Multi-Component Sequential Synthesis of Dihydroorotic Acid-Based Amphiphilic Molecules

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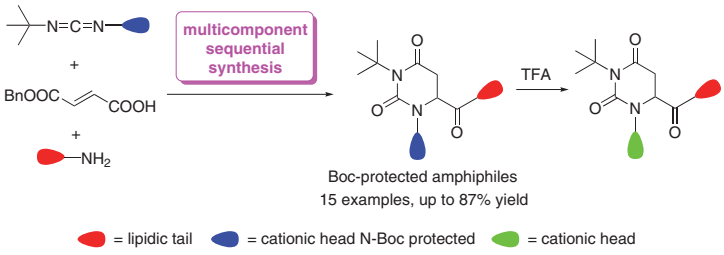
Dedicated to the memory of Tommaso Marcelli



Received: 27.06.2022
Accepted after revision: 29.07.2022
Published online:
DOI: 10.1055/a-1913-3105; Art ID: SS-2022-06-0316-OP

Abstract An efficient multicomponent sequential process, which occurs in mild condition has been exploited for the synthesis of systematically modified amphiphilic molecules where the cationic head is tethered to a lipophilic tail through a dihydroorotic acid linker. The process is operatively simple, high yielding, and flexible. Such a strategy could impact combinatorial synthesis of wide libraries of amphiphilic molecules to be tested as transfection agents and/or as antimicrobials.

Key words multicomponent reactions, domino process, amphiphilic molecules, cationic lipids, antibacterial

Amphiphilic molecules are commonly defined as molecules having a hydrophobic domain spatially separated from a hydrophilic region.[1] The hydrophobic domain is generally constituted by apolar hydrocarbon chains of different length or by (poly)aromatic rings, while the hydrophilic moiety by polar functional groups. When the hydrophilic polar head is a cation, the amphiphilic molecule is often referred to as cationic lipid.[2]

Cationic lipids have been found wide application in the field of gene/drug delivery and as membrane-active antibacterials due to their intrinsic ability to interact with cell membrane.[3] Indeed, although the processes of cell internalization and membrane destruction are diverse and often complex, it is widely accepted that a general mechanism of action relies on a first electrostatic interaction between the cationic head of the amphiphilic molecules and the negatively charged surface of both bacterial and mammalian cells followed by membrane penetration through the intercalation of the lipophilic tails into the lipidic bilayer (Figure 1).[4] Since the optimal synthetic vector as well as efficient and selective antibiotic agents able to fight drug-resistant bacteria are not yet available, there is a great interest in the development of both new generations of antibacterials and highly efficient liposome delivery vehicles based on amphiphilic molecules. However, this task has been hampered also by the lack of efficient synthetic procedures, which would not require multistep synthesis, extensive purification procedures, and individual optimization.[5]

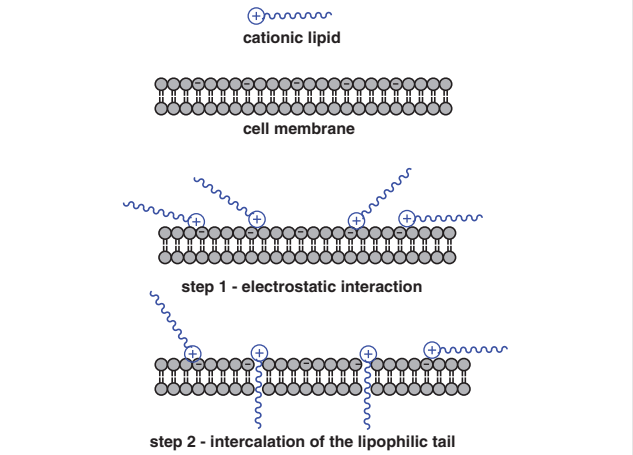
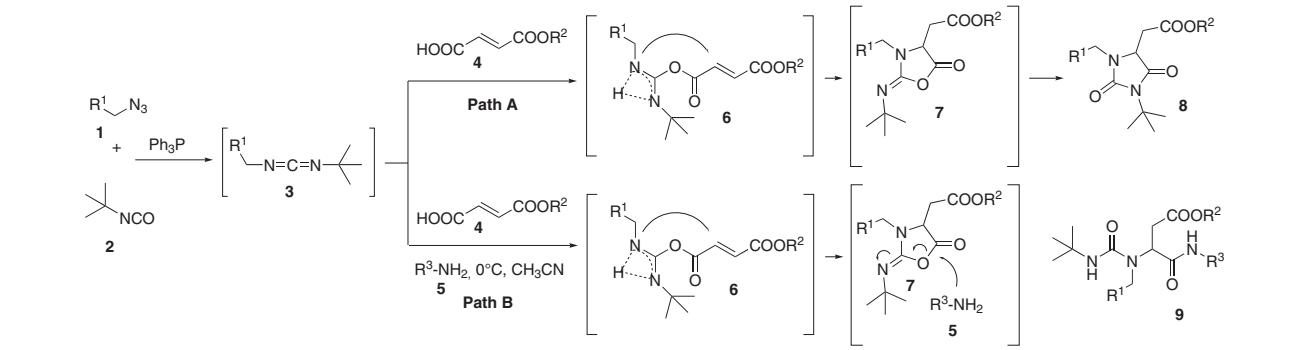


Figure 1 General mechanism of action of cationic lipid on cell membrane

Multicomponent reactions (MCRs) are one-pot processes where three or more reactants are combined to afford a kind of complex scaffold incorporating moieties from all the starting materials.[6] Due to their efficiency both in term of atom economy, time saving, diversity generating, convergence, MCRs have been exploited not only to prepare libraries of small molecules, typically heterocycles, peptidomimetics, or natural products, but also in the synthesis of polymers or ligation and conjugation of (macro)biomolecules.[7] However, in order to produce libraries having new structural diversity, there is still a need and great interest in



Scheme 1 MC domino process to access hydantoins **8** or urea-amides **9**

finding new MC processes or in varying known MCRs by using different reaction conditions, different catalysts, or by slightly changing the structure of the building blocks.[8] For instance, in the last years, well-known isocyanide-based MCRs, such as Passerini and Ugi-type MCRs, have been exploited for the lipidation of biomolecules such as sugars and peptides by using simple lipidic isocyanides[7c] and likewise some example of new MC processes for the synthesis of libraries of amphiphilic molecules have been recently appeared in literature.[9]

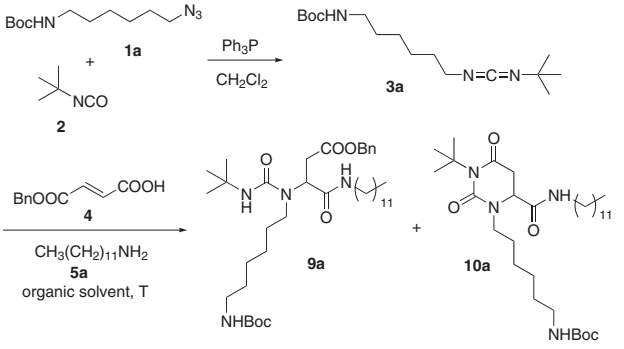
Herein, we describe the application of an MC reaction developed by us for the synthesis of a library of amphiphilic molecules where the lipophilic tail is connected to the cationic head through a dihydroorotic acid heterocycle. The process is operatively simple, high-yielding, flexible, and undergoes under mild conditions, thus possessing all the required features to be applied in future for the preparation of different libraries of such interesting compounds in a very efficient way.

During the last years, we have developed a sequential MC domino process, which was used efficiently for the synthesis of libraries of small molecules of biological interest, such as glycomimetics and peptide-sugar conjugates,[10] aminoglycoside-sugar conjugates,[11] and universal peptidomimetics.[12] Briefly, primary azides **1** reacts with *tert*-butyl isocyanate (**2**) producing carbodiimides **3**, which can be isolated or treated in situ with fumaric acid monoester **4** in the presence of trimethylpyridine (TMP) to yield hydantoin heterocycle **8** through a regiospecific condensation/aza-Michael/O→N acyl migration domino process (Path A, Scheme 1). When the reaction is carried out in the presence of an amine or α-aminoester **5** at 0 °C in CH_3CN as solvent the 2-imino-oxazolidin-2-one intermediate **7** is captured by the nucleophile producing the four-component urea-amide or urea peptide conjugate **9** (Path B, Scheme 1).

We envisaged to exploit this process for the preparation of amphiphilic molecules by anchoring a lipophilic tail and a cationic moiety to the components of the process. Since we presumed that the difficult part would be the introduction of the lipophilic tail due to solubility and steric hinderance concerns, we conceived to exploit path b in Scheme 1 for the synthesis of amphiphilic urea-amides **9** by using fatty amines and azides bearing opportunely protected amino groups that would be transformed into unprotected cationic ammonium groups after the MC process has occurred.

Accordingly, we decided to fine tune the reaction conditions by reacting dodecylamine (**5a**) as lipophilic amine and carbodiimide **3a** derived from the reaction between *tert*-butyl isocyanate (**2**) and commercially available *N*-Boc-6-azido-1-hexylammine **1a** (Table 1). However, the first attempt by using the required conditions, namely CH_3CN as solvent and 0 °C, failed giving rise to the formation of the desired product **9a** in very low yields (Table 1, entry 1).

Table 1 Reaction Condition Optimization

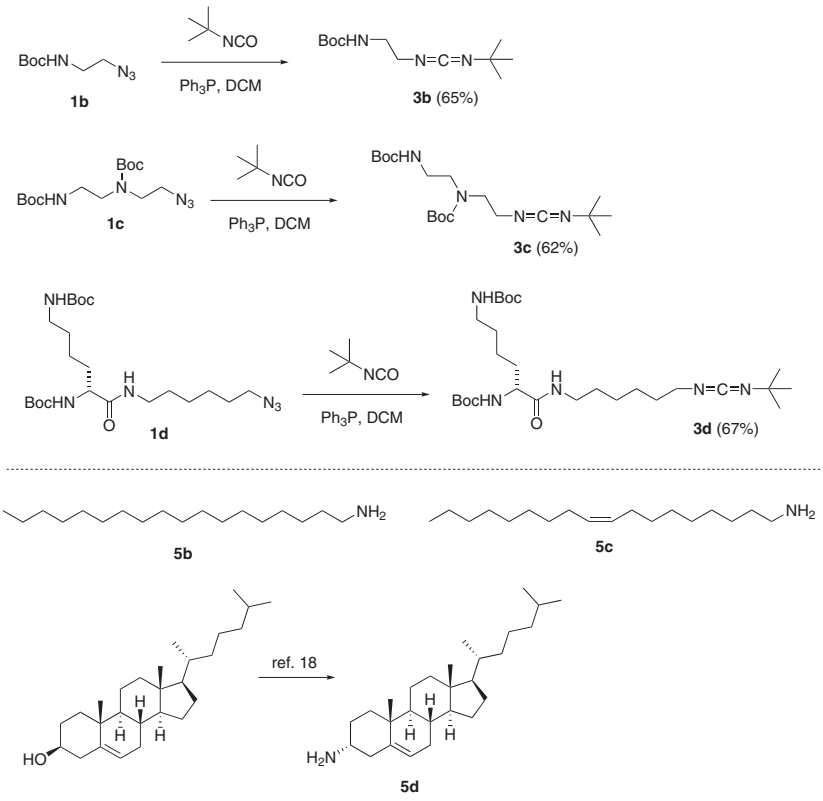


Entry	CH_3CN (%)	$CHCl_3$ (%)	Temp (°C)	Yield (%)[a] of **9a**	Yield (%)[a] of **10a**
1	100	–	0	25	0
2	80	20	0	40	0
3	50	50	0	63	0
4	–	100	0	0	0
5	50	50	20	81	15
6[b]	50	50	20	0	87

[a] Isolated yields.
[b] Reaction quenched with aq 1 N NaOH.

