td>
<td>0.749 0.685 0.046</td>
<td>15</td>
<td>2.376 0.045</td>
<td>0.046</td>
</tr>
</tbody>
</table>

Fig. 2.9 NCI-60 screening results for derivative 2.51c
Fig. 2.10 NCI-60 screening results for derivative 2.42d
Fig. 2.11 NCI-60 screening results for derivative ent-Pactamycin
Fig. 2.12 NCI-60 screening results for derivative 2.29f
Fig. 2.13 NCI-60 screening results for derivative 2.57a
Table 2.8 Physical Characteristics of 80 × 320 nm PRINT-Therapeutic NPs as Measured by DLS and ζ-Potential

<table>
<thead>
<tr>
<th>NP containing therapeutic#</th>
<th>Hydrodynamic size (nm)</th>
<th>Polydispersity Index</th>
<th>Zetapotential (mV)</th>
<th>Drug Loading (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>253 ± 8</td>
<td>0.07 ± 0.02</td>
<td>−9</td>
<td>10</td>
</tr>
<tr>
<td>2.29e</td>
<td>262 ± 5</td>
<td>0.09 ± 0.02</td>
<td>−9</td>
<td>9</td>
</tr>
<tr>
<td>2.42dc</td>
<td>243 ± 2</td>
<td>0.09 ± 0.02</td>
<td>−9</td>
<td>9</td>
</tr>
</tbody>
</table>

References

5. S. Schreiber, Science 2000, 287 (1964)
22. S. Ozaki, Chem. Rev. 72, 457 (1972)
References

26. C. Han, J.A. Porco, Org. Lett. 9, 1517 (2007)
51. See Table 2.8 in Section 2.5 of this Chapter
52. See Figure 2.8 in Section 2.5 of this Chapter
Stereoselective Desymmetrization Methods in the Assembly of Complex Natural Molecules
Sharpe, R.J.
2016, XXIX, 266 p. 367 illus., 11 illus. in color., Hardcover
ISBN: 978-3-319-39024-6