Actually, after the introduction of the amine **5a** followed by solid fumaric acid monobenzyl ester **4** to the reaction solution, a solid precipitated. In a second experiment, we recovered by filtration the solid and through ^{1}H NMR we found it was the ammonium salt formed by protonation of the fatty amine **5a** by fumaric acid **4**. Since this salt is insoluble in CH_3CN but soluble in $CDCl_3$ (the NMR solvent), we thought to use chloroform as solvent or co-solvent in the MC process. When the reaction was performed at 0 °C, the best yields were obtained by using a 1:1 (v/v) mixture of $CH_3CN/CHCl_3$ (entry 3), while a decreasing amount of chloroform resulted in a decrease of the yield (entry 2). As expected, the reaction did not occur in pure $CHCl_3$ (entry 4). Indeed, we have already seen the necessity to use CH_3CN as solvent for this MC process in order to avoid all the possible side reactions, in particular the coupling reaction between carboxylic acids and amines promoted by carbodiimides and the formation of hydantoin according to path A depicted in Scheme 1. Mixtures of other not nucleophilic organic solvents, such as CH_2Cl_2, THF, and DMF in different amounts did not improve the yield of the process (data not shown).

In order to further increase the yield of the MC process, we tried the reaction at room temperature. Interestingly, in contrast to what was reported in the previous works,[10–12] we got better yields of the derivative **9a**, along with the formation of cyclic dihydroorotic acid derivative **10a** in a not marginal quantity (Table 1, entry 5). Indeed, urea-aspartic amide conjugates **9** are very prone to cyclization, which occurs by the nucleophilic attack of the urea NH to the benzyl ester under slightly basic conditions.[13] Probably, the presence of a lower polar solvent such as $CHCl_3$ facilitate the solubility of all the components increasing the yields of **9a**, but at the same moment the higher temperature trigger to a certain extent the cyclization reaction. In order to avoid the formation of two products, we tried to push the cyclization step by quenching the reaction with a base, either a 1 M aqueous solution of NaOH or an ethanolic solution of methylamine. Moreover, it has been demonstrated that both cyclic antimicrobial peptides and cyclic cationic lipid transfectants could be more efficient than their acyclic counterparts due to the conformational constraint.[14] We were delighted to find out that in both conditions we obtained the formation of the only dihydroorotate derivative **10a** in good yields (entry 6), the use of NaOH being the best choice both in terms of yields and workout conditions. It is worth noting that the process occurs also one-pot without the recovery of the carbodiimide intermediate **3**, that is, by adding to the CH_3CN solution a solution of the amine **5**, fumaric acid monobenzyl ester **4**, and TMP dissolved in chloroform once carbodiimide **3** is formed. However, due to the presence of



Scheme 2 Synthesis of carbodiimides **3b–d**

by-products such as triphenylphosphine oxide and the urea of the carbodiimide, the recovery of the clean dihydroorotic acid derivative **10** resulted quite demanding.

Once the reaction conditions have been optimized, we decided to test the scope and limitation of the MC process by using carbodiimides **3b–d**, prepared starting from azides **1b–d** bearing one or two protected amino groups. Briefly, azides **1b**,[15] **1c**,[16] and **1d**[17] were treated with *tert*-butyl isocyanate (**2**) in the presence of Ph_3P in DCM overnight. After short-path flash-chromatography, carbodiimides **3b–d**, respectively, were obtained in acceptable yields (Scheme 2).

As lipophilic nucleophiles, other than dodecyl amine **5a**, we used commercially available stearyl and oleyl amine **5b**,**c** and cholesteryl amine **5d** prepared according to the literature (Scheme 2).[18]

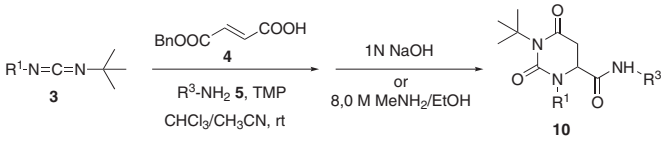
The MC-cyclization sequential process worked nicely in all cases, providing the formation of a collection of 14 new systematically modified Boc-protected dihydroorotic acid-based amphiphilic compounds (Table 2). Indeed, by reacting carbodiimides **3a**,**b** with lipophilic amines **5b–d** we obtained seven derivatives **10b–h** having only one amino group protected as Boc (Table 2, entries 1–7), while by reacting spermine-carbodiimide **3c** and lysine-carbodiimide **3d** we collected other six derivatives, namely compounds **10i–l** (entries 8–11) and **10m–o** (entries 12–14), respectively, with two amino groups Boc-protected. All the reactions occurred in good yields, in particular when the nucleophiles are linear amines like **5a–c**, while the yields are slightly lower with bulkier cholesteryl amine **5d**.

The obtained derivatives **10** were treated with a 10% TFA solution in DCM for three hours at room temperature to remove the Boc protecting group producing in quantitative yields the final dihydroorotic acid-based lipidic cations **11a–o** depicted in Chart 1 as trifluoroacetate salts.

Since the presence of either two lipophilic alkyl chains or guanidino groups could impact on the ability of amphiphilic molecules to interact with the cell membrane,[19] we investigated the possibility to introduce these groups in the scaffolds obtained with our MC process. Accordingly, we tried to perform the MC process with secondary didecylamine (**5e**) and carbodiimides **3a–c** (Scheme 3). In all these cases, the MC process worked nicely producing urea-aspartic amide conjugates **12a–c**, respectively, in good yields. However, any attempt to trigger the cyclization leading to the corresponding dihydroorotate derivative failed.

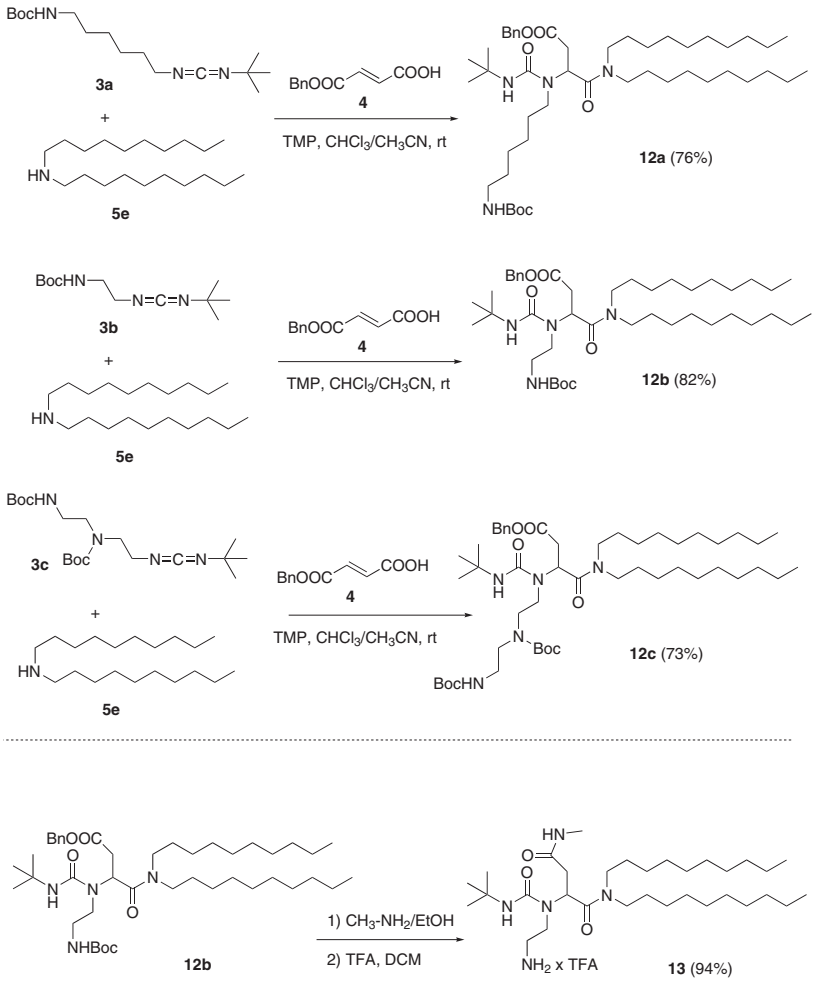
For instance, by treating derivative **12b** with an ethanolic solution of methylamine we obtained instead, after Boc-deprotection, the corresponding methyl amide **13**. These results were not unexpected since we have already seen that when the amide in urea-aspartic amides of type **12** is a secondary amide the cyclization process does not occur.[10] It is worth noting that compounds **12**, as well as compounds **9**, are *per se* interesting amphiphilic molecules since they contain two (or one) lipophilic tails tethered to a moiety

Table 2 MC Sequential Process Producing *N*-Boc Protected Dihydroorotate Derivatives **10**



Entry	R^1, carbodiimide	R^3, amine	Product (yield %)[a]
1	BocNH$(CH_2)_6$, **3a**	Stearyl, **5b**	**10b** (86)
2	BocNH$(CH_2)_6$, **3a**	Oleyl, **5c**	**10c** (82)
3	BocNH$(CH_2)_6$, **3a**	Cholesteryl, **5d**	**10d** (71)
4	BocNH$(CH_2)_2$, **3b**	Dodecyl, **5a**	**10e** (84)
5	BocNH$(CH_2)_2$, **3b**	Stearyl, **5b**	**10f** (75)
6	BocNH$(CH_2)_2$, **3b**	Oleyl, **5c**	**10g** (91)
7	BocNH$(CH_2)_2$, **3b**	Cholesteryl, **5d**	**10h** (69)
8	BocNH$(CH_2)_2$-NBoc-$(CH_2)_2$, **3c**	Dodecyl, **5a**	**10i** (77)
9	BocNH$(CH_2)_2$-NBoc-$(CH_2)_2$, **3c**	Stearyl, **5b**	**10j** (73)
10	BocNH$(CH_2)_2$-NBoc-$(CH_2)_2$, **3c**	Oleyl, **5c**	**10k** (80)
11	BocNH$(CH_2)_2$-NBoc-$(CH_2)_2$, **3c**	Cholesteryl, **5d**	**10l** (66)
12	diBoc-Lys-NH$(CH_2)_6$, **3d**	Stearyl, **5b**	**10m** (82)
13	diBoc-Lys-NH$(CH_2)_6$, **3d**	Oleyl, **5c**	**10n** (87)
14	diBoc-Lys-NH$(CH_2)_6$, **3d**	Cholesteryl, **5d**	**10o** (70)

[a] Isolated yields.

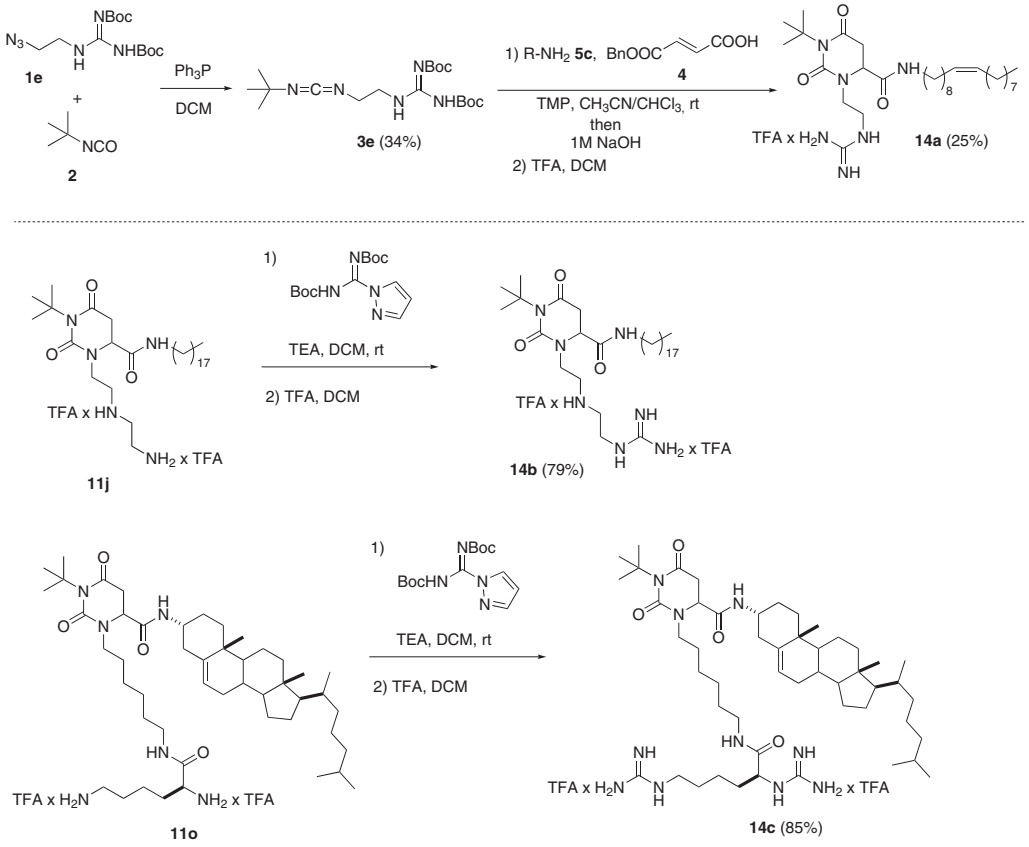


Scheme 3 Reaction with didecylamine (**5e**)

having one or two NBoc groups through a linker possessing a benzyl ester functional group that could be exploited for a further functionalization.

Analogously, we tried the MC process with carbodiimide **3e**, obtained by reacting the diBoc protected guanidino derivative of 2-azido-1-ethylamine (**1e**)[20] with *tert*-butyl isocyanate (**2**), lipophilic amine **5c** and fumaric acid monobenzyl ester (**4**) in the optimized reaction conditions, followed by cyclization by quenching the reaction with aqueous 1 M NaOH (Scheme 4). Unfortunately, in this case both the formation of carbodiimide **3e** and dihydroorotic acid derivative **14a** occurred in low yield, probably due to the steric hindrance of the diBoc protected guanidino groups. However, the guanidino derivatives of dihydroorotic acid-based amphiphilic derivatives could be easily obtained in high yields by reacting compounds **11** with *N*,*N*′-bis-boc-1-guanylpyrazole followed by Boc-deprotection. For instance, with the latter procedure, starting from derivatives **11j**,**o** we obtained guanidino derivatives **14b**,**c**, respectively, having one and two guanidino groups in very good yields.

In conclusion, by applying a novel MC domino process followed by selective intramolecular cyclization reaction we were able to develop an efficient way to prepare a library of systematically modified dihydroorotic acid-based amphiphilic molecules starting from easily accessible starting materials. The process occurs in mild conditions and in overall good yields, providing a new procedure for the efficient synthesis of new libraries of cationic lipids to be tested in the field of gene/drug delivery and as membrane-active antibacterials. The synthesis of wider libraries of amphiphilic molecules with different cationic groups and lipophilic tails and the evaluation of their ability as transfection agents and/or as antimicrobials are under investigation in our laboratory.



Scheme 4 Synthesis of guanidino derivatives **14**

Commercially available reagent-grade solvents were employed without purification. TLC were run on silica gel 60 F254 Merck. Flash chromatography (FC) was performed with silica gel 60 (60–200 mm, Merck). ^{1}H NMR spectra were recorded on 400 MHz spectrometers. Chemical shifts are expressed in ppm (δ), using TMS as internal standard for ^{1}H and ^{13}C nuclei (δ_H and δ_C = 0.00). ESIMS was performed with an Esquire 3000 plus ion-trap mass spectrometer equipped with an ESI source. Elemental analyses were obtained on FlashEA 1112 NC Analyzers. Azides **1b–d** were prepared as described in the literature,[15–17] respectively.

Carbodiimides 3a–d; General Procedure

To a stirred solution of azide **1** (■■?■■ mmol, 1 equiv) in DCM (0.1 M solution) were added *tert*-butyl isocyanate (**2**; 1.05 equiv) followed by solid Ph_3P (1.05 equiv) at rt. The solution was stirred until complete formation of the corresponding carbodiimide **3** was achieved (TLC monitoring). The organic solvent was evaporated, and the crude purified by short-path flash chromatography.

tert-Butyl (6-Azidohexyl)carbamate (3a)

Yield: 68%; R_f = 0.32 (hexane–EtOAc 80:20).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.57 (br s, 1 H), 3.21 (t, *J* = 6.8 Hz, 2 H), 3.12 (q, *J* = 6.4 Hz, 2 H), 1.59–1.55 (m, 2 H), 1.48–1.31 (m, 6 H), 1.46 (s, 9 H), 1.29 (s, 9 H).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 155.7, 139.6, 79.2, 77.1, 55.2, 41.8, 32.1,31.2, 28.2, 27.3, 27.1.

ESI-MS: *m*/*z* (%) = 298.0 ([M + H]$^+$, 100).

tert-Butyl (2-(((*tert*-Butylimino)methylene)amino)ethyl)carbamate (3b)

Yield: 65%; R_f = 0.28 (hexane–EtOAc 80:20).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.83 (br s, 1H), 3.32–3.26 (m, 2H), 3.25–3.18 (m, 2H), 1.41 (s, 9H), 1.25 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 155.8, 139.7, 79.5, 55.3, 47.0, 41.9, 31.4, 28.5

ESI-MS: *m*/*z* (%) = 264.0 ([M + Na]$^+$, 100), 242.0 ([M + H]$^+$, 23).

tert-Butyl (2-((*tert*-Butoxycarbonyl)amino)ethyl)(2-(((*tert*-butylimino)methylene)amino)ethyl)carbamate (3c)

Yield: 62%; R_f = 0.25 (hexane–EtOAc 80:20).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.92 (br s, 1 H), 3.33–3.03 (m, 8 H), 1.41 (s, 9 H), 1.38 (s, 9 H), 1.22 (s, 9 H).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 155.9, 155.4, 139.7, 80.1, 79.1, 55.1, 49.0, 45.5, 39.7, 31.3, 28.4.

Di-*tert*-butyl ((5*R*)-6-((6-(((*tert*-Butylimino)methylene)amino)hexyl)amino)-6-oxohexane-1,5-diyl)dicarbamate (3d)

Yield: 67%; R_f = 0.33 (hexane–EtOAc 20:80).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.22 (br s, 1 H), 5.14 (br s, 1 H), 4.63 (br s, 1 H), 4.00 (q, *J* = 6.0 Hz, 1 H), 3.24–3.20 (m, 4 H), 3.12–3.09 (m, 2 H), 1.82–1.80 (m, 2 H), 1.69–1.37 (m, 12 H), 1.44 (s, 18 H), 1.26 (s, 9 H).

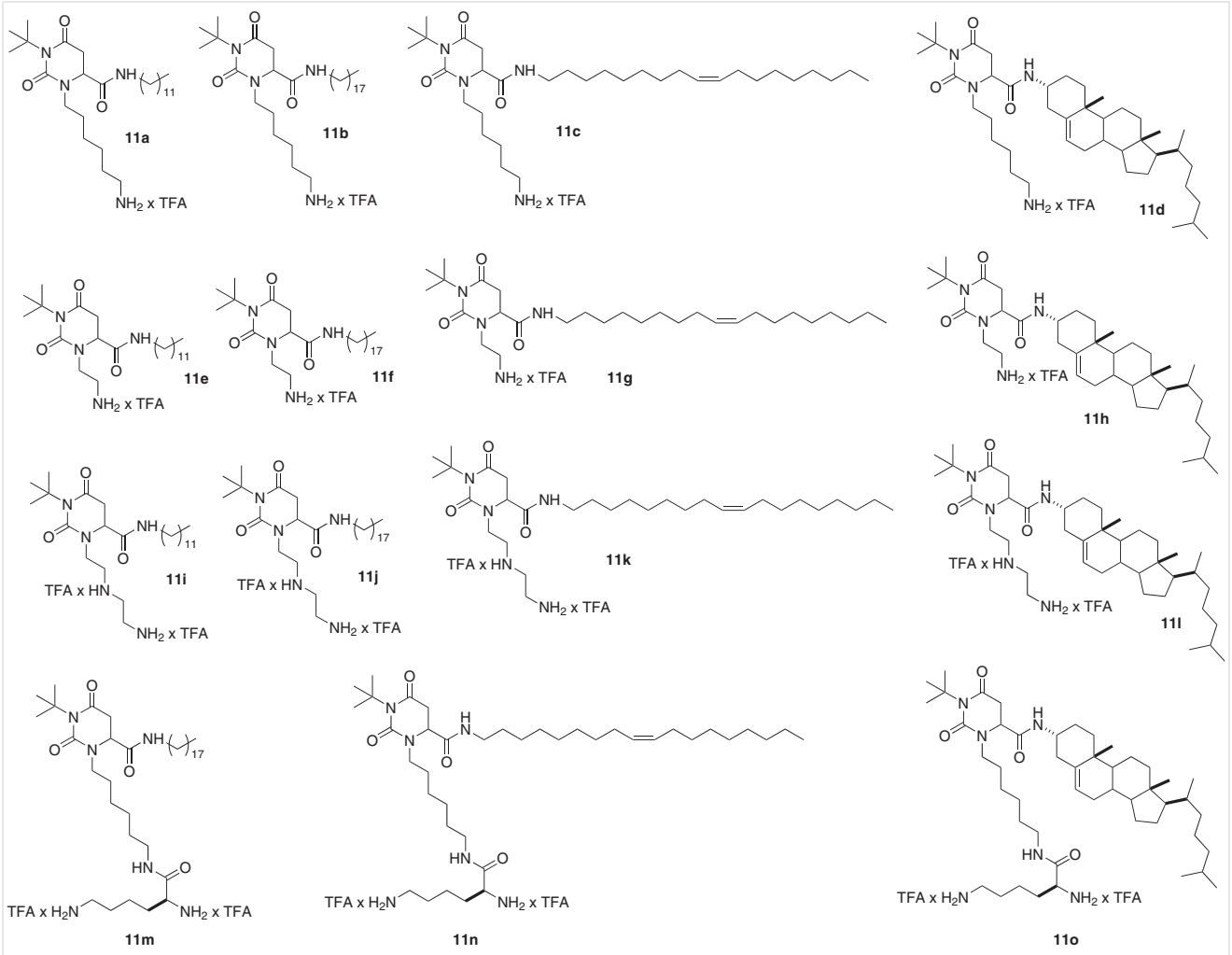


Chart 1 Dihydroorotate-based amphiphilic derivatives **11a–o**

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 172.3, 156.2, 155.8, 139.7, 79.7, 78.9, 54.4, 54.2, 51.3, 39.9, 39.2, 38.5, 32.1, 29.6, 29.3, 29.13, 29.06, 28.6, 28.4, 28.3, 26.3, 24.8, 24.5, 22.6.

ESI-MS: *m*/*z* (%) = 526.3 ([M + H]$^+$, 100).

N-Boc-Protected Dihydroorotic Acid-Based Amphiphilic 10a–o; General Procedure

To a stirred solution of carbodiimide **3** (■■?■■ mmol, 1.0 equiv) in CH_3CN (0.1 M solution) were added lipophilic amine **5** (1.05 equiv) followed by TMP (1.05 equiv) and a solution of fumaric acid monobenzyl ester (**4**; 1.2 equiv) in $CHCl_3$ (same volume of CH_3CN) at rt. The solution was stirred until complete formation of the corresponding urea-amide **9** was achieved (TLC monitoring). Aq 1 M NaOH was added (20% of the total volume) and the mixture vigorously stirred for 30 min. The mixture was diluted with aq 1 M HCl until acidic pH was reached and extracted with EtOAc (3 ×). The collected organic layers were washed with brine (1 ×), with sat. $NaHCO_3$ (1 ×), and with brine (3 ×). The organic layer was dried (Na_2SO_4), filtered, and the solvent evaporated. The crude was purified by flash chromatography.

Benzyl 3-(1-(6-((*tert*-Butoxycarbonyl)amino)hexyl)-3-(*tert*-butyl)ureido)-4-(dodecylamino)-4-oxobutanoate (9a)

Yield: 81%; R_f = 0.30 (hexane–EtOAc 65:35).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.32–7.28 (m, 6 H), 6.71 (br t, *J* = 5.6 Hz, 1 H), 5.13 (d, *J* = 12.4 Hz, 1 H), 5.04 (d, *J* = 12.4 Hz, 1 H), 4.96 (br t, *J* = 5.8 Hz, 1 H), 4.66–4.61 (m, 2 H), 3.24–3.20 (m, 1 H), 3.14–3.04 (m, 6 H), 2.65 (dd, *J* = 16.8, 7.6 Hz, 1 H), 1.42–1.40 (m, 10 H), 1.32 (s, 9 H), 1.25–1.21 (m, 27 H), 0.86 (t, *J* = 6.8 Hz).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 171.0, 170.8, 157.7, 156.0, 135.7, 128.5, 128.3, 128.21, 128.16, 78.9, 77.3, 66.5, 55.3, 51.0, 45.5, 40.3, 39.4, 34.3, 31.9, 29.58, 29.56, 29.51, 29.5, 29.4, 29.35, 29.30, 29.2, 28.4, 26.8, 26.7, 26.3, 22.6, 14.1.

ESI-MS: *m*/*z* (%) = 711.4 ([M + Na]$^+$, 100), 727.4 ([M + K]$^+$, 4).

Anal. Calcd for $C_{39}H_{68}N_4O_6$: C, 67.99; H, 9.95; N, 8.13. Found: C, 68.01; H, 9.97; N, 8.11.

tert-Butyl (6-(3-(*tert*-Butyl)-6-(dodecylcarbamoyl)-2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)hexyl)carbamate (10a)

Yield: 87%; R_f = 0.43 (hexane–EtOAc 40:60).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.22 (br s, 1 H), 4.68 (br s, 1 H), 4.15 (t, *J* = 6.0 Hz, 1 H), 3.62–3.42 (m, 1 H), 3.23–3.18 (m, 2 H), 3.13–2.95 (m, 3 H), 2.72 (dd, *J* = 15.5, 4.6 Hz, 1 H), 2.50 (dd, *J* = 15.5, 6.0 Hz, 1 H), 1.58 (s, 9 H), 1.54–1.24 (m, 37 H), 0.92–0.75 (t, *J* = 6.7 Hz, 3 H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 173.8, 168.2, 157.0, 156.1, 79.0, 77.3, 55.8, 41.0, 40.3, 39.8, 37.1, 31.9, 29.61, 29.58, 29.55, 29.51, 29.30, 29.26, 28.6, 28.4, 27.5, 26.9, 26.11, 26.07, 22.6, 14.1.

ESI-MS: *m*/*z* (%) = 603.0 ([M + Na]$^+$, 100).

Anal. Calcd for $C_{32}H_{60}N_4O_5$: C, 66.17; H, 10.41; N, 9.65. Found: C, 66.16; H, 10.40; N, 9.65.

tert-Butyl (6-(3-(_tert_-Butyl)-6-(octadecylcarbamoyl)-2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)hexyl)carbamate (10b)

Yield: 86%; R_f = 0.45 (hexane–EtOAc 40:60).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.18 (br s, 1 H), 4.64 (br s, 1 H), 4.16 (t, *J* = 6.7 Hz, 1 H), 3.60–3.47 (m, 1 H), 3.27–3.17 (m, 2 H), 3.11–3.02 (m, 3 H), 2.72 (dd, *J* = 15.5, 4.7 Hz, 1 H), 2.51 (dd, *J* = 15.5, 5.9 Hz, 1 H), 1.59 (s, 9 H), 1.52–1.22 (m, 49 H), 0.90–0.84 (t, *J* = 6.5 Hz, 3 H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 173.8, 168.2, 157.0, 156.1, 79.1, 77.3, 57.9, 55.8, 41.0, 40.4, 39.8, 37.1, 31.9, 29.8, 29.7, 29.63, 29.57, 29.5, 29.32, 29.27, 28.7, 28.4, 27.5, 26.9, 26.12, 26.08, 22.7, 14.1.

ESI-MS: *m*/*z* (%) = 687.4 ([M + Na]$^+$, 100).

Anal. Calcd for $C_{38}H_{72}N_4O_5$: C, 68.63; H, 10.91; N, 8.43. Found: C, 68.62; H, 10.93; N, 8.44.

tert-Butyl (_Z_)-(6-(3-(_tert_-Butyl)-6-(octadec-9-en-1-ylcarbamoyl)-2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)hexyl)carbamate (10c)

Yield: 82%; R_f = 0.46 (hexane–EtOAc 40:60).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.26 (br s, 1 H), 5.40–5.28 (m, 2 H), 4.69 (br s, 1 H), 4.14 (t, *J* = 5.9 Hz, 1 H), 3.58–3.42 (m, 1 H), 3.25–3.15 (m, 2 H), 3.11–3.03 (m, 3 H), 2.71 (dd, *J* = 15.5, 4.6 Hz, 1 H), 2.50 (dd, *J* = 15.5, 6.0 Hz, 1 H), 2.04–1.89 (m, 4 H), 1.57 (s, 9 H), 1.52–1.12 (m, 41 H), 0.86 (t, *J* = 6.8 Hz, 3 H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 173.7, 168.2, 157.0, 156.1, 129.9, 129.7, 79.0, 77.3, 57.9, 55.8, 41.0, 40.3, 39.8, 37.0, 31.9, 29.7, 29.6, 29.6, 29.5, 29.4, 29.23, 29.20, 29.2, 28.6, 28.4, 27.5, 27.18, 27.16, 26.91, 26.16, 22.63, 14.1.

ESI-MS: *m*/*z* (%) = 685.6 ([M + Na]$^+$, 100).

Anal. Calcd for $C_{38}H_{70}N_4O_5$: C, 68.84; H, 10.64; N, 8.45. Found: C, 68.84; H, 10.66; N, 8.45.

tert-Butyl (6-(3-(_tert_-Butyl)-6-(((3*R*,10*R*,13*R*,17*R*)-10,13-dimethyl-17-((*S*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl)carbamoyl)-2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)hexyl)carbamate (10d)

Yield: 71%; R_f = 0.33 (hexane–EtOAc 40:60).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.33 (s, 1 H), 5.22–5.03 (m, 1 H), 4.60 (s, 1 H), 4.43–4.07 (m, 1 H), 4.07–3.82 (m, 1 H), 3.82–3.45 (m, 1 H), 3.10 (s, 5 H), 2.98–2.76 (m, 2 H), 2.62–2.39 (m, 1 H), 2.35–2.07 (m, 1 H), 2.07–1.72 (m, 5 H), 1.72–0.79 (m, 66 H), 0.68 (s, 3 H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 176.4, 175.0, 173.7, 157.0, 156.0, 155.8, 128.6, 128.6, 128.5, 128.5, 128.4, 122.1, 79.0, 67.0, 56.7, 56.1, 51.0, 50.1, 42.3, 39.5, 36.8, 36.2, 35.8, 31.9, 31.8, 29.4, 28.7, 28.6, 28.4, 28.2, 28.0, 24.3, 23.8, 22.8, 22.6, 19.4, 18.7, 11.9.

ESI-MS: *m*/*z* (%) = 803.7 ([M + Na]$^+$, 100).

Anal. Calcd for $C_{47}H_{80}N_4O_5$: C, 72,26; H, 10.32; N, 7.17. Found: C, 72,25; H, 10.31; N, 7.18.

tert-Butyl (2-(3-(_tert_-Butyl)-6-(dodecylcarbamoyl)-2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)ethyl)carbamate (10e)

Yield: 84%; R_f = 0.24 (hexane–EtOAc 40:60).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.01 (br t, *J* = 5.7 Hz, 1 H), 5.10 (br s, 1 H), 4.19 (dd, *J* = 5.9, 4.1 Hz, 1 H), 3.53–3.42 (m, 1 H), 3.40–3.11 (m, 5 H), 2.75 (dd, *J* = 15.5, 4.2 Hz, 1 H), 2.57 (dd, *J* = 15.5, 6.0 Hz, 1 H), 1.57 (s, 9 H), 1.51–1.43 (m, 2 H), 1.40 (s, 9 H), 1.24 (s, 18 H), 0.86 (t, *J* = 6.8 Hz, 3 H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 173.5, 168.3, 157.7, 156.2, 79.4, 58.0, 57.0, 41.7, 39.8, 39.4, 36.9, 31.8, 29.6, 29.51, 29.46, 29.3, 29.2, 28.6, 28.4, 26.9, 22.6, 14.0.

ESI-MS: *m*/*z* (%) = 563.3 ([M + K]$^+$, 5); 547.3 ([M + Na]$^+$, 23).

Anal. Calcd for $C_{28}H_{52}N_4O_5$: C, 64.09; H, 9.99; N, 10.68. Found: C, 64.10; H, 8.00; N, 10.68.

tert-Butyl (2-(3-(_tert_-Butyl)-6-(octadecylcarbamoyl)-2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)ethyl)carbamate (10f)

Yield: 73%■■*75% in Table 2?*■■; R_f = 0.27 (hexane–EtOAc 40:60).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.02 (br t, *J* = 5.7 Hz, 1 H), 5.13 (br s, 1 H), 4.21 (dd, *J* = 6.0, 4.1 Hz, 1 H), 3.56–3.44 (m, 1 H), 3.43–3.14 (m, 5 H), 2.77 (dd, *J* = 15.5, 4.1 Hz, 1 H), 2.58 (dd, *J* = 15.6, 5.9 Hz, 1 H), 1.59 (s, 9 H), 1.52–1.44 (m, 2 H), 1.42 (s, 9 H), 1.25 (s, 30 H), 0.87 (t, *J* = 6.6 Hz, 3 H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 173.5, 168.3, 157.7, 156.2, 79.3, 58.0, 57.0, 41.7, 39.8, 39.3, 36.9, 31.9, 29.62, 29.58, 29.52, 29.47, 29.3, 29.2, 28.6, 28.4, 26.9, 22.6, 14.0.

ESI-MS: *m*/*z* (%) = 739.4 ([M + Na]$^+$, 100).

Anal. Calcd for $C_{34}H_{64}N_4O_5$: C, 67.07; H, 10.59; N, 9.20. Found: C, 67.08; H, 10.60; N, 9.20.

tert-Butyl (_Z_)-(2-(3-(_tert_-Butyl)-6-(octadec-9-en-1-ylcarbamoyl)-2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)ethyl)carbamate (10g)

Yield: 78%■■*91% in Table 2?*■■; R_f = 0.30 (hexane–EtOAc 40:60).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.14 (br s, 1 H), 5.48–5.30 (m, 2 H), 5.11 (br s, 1 H), 4.23 (t, *J* = 6.0 Hz, 1 H), 3.29–3.01 (m, 7 H), 2.64 (dd, *J* = 16.2, 7.0 Hz, 1 H), 2.10–1.92 (m, 4 H), 1.51–1.13 (m, 42 H), 0.88 (t, *J* = 6.6 Hz, 3 H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 171.0, 168.3, 157.9, 156.7, 129.9, 129.8, 79.9, 54.5, 51.3, 44.3, 40.2, 39.4, 34.4, 32.5, 31.8, 29.7, 29.6, 29.5, 29.4, 29.35, 29.25, 29.2, 28.3, 27.2, 26.8, 22.6, 14.0.

ESI-MS: *m*/*z* (%) = 737.4 ([M + Na]$^+$, 100).

Anal. Calcd for $C_{34}H_{62}N_4O_5$: C, 67.29; H, 10.30; N, 9.23. Found: C, 67.27; H, 10.31; N, 9.24.

tert-Butyl (2-(3-(_tert_-Butyl)-6-(((3*R*,10*R*,13*R*,17*R*)-10,13-dimethyl-17-((*S*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl)carbamoyl)-2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)ethyl)carbamate (10h)

Yield: 65%■■*69% in Table 2?*■■; R_f = 0.32 (hexane–EtOAc 40:60).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.99 (d, *J* = 7.9 Hz, 1 H), 5.32 (br s, 1 H), 5.17 (br s, 1 H), 4.23–4.14 (m, 1 H), 3.72–3.55 (m, 1 H), 3.55–3.42 (m, 1 H), 3.42–3.18 (m, 3 H), 2.74 (dd, *J* = 15.5, 4.1 Hz, 1 H), 2.62–2.46 (m, 1 H), 2.30–2.02 (m, 2 H), 2.02–1.87 (m, 2 H), 1.87–1.72 (m, 3 H), 1.57–0.84 (m, 51 H), 0.65 (s, 3 H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 173.4, 167.6, 167.5, 157.7, 156.2, 140.0, 122.0, 79.3, 58.0, 56.9, 56.7, 56.2, 50.1, 50.0, 42.3, 41.6, 39.8, 39.5, 39.3, 39.1, 39.0, 37.8, 36.5, 36.2, 35.7, 31.9, 31.8, 28.9, 28.6, 28.4, 28.2, 27.9, 24.2, 23.8, 22.7, 22.5, 21.0, 19.3, 18.7, 11.8.

ESI-MS: *m*/*z* (%) = 725.5 ([M + Na]$^+$, 4); 747.5 ([M + Na]$^+$, 100).

Anal. Calcd for $C_{43}H_{72}N_4O_5$: C, 71.23; H, 10.01; N, 7.73. Found: C, 71.23; H, 9.99; N, 7.72.

tert-Butyl (2-((_tert_-Butoxycarbonyl)amino)ethyl)(2-(3-(_tert_-butyl)-6-(dodecylcarbamoyl)-2,4-dioxotetrahydropyrimidin-1(2_H_)-yl)ethyl)carbamate (10i)

Yield: 77%; R_f = 0.21 (hexane–EtOAc 40:60).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.08 (br s, 1 H), 5.26–5.04 (m, 1 H), 4.33–4.14 (m, 1 H), 3.69–3.57 (m, 1 H), 3.57–3.41 (m, 1 H), 3.38–3.02 (m, 8 H), 2.71 (dd, *J* = 15.6, 4.5 Hz, 1 H), 2.65–2.43 (m, 1 H), 1.53 (s, 9 H), 1.42–1.20 (m, 38 H), 0.90–0.71 (m, 3 H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 173.6, 168.3, 155.8, 80.2, 79.1, 57.8, 56.3, 45.4, 39.73, 39.66, 31.8, 29.6, 29.55, 29.50, 29.46, 29.24, 29.20, 28.6, 28.4, 28.3, 26.9, 22.6, 14.0.

ESI-MS: *m*/*z* (%) = 798.4 ([M + Na]$^+$, 100).

Anal. Calcd for $C_{35}H_{65}N_5O_7$: C, 62.94; H, 9.81; N, 10.49. Found: C, 62.94; H, 9.82; N, 10.50.

tert-Butyl (2-((_tert_-Butoxycarbonyl)amino)ethyl)(2-(3-(_tert_-butyl)-6-(octadecylcarbamoyl)-2,4-dioxotetrahydropyrimidin-1(2_H_)-yl)ethyl)carbamate (10j)

Yield: 78%■■*73% in Table 2?*■■; R_f = 0.23 (hexane–EtOAc 40:60).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.15 (br s, 1 H), 5.17 (br s, 1 H), 4.24 (br s, 1 H), 3.72–3.56 (m, 1 H), 3.56–3.42 (m, 1 H), 3.34–2.99 (m, 8 H), 2.70 (dd, *J* = 15.7, 4.3 Hz, 1 H), 2.64–2.44 (m, 1 H), 1.51 (s, 9 H), 1.41–1.15 (m, 50 H), 0.88 (t, *J* = 6.7 Hz, 3 H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 173.4, 168.1, 157.1, 155.6, 80.0, 78.9, 57.6, 56.1, 47.7, 45.2, 39.66, 39.67, 36.5, 31.6, 29.8, 29.50, 29.46, 29.24, 29.20, 29.0, 28.4, 28.2, 28.1, 26.7, 22.4, 13.8.

ESI-MS: *m*/*z* (%) = 882.5 ([M + Na]$^+$, 100).

Anal. Calcd for $C_{41}H_{77}N_5O_7$: C, 65.48; H, 10.32; N, 9.31. Found: C, 65.50; H, 10.31; N, 9.30.

tert-Butyl (_Z_)-(2-((_tert_-Butoxycarbonyl)amino)ethyl)(2-(3-(_tert_-butyl)-6-(octadec-9-en-1-ylcarbamoyl)-2,4-dioxotetrahydropyrimidin-1(2_H_)-yl)ethyl)carbamate (10k)

Yield: 80%; R_f = 0.35 (hexane–EtOAc 40:60).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.11 (br s, 1 H), 5.39–5.22 (m, 2 H), 5.15 (br s, 1 H), 4.31–4.07 (m, 1 H), 3.68–3.55 (m, 1 H), 3.55–3.42 (m, 1 H), 3.39–3.07 (m, 8 H), 2.71 (dd, *J* = 15.5, 4.6 Hz, 1 H), 2.66–2.43 (m, 1 H), 2.03–1.82 (m, 3 H), 1.53 (s, 9 H), 1.39–1.17 (m, 42 H), 0.82 (t, *J* = 5.9 Hz, 3 H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 173.7, 168.3, 155.8, 129.9, 129.7, 128.4, 127.4, 126.9, 80.2, 79.2, 65.1, 56.3, 45.4, 39.8, 32.5, 31.8, 29.7, 29.61, 29.58, 29.47, 29.44, 29.4, 29.4, 29.25, 29.22, 29.19, 29.17, 28.6, 28.4, 28.3, 27.17, 27.15, 26.9, 22.6, 14.0.

ESI-MS: *m*/*z* (%) = 880.5 ([M + Na]$^+$, 100).

Anal. Calcd for $C_{41}H_{75}N_5O_7$: C, 65.65; H, 10.08; N, 9.34. Found: C, 65.65; H, 10.10; N, 9.32.

tert-Butyl (2-((_tert_-Butoxycarbonyl)amino)ethyl)(2-(3-(_tert_-butyl)-6-(((3_R_,10_R_,13_R_,17_R_)-10,13-dimethyl-17-((_S_)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1_H_-cyclopenta[_a_]phenanthren-3-yl)carbamoyl)-2,4-dioxotetrahydropyrimidin-1(2_H_)-yl)ethyl)carbamate (10l)

Yield: 69%■■*66% in Table 2?*■■; R_f = 0.28 (hexane–EtOAc 40:60).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.99 (br s, 1 H), 5.35–5.21 (m, 1 H), 5.15 br (s, 1 H), 4.24 (br s, 1 H), 3.71–3.40 (m, 3 H), 3.36–3.06 (m, 6 H), 2.70 (dd, *J* = 15.4, 4.2 Hz, 1 H), 2.63–2.37 (m, 1 H), 2.28–2.12 (m, 1 H), 2.12–1.83 (m, 3 H), 1.83–1.66 (m, 3 H), 1.53 (s, 9 H), 1.47–0.80 (m, 60 H), 0.62 (s, 3 H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 173.6, 167.5, 155.8, 140.1, 122.0, 80.2, 79.2, 56.7, 56.4, 56.2, 50.1, 49.9, 45.5, 42.3, 39.8, 39.5, 39.13, 39.05, 37.8, 36.5, 36.2, 35.7, 31.9, 31.8, 28.9, 28.6, 28.40, 28.36, 28.3, 28.2, 27.9, 24.2, 23.8, 22.7, 22.5, 21.0, 19.2, 18.7, 11.8.

ESI-MS: *m*/*z* (%) = 998.7 ([M + Na]$^+$, 100).

Anal. Calcd for $C_{50}H_{85}N_5O_7$: C, 69.17; H, 9.87; N, 8.07. Found: C, 69.18; H, 9.88; N, 8.05.

Di-_tert_-butyl ((5_R_)-6-((6-(3-(_tert_-Butyl)-6-(octadecylcarbamoyl)-2,4-dioxotetrahydropyrimidin-1(2_H_)-yl)hexyl)amino)-6-oxohexane-1,5-diyl)dicarbamate (10m)

Yield: 71%■■*82% in Table 2?*■■; R_f = 0.21 (hexane–EtOAc 20:80).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.46–6.44 (m, 1 H), 6.26–6.24 (m, 1 H), 5.29 (br s, 1 H), 4.73 (br s, 1 H), 4.16 (br s, 1 H), 4.02 (br s, 1 H), 3.55–3.52 (m, 1 H), 3.23–3.20 (m, 4 H), 3.11–3.08 (m, 3 H), 2.55 (dd, *J* = 8.8, 5.2 Hz, 1 H), 2.52–2.50 (m, 1 H), 1.65–1.60 (m, 1 H), 1.59–1.25 (m, 72 H), 0.88 (t, *J* = 6.8 Hz, 3 H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 173.8, 172.2, 168.3, 157.0, 156.2, 155.8, 79.9, 79.1, 57.95, 57.93, 55.9, 55.8, 40.9, 39.8, 39.0, 37.1, 37.0, 29.7, 29.64, 29.63, 29.3, 28.7, 28.4, 28.3, 22.7, 14.1.

ESI-MS: *m*/*z* (%) = 915.9 ([M + Na]$^+$, 100).

Anal. Calcd for $C_{49}H_{92}N_6O_8$: C, 65.88; H, 10.38; N, 9.41. Found: C, 65.90; H, 10.39; N, 9.41.

Di-_tert_-butyl ((5_R_)-6-((6-(3-(_tert_-Butyl)-6-(((_Z_)-octadec-9-en-1-yl)carbamoyl)-2,4-dioxotetrahydropyrimidin-1(2_H_)-yl)hexyl)amino)-6-oxohexane-1,5-diyl)dicarbamate (10n)

Yield: 73%■■*87% in Table 2?*■■; R_f = 0.24 (hexane–EtOAc 20:80).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.36–6.34 (m, 1 H), 6.16–6.14 (m, 1 H), 5.35–5.33 (m, 2 H), 5.22 (br s, 1 H), 4.67 (br s, 1 H), 4.16 (q, *J* = 3.2 Hz, 1 H), 4.01 (br s, 1 H), 3.55–3.52 (m, 1 H), 3.23–3.20 (m, 4 H), 3.11–3.08 (m, 3 H), 2.72 (dd, *J* = 12.4, 3.2 Hz, 1 H), 2.55–2.51 (m, 1 H), 2.05–2.00 (m, 4 H), 1.85–1.80 (m, 1 H), 1.60–1.25 (m, 64 H), 0.88 (t, *J* = 5.2 Hz, 3 H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 174.1, 172.3, 168.5, 157.3, 156.5, 130.3, 130.0, 80.2, 79.4, 58.2, 56.2, 56.1, 54.9, 30.0, 29.9, 29.8, 29.7, 29.6, 29.55, 29.52, 29.4, 29.0, 28.7, 28.6, 27.5, 27.2, 26.1, 22.9, 14.4.

ESI-MS: *m*/*z* (%) = 913.9 ([M + Na]$^+$, 100).

Anal. Calcd for $C_{49}H_{90}N_6O_8$: C, 66.03, H, 10.18; N, 9.43. Found: C, 66.04; H, 10.20, N, 9.44.

Di-_tert_-butyl ((5_S_)-6-((6-(3-(_tert_-Butyl)-6-(((3_R_,10_R_,13_R_,17_R_)-10,13-dimethyl-17-((_S_)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1_H_-cyclopenta[_a_]phenanthren-3-yl)carbamoyl)-2,4-dioxotetrahydropyrimidin-1(2_H_)-yl)hexyl)amino)-6-oxohexane-1,5-diyl)dicarbamate (10o)

Yield: 70%; R_f = 0.33 (hexane–EtOAc 30:70).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.34–6.32 (m, 1 H), 6.01–5.94 (m, 1 H), 5.30–5.28 (m, 1 H), 5.17 (br s, 1 H), 4.61 (br s, 1 H), 4.10–4.08 (m, 1 H), 3.95–3.93 (m, 1 H), 3.63–3.60 (m, 1 H), 3.48–3.45 (m, 1 H), 3.37–3.33 (m, 2 H), 3.10–2.98 (m, 4 H), 2.65–2.62 (m, 1 H), 2.46–2.43 (m, 1 H), 2.25–2.22 (m, 1 H), 2.05–1.77 (m, 11 H), 1.53–0.78 (m, 68 H), 0.60 (s, 3 H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 173.7, 167.4, 157.0, 156.0, 140.0, 122.1, 57.9, 56.7, 56.1, 55.9, 50.1, 50.0, 42.3, 39.5, 36.6, 35.8, 31.8, 28.7, 28.6, 28.4, 28.0, 23.8, 22.8, 22.6, 19.3, 18.7, 11.8. The two quaternary carbons of the *tert*-butyl group did not appear due to low intensity.

ESI-MS: *m*/*z* (%) = 1032.0 ([M + Na]$^+$, 100).

Anal. Calcd for $C_{58}H_{100}N_6O_8$: C, 69.01; H, 9.99; N, 8.33. Found: C, 69.00; H, 9.99; N, 8.34.

Benzyl 3-(1-(6-((*tert*-Butoxycarbonyl)amino)hexyl)-3-(*tert*-butyl)ureido)-4-(didecylamino)-4-oxobutanoate (12a)

Yield: 76%; R_f = 0.39 (hexane–EtOAc 65:35).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.38–7.24 (m, 5 H), 6.72 br (t, *J* = 5.7 Hz, 1 H), 5.18–5.02 (m, 2 H), 5.01–4.92 (m, 1 H), 4.67–4.57 (m, 2 H), 3.30–3.18 (m, 1 H), 3.17–3.01 (m, 6 H), 2.98–2.84 (m, 1 H), 2.65 (dd, *J* = 16.5, 7.3 Hz, 1 H), 1.51–1.35 (m, 20 H), 1.36–1.11 (m, 46 H), 0.86 (t, *J* = 6.8 Hz, 3 H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 171.2, 169.4, 155.9, 155.8, 135.8, 128.4, 128.2, 128.1, 79.0, 66.4, 51.3, 50.9, 47.4, 46.4, 43.8, 40.4, 35.5, 31.8, 30.0, 29.6, 29.5, 29.4, 29.3, 29.2, 28.9, 28.7, 28.4, 27.4, 27.1, 26.9, 26.8, 26.4, 26.3, 22.6, 14.0.

ESI-MS: *m*/*z* (%) = 823.5 ([M + Na]$^+$, 100).

Anal. Calcd for $C_{47}H_{84}N_4O_6$: C, 70.46; H, 10.57; N, 6.99. Found: C, 70.45; H, 10.57; N, 7.00.

Benzyl 3-(1-(2-((*tert*-Butoxycarbonyl)amino)ethyl)-3-(*tert*-butyl)ureido)-4-(didecylamino)-4-oxobutanoate (12b)

Yield: 82%; R_f = 0.34 (hexane–EtOAc 65:35).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.37–7.25 (m, 5 H), 6.15 (br s, 1 H), 5.64 (br s, 1 H), 5.18–4.96 (m, 2 H), 4.87 (br s, 1 H), 3.40–2.91 (m, 9 H), 2.48 (dd, *J* = 15.9, 5.0 Hz, 1 H), 1.63–1.16 (m, 54 H), 0.89 (t, *J* = 6.6 Hz, 6 H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 171.0, 170.2, 156.7, 156.1, 136.1, 128.6, 128.4, 128.2, 80.1, 77.4, 66.5, 51.4, 50.0, 47.7, 46.6, 42.3, 40.3, 35.5, 32.0, 29.8, 29.7, 29.5, 29.4, 29.1, 28.4, 27.6, 27.3, 27.1, 22.8, 14.2.

ESI-MS: *m*/*z* (%) = 767.5 ([M + Na]$^+$, 100), 745.5 ([M + H]$^+$, 21).

Anal. Calcd for $C_{43}H_{76}N_4O_6$: C, 69.32; H, 10.28; N, 7.52. Found: C, 69.33; H, 10.30; N, 7.50.

Benzyl 8-(*tert*-Butoxycarbonyl)-11-(*tert*-butylcarbamoyl)-12-(didecylcarbamoyl)-2,2-dimethyl-4-oxo-3-oxa-5,8,11-triazatetradecan-14-oate (12c)

Yield: 73%; R_f = 0.35 (hexane–EtOAc 60:40).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.45–7.07 (m, 5 H), 6.33 (br s, 1 H), 5.66 (br s, 1 H), 5.15–4.79 (m, 2 H), 4.66 (s, 1 H), 3.41–2.95 (m, 12 H), 2.95–2.69 (m, 1 H), 2.50–2.31 (m, 1 H), 1.61–1.03 (m, 67 H), 0.96–0.60 (m, 6 H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 170.7, 170.2, 155.9, 155.8, 135.9, 128.4, 128.3, 128.0, 80.7, 79.2, 66.3, 51.2, 49.3, 48.3, 47.6, 47.2, 46.5, 40.3, 39.7, 35.3, 31.8, 29.6, 29.5, 29.42, 29.40, 29.3, 29.24, 29.21, 29.0, 28.4, 28.3, 27.5, 27.1, 27.0, 22.6, 14.0.

ESI-MS: *m*/*z* (%) = 910.7 ([M + Na]$^+$, 100), 888.7 ([M + H]$^+$, 5).

Anal. Calcd for $C_{50}H_{89}N_5O_8$: C, 67.61; H, 10.10; N, 7.88. Found: C, 67.60; H, 10.12; N, 7.90.

Dihydroorotic Acid-Based Amphiphilic 11a–o; General Procedure

To a stirred solution of *N*-Boc-protected derivative **10** (■■?■■ mmol, 1 equiv) in DCM (0.1 M solution) was added neat TFA (10% in volume) at rt and the solution was stirred for 2 h. The solvents were evaporated under reduced pressure and co-evaporated with toluene (3 ×), providing pure dihydroorotic acid-based amphiphilic **11a–o** as trifluoroacetate salts in quantitative yields.

3-(6-Aminohexyl)-1-(*tert*-butyl)-*N*-dodecyl-2,6-dioxohexahydropyrimidine-4-carboxamide x TFA (11a)

^{1}H NMR (CD_3OD, 400 MHz): δ = 4.13 (t, *J* = 4.8 Hz, 1 H), 3.39–3.54 (m, 1 H), 3.21–3.02 (m, 3 H), 2.92 (t, *J* = 7.7 Hz, 2 H), 2.76 (dd, *J* = 15.8, 4.6 Hz, 1 H), 2.67 (dd, *J* = 15.7, 5.2 Hz, 1 H), 1.73–1.29 (m, 37 H), 0.89 (t, *J* = 6.6 Hz, 3 H).

^{13}C NMR (CD_3OD, 101 MHz): δ = 175.3, 170.8, 160.64 (q, *J* = 38.9 Hz), 159.1, 129.6, 116.94 (q, *J* = 287.9 Hz), 58.8, 57.2, 41.7, 40.6, 40.5, 36.5, 33.0, 30.8, 30.73, 30.70, 30.67, 30.44, 30.41, 30.37, 29.0, 28.44, 28.37, 28.0, 27.99, 27.1, 26.8, 23.7, 14.4.

ESI-MS: *m*/*z* (%) = 481.4 ([M + H]$^+$, 100).

Anal. Calcd for $C_{27}H_{52}N_4O_3$: C, 67.46; H, 10.90; N, 11.65. Found: C, 67.47; H, 10.92; N, 11.64.

3-(6-Aminohexyl)-1-(*tert*-butyl)-*N*-ethyl-2,6-dioxohexahydropyrimidine-4-carboxamide x TFA (11b)

^{1}H NMR (CD_3OD, 400 MHz): δ = 4.13 (t, *J* = 4.8 Hz, 1 H), 3.59–3.46 (m, 1 H), 3.20–3.01 (m, 3 H), 2.91 (t, *J* = 7.7 Hz, 2 H), 2.81–2.62 (m, 2 H), 1.71–1.28 (m, 45 H), 0.89 (t, *J* = 6.7 Hz, 3 H)..

^{13}C NMR (CD_3OD, 101 MHz): δ = 173.9, 169.5, 160.54 (q, *J* = 38.8 Hz), 157.7, 116.96 (q, *J* = 287.7 Hz), 57.4, 55.8, 40.3, 39.2, 39.1, 35.2, 31.7, 29.4, 29.32, 29.29, 29.1, 29.0, 28.98, 27.6, 27.0, 26.98, 26.6, 25.7, 25.4, 22.3, 14.7.

ESI-MS: *m*/*z* (%) = 565.5 ([M + H]$^+$, 100).

Anal. Calcd for $C_{33}H_{64}N_4O_3$: C, 70.17; H, 11.42; N, 9.92. Found: C, 70.18; H, 11.44; N, 9.92.

(*Z*)-3-(6-Aminohexyl)-1-(*tert*-butyl)-*N*-(octadec-9-en-1-yl)-2,6-dioxohexahydropyrimidine-4-carboxamide x TFA (11c)

^{1}H NMR (CD_3OD, 500 MHz): δ = 5.43–5.26 (m, 2 H), 4.20 (t, *J* = 4.5 Hz, 1 H), 3.67–3.57 (m, 3 H), 3.48–3.26 (m, 5 H), 3.21–3.10 (m, 2 H), 2.76 (dd, *J* = 15.6, 4.7 Hz, 1 H), 2.03 (dd, *J* = 15.6, 5.1 Hz, 1 H), 1.58 (s, 9 H), 1.53–1.46 (m, 2 H), 1.43–1.25 (m, 30 H), 0.90 (t, *J* = 6.8 Hz, 3 H).

^{13}C NMR (CD_3OD, 126 MHz): δ = 174.4, 171.1, 160.3, 130.85, 130.78, 59.2, 58.8, 48.0, 47.3, 40.7, 40.1, 39.0, 36.0, 33.0, 30.83, 30.80, 30.7, 30.6, 30.5, 30.4, 30.3, 29.0, 28.9, 28.2, 28.13, 28.10, 28.0, 23.7, 14.4.

ESI-MS: *m*/*z* (%) = 563.5 ([M + H]$^+$, 100).

Anal. Calcd for $C_{33}H_{62}N_4O_3$: C, 70.42; H, 11.10; N, 9.95. Found: C, 70.41; H, 11.11; N, 9.97.

3-(6-Aminohexyl)-1-(*tert*-butyl)-*N*-((3*R*,10*R*,13*R*,17*R*)-10,13-dimethyl-17-((*S*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl)-2,6-dioxohexahydropyrimidine-4-carboxamide x TFA (11d)

^{1}H NMR (CD_3OD, 400 MHz): δ = 5.31 (dd, *J* = 12.7, 4.9 Hz, 1 H), 4.23–4.08 (m, 1 H), 3.73–3.57 (m, 2 H), 3.57–3.22 (m, 7 H), 2.86–2.63 (m, 2 H), 1.63–0.89 (m, 47 H), 0.84 (dd, *J* = 6.6, 1.3 Hz, 6 H), 0.69 (s, 3 H).

^{13}C NMR (CD_3OD, 101 MHz): δ = 174.32, 170.4, 160.2, 141.8, 122.7, 58.2, 57.6, 48.1, 45.7, 43.5, 41.1, 40.7, 39.1, 37.8, 37.4, 37.0, 33.2, 33.0, 29.3, 29.1, 28.9, 27.9, 25.3, 25.0, 23.2, 23.0, 22.1, 19.8, 19.3, 12.4.

ESI-MS: *m*/*z* (%) = 681.6 ([M + H]$^+$, 100).

Anal. Calcd for $C_{42}H_{72}N_4O_3$: C, 74.07; H, 10.66; N, 8.23. Found: C, 74.08; H, 10.68; N, 8.22.

3-(2-Aminoethyl)-1-(*tert*-butyl)-*N*-dodecyl-2,6-dioxohexahydropyrimidine-4-carboxamide x TFA (11e)

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.42 (t, *J* = 5.7 Hz, 1 H), 4.17 (t, *J* = 5.1 Hz, 1 H), 3.52–3.41 (m, 1 H), 3.37–3.23 (m, 1 H), 3.22–3.09 (m, 2 H), 2.96–2.83 (m, 2 H), 2.75 (dd, *J* = 15.4, 4.4 Hz, 1 H), 2.56 (dd, *J* = 15.3, 5.7 Hz, 1 H), 2.38 (s, 2 H), 1.56 (s, 9 H), 1.52–1.38 (m, 2 H), 1.35–1.10 (m, 18 H), 0.91–0.77 (m, 3 H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 173.6, 168.4, 157.8, 58.0, 57.1, 44.2, 40.2, 39.8, 36.9, 31.7, 29.4, 29.1, 29.1, 28.5, 26.9, 22.5, 13.9.

ESI-MS: *m*/*z* (%) = 465.4 ([M + K]$^+$, 56), 447.4 ([M + Na]$^+$, 4), 425.4 ([M + H]$^+$, 100).

Anal. Calcd for $C_{23}H_{44}N_4O_3$: C, 65.06; H, 10.44; N, 13.19. Found: C, 65.05; H, 10.44; N, 13.20.

3-(2-Aminoethyl)-1-(*tert*-butyl)-*N*-octadecyl-2,6-dioxohexahydropyrimidine-4-carboxamide x TFA (11f)

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.29 (br t, *J* = 5.8 Hz, 1 H), 4.17 (t, *J* = 5.1 Hz, 1 H), 3.52–3.39 (m, 1 H), 3.36–3.24 (m, 1 H), 3.21–3.09 (m, 2 H), 2.96–2.82 (m, 2 H), 2.75 (dd, *J* = 15.3, 4.5 Hz, 1 H), 2.56 (dd, *J* = 15.3, 5.5 Hz, 1 H), 2.45 (br s, 2 H), 1.57 (s, 9 H), 1.50–1.38 (m, 2 H), 1.24 (s, 32 H), 0.86 (t, *J* = 6.6 Hz, 3 H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 173.7, 168.5, 157.9, 77.1, 58.2, 57.3, 44.4, 40.3, 40.0, 39.8, 37.1, 32.0, 29.8, 29.79, 29.75, 29.65, 29.61, 29.44, 29.39, 28.7, 27.1, 22.8, 14.2.

ESI-MS: *m*/*z* (%) = 509.4 ([M + H]$^+$, 100).

Anal. Calcd for $C_{29}H_{56}N_4O_3$: C, 68.46; H, 11.09; N, 11.01. Found: C, 68.47; H, 11.11; N, 11.00.

(*Z*)-3-(2-Aminoethyl)-1-(*tert*-butyl)-*N*-(octadec-9-en-1-yl)-2,6-dioxohexahydropyrimidine-4-carboxamide x TFA (11g)

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.35–5.29 (m, 2 H), 4.14 (t, *J* = 5.6, Hz, 1 H), 3.49–3.40 (m, 1 H), 3.37–3.29 (m, 1 H), 3.21–3.08 (m, 2 H), 2.97–2.82 (m, 2 H), 2.78–2.70 (m, 2 H), 2.56 (dd, *J* = 15.4, 5.7 Hz, 1 H), 2.05–1.85 (m, 4 H), 1.55 (s, 9 H), 1.48–1.37 (m, 2 H), 1.24 (s, 22 H), 0.85 (t, *J* = 6.6 Hz, 3 H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 173.5, 168.5, 157.9, 129.9, 129.7, 58.0, 57.8, 57.2, 43.9, 39.9, 39.6, 36.6, 32.5, 31.8, 29.7, 29.6, 29.4, 29.3, 29.19, 29.16, 28.5, 27.1, 26.9, 22.6, 18.2, 13.9.

ESI-MS: *m*/*z* (%) = 507.4 ([M + H]$^+$, 100).

Anal. Calcd for $C_{29}H_{54}N_4O_3$: C, 68.73; H, 10.74; N, 11.06. Found: C, 68.75; H, 10.74; N, 11.05.

3-(2-Aminoethyl)-1-(*tert*-butyl)-*N*-((3*R*,10*R*,13*R*,17*R*)-10,13-dimethyl-17-((*S*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl)-2,6-dioxohexahydropyrimidine-4-carboxamide x TFA (11h)

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.41 (d, *J* = 7.9 Hz, 1 H), 6.30 (br s, 2 H), 5.33 (br s, 1 H), 4.12 (t, *J* = 5.6 Hz, 1 H), 3.70–3.47 (m, 3 H), 3.32–3.02 (m, 2 H), 2.87 (dd, *J* = 15.3, 4.3 Hz, 1 H), 2.69–2.59 (m, 1 H), 2.29–2.16 (m, 2 H), 2.14–2.07 (m, 2 H), 2.00–1.92 (m, 2 H), 1.88–1.71 (m, 3 H), 1.57 (s, 9 H), 1.52–0.82 (m, 30 H), 0.67 (s, 3 H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 172.8, 168.1, 161.9 (q, *J* = 37.5 Hz), 158.3, 140.0, 139.9, 122.1, 60.3, 58.3, 58.2, 56.7, 56.2, 50.3, 50.1, 42.3, 39.8, 39.5, 39.0, 38.7, 37.8, 36.5, 36.2, 36.0, 35.8, 31.8, 28.8, 28.5, 28.2, 28.0, 24.2, 23.9, 22.7, 22.5, 21.0, 19.2, 18.7, 14.1, 11.8.

ESI-MS: *m*/*z* (%) = 665.6 ([M + K]$^+$, 28), 625.5 ([M + H]$^+$, 100).

Anal. Calcd for $C_{38}H_{64}N_4O_3$: C, 73.03; H, 10.32; N, 8.97. Found: C, 73.01; H, 10.30; N, 8.98.

3-(2-((2-Aminoethyl)amino)ethyl)-1-(*tert*-butyl)-*N*-dodecyl-2,6-dioxohexahydropyrimidine-4-carboxamide x 2TFA (11i)

^{1}H NMR (CD_3OD, 400 MHz): δ = 4.27 (br t, *J* = 4.5 Hz, 1 H), 3.86–3.66 (m, 2 H), 3.56–3.38 (m, 6 H), 3.29–3.14 (m, 2 H), 2.87 (qd, *J* = 15.6, 4.5 Hz, 2 H), 1.64 (s, 9 H), 1.60–1.49 (m, 2 H), 1.37 (s, 18 H), 0.96 (m, 3 H).

^{13}C NMR (CD_3OD, 101 MHz): δ = 174.3, 171.3, 161.0 (q, *J* = 38.4 Hz), 160.3, 117.5 (q, *J* = 290.9 Hz), 59.2, 59.0, 48.1, 45.7, 40.7, 40.2, 37.0, 36.0, 30.4, 30.2, 28.9, 28.0, 23.6, 14.3.

ESI-MS: *m*/*z* (%) = 480.4 ([M + Na]$^+$, 32), 468.4 ([M + H]$^+$, 100).

Anal. Calcd for $C_{25}H_{49}N_5O_3$: C, 64.20; H, 10.56; N, 14.97. Found: C, 64.21; H, 10.54; N, 14.98.

3-(2-((2-Aminoethyl)amino)ethyl)-1-(*tert*-butyl)-*N*-octadecyl-2,6-dioxohexahydropyrimidine-4-carboxamide x 2TFA (11j)

^{1}H NMR (CD_3OD, 400 MHz): δ = 4.21 (t, *J* = 4.5 Hz, 1 H), 3.78–3.60 (m, 2 H), 3.51–3.32 (m, 6 H), 3.22–3.05 (m, 2 H), 2.80 (qd, *J* = 15.6, 4.6 Hz, 2 H), 1.56 (s, 9 H), 1.54–1.45 (m, 2 H), 1.28 (s, 32 H), 0.89 (t, *J* = 6.9 Hz, 3 H).

^{13}C NMR (CD_3OD, 101 MHz): δ = 174.3, 171.2, 161.7 (q, *J* = 37.4 Hz), 160.2, 117.3 (q, *J* = 290.9 Hz), 59.2, 58.8, 48.0, 45.7, 40.7, 40.1, 37.0, 36.0, 33.0, 30.7, 30.64, 30.60, 30.3, 30.2, 28.9, 28.0, 23.6, 14.4.

ESI-MS: *m*/*z* (%) = 552.5 ([M + H]$^+$, 100).

Anal. Calcd for $C_{31}H_{61}N_5O_3$: C, 67.47; H, 11.14; N, 12.69. Found: C, 67.48; H, 11.12; N, 12.70.

(*Z*)-3-(2-((2-Aminoethyl)amino)ethyl)-1-(*tert*-butyl)-*N*-(octadec-9-en-1-yl)-2,6-dioxohexahydropyrimidine-4-carboxamide x 2TFA (11k)

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.42–5.29 (m, 2 H), 4.21 (t, *J* = 4.5 Hz, 1 H), 3.79–3.60 (m, 2 H), 3.49–3.31 (m, 6 H), 3.22–3.05 (m, 2 H), 2.80 (qd, *J* = 15.6, 4.6 Hz, 2 H), 2.09–1.92 (m, 3 H), 1.57 (s, 9 H), 1.48 (m, 3 H), 1.39–1.10 (m, 24 H), 0.89 (t, *J* = 7.3 Hz, 3 H).

^{13}C NMR (CD_3OD, 101 MHz): δ = 174.3, 171.2, 161.9 (q, *J* = 37.4 Hz), 160.2, 130.8, 130.7, 117.5 (q, *J* = 289.9 Hz), 59.2, 58.9, 48.0, 45.7, 40.7, 40.2, 37.0, 36.0, 33.5, 33.0, 30.8, 30.7, 30.6, 30.59, 30.54, 30.51, 30.4, 30.37, 30.34, 30.31, 30.2, 30.15, 30.11, 28.9, 28.14, 28.10, 28.0, 23.6, 14.3.

ESI-MS: *m*/*z* (%) = 550.5 ([M + H]$^+$, 100).

Anal. Calcd for $C_{31}H_{59}N_5O_3$: C, 67.72; H, 10.82; N, 12.74. Found: C, 67.70; H, 10.82; N, 12.75.

3-(2-((2-Aminoethyl)amino)ethyl)-1-(*tert*-butyl)-*N*-((3*R*,10*R*,13*R*,17*R*)-10,13-dimethyl-17-((*S*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl)-2,6-dioxohexahydropyrimidine-4-carboxamide x 2TFA (11l)

^{1}H NMR (CD_3OD, 400 MHz): δ = 5.31 (dd, *J* = 12.7, 4.9 Hz, 1 H), 4.23–4.08 (m, 1 H), 3.73–3.57 (m, 2 H), 3.57–3.22 (m, 7 H), 2.86–2.63 (m, 2 H), 2.25–1.62 (m, 9 H), 1.63–0.84 (m, 40 H), 0.69 (s, 3 H).

^{13}C NMR (CD_3OD, 101 MHz): δ = 174.32, 174.27, 170.4, 161.7 (q, *J* = 38.3 Hz), 160.2, 141.8, 141.7, 122.73, 122.68, 117.2 (q, *J* = 289.5 Hz), 59.2, 58.9, 58.8, 58.2, 57.6, 51.7, 51.4, 48.1, 45.7, 43.5, 41.1, 40.7, 39.1, 37.8, 37.4, 37.0, 33.2, 33.0, 29.3, 29.1, 28.9, 27.9, 25.3, 25.0, 23.2, 23.0, 22.1, 19.8, 19.3, 12.4.

ESI-MS: *m*/*z* (%) = 668.6 ([M + H]$^+$ (100).

Anal. Calcd for $C_{40}H_{69}N_5O_3$: C, 71.92; H, 10.41; N, 10.48. Found: C, 71.92; H, 10.42; N, 10.50.

1-(*tert*-Butyl)-3-(6-((*S*)-2,6-diaminohexanamido)hexyl)-*N*-octadecyl-2,6-dioxohexahydropyrimidine-4-carboxamide x 2TFA (11m)

^{1}H NMR (CD_3OD, 400 MHz): δ = 4.15 (t, *J* = 4.8 Hz, 1 H), 3.85 (t, *J* = 6.8 Hz, 1 H), 3.57–3.52 (m, 1 H), 3.27–3.24 (m, 2 H), 3.18–3.15 (m, 2 H), 3.09–3.04 (m, 1 H), 2.98–2.96 (m, 2 H), 2.76 (dd, *J* = 15.6, 4.8 Hz, 1 H), 2.67 (dd, *J* = 15.6, 5.2 Hz, 1 H), 1.90–1.88 (m, 2 H), 1.74–1.70 (m, 2 H), 1.60 (s, 9 H), 1.58–1.48 (m, 8 H), 1.33–1.28 (m, 34 H), 0.91 (t, *J* = 6.8 Hz, 3 H).

^{13}C NMR (CD_3OD, 101 MHz): δ = 174.0, 169.5, 168.4, 160.4 (q, *J* = 36.4 Hz), 157.7, 116.2 (q, *J* = 291.9 Hz), 57.4, 55.7, 52.9, 39.1, 38.9, 31.7, 29.4, 29.34, 29.32, 29.28, 29.1, 29.0, 28.9, 28.7, 27.6, 26.7, 26.6, 22.3, 21.6, 13.0.

ESI-MS: *m*/*z* (%) = 717.8 ([M + K]$^+$, 100), 705.8 ([M + Na]$^+$, 75), 693.8 ([M + H]$^+$, 21).

Anal. Calcd for $C_{39}H_{76}N_6O_4$: C, 67.59; H, 11.05; N, 12.13. Found: C, 67.58; H, 11.03; N, 12.14.

1-(*tert*-Butyl)-3-(6-((*R*)-2,6-diaminohexanamido)hexyl)-*N*-((*Z*)-octadec-9-en-1-yl)-2,6-dioxohexahydropyrimidine-4-carboxamide x 2TFA (11n)

^{1}H NMR (CD_3OD, 400 MHz): δ = 5.40–5.33 (m, 2 H), 4.16 (t, *J* = 5.2 Hz, 1 H), 3.84 (t, *J* = 6.4 Hz, 1 H), 3.58–3.53 (m, 1 H), 3.27–3.24 (m, 2 H), 3.18–3.15 (m, 2 H), 3.09–3.04 (m, 1 H), 2.98–2.94 (m, 2 H), 2.75 (dd, *J* = 16.0, 4.8 Hz, 1 H), 2.67 (dd, *J* = 16.0, 5.2 Hz, 1 H), 2.08–2.04 (m, 3 H), 1.93–1.89 (m, 2 H), 1.79–1.74 (m, 2 H), 1.61 (s, 9 H), 1.58–1.50 (m, 8 H), 1.34–1.29 (m, 27 H), 0.92 (t, *J* = 6.4 Hz, 3 H).

^{13}C NMR (CD_3OD, 101 MHz): δ = 174.0, 169.5, 168.4, 160.7 (q, *J* = 36.4 Hz), 157.7, 129.5, 129.4, 57.4, 55.7, 52.9, 39.1, 38.9, 31.6, 30.7, 29.4, 29.3, 29.2, 29.0, 28.9, 28.8, 28.7, 27.6, 26.7, 26.6, 25.9, 22.3, 21.6, 13.0.

ESI-MS: *m*/*z* (%) = 691.9 ([M + H]$^+$, 100).

Anal. Calcd for $C_{39}H_{74}N_6O_4$: C, 67.78; H, 10.79; N, 12.16. Found: C, 67.77; H, 10.80; N, 12.16.

1-(*tert*-Butyl)-3-(6-((*S*)-2,6-diaminohexanamido)hexyl)-*N*-((3*R*,10*R*,13*R*,17*R*)-10,13-dimethyl-17-((*S*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl)-2,6-dioxohexahydropyrimidine-4-carboxamide x 2TFA (11o)

^{1}H NMR (CD_3OD, 400 MHz): δ = 5.37 (br s, 1 H), 4.16 (t, *J* = 4.8 Hz, 1 H), 3.84 (t, *J* = 6.4 Hz, 1 H), 3.57–3.51 (m, 2 H), 3.26–3.23 (m, 2 H), 3.09–3.04 (m, 1 H), 2.96 (t, *J* = 7.6 Hz, 2 H), 2.75 (dd, *J* = 16.0, 4.8 Hz, 1 H), 2.65 (dd, *J* = 16.0, 5.2 Hz, 1 H), 2.22–2.18 (m, 2 H), 2.08–0.88 (m, 61 H), 0.74 (s, 3 H).

^{13}C NMR (CD_3OD, 101 MHz): δ = 173.9, 168.6, 168.4, 157.7, 140.4, 121.3, 57.4, 56.8, 55.8, 52.9, 50.3, 49.8, 42.1, 39.3, 38.9, 36.4, 31.8, 30.7, 27.7, 27.6, 26.7, 26.6, 21.8, 21.5, 18.4, 17.9, 10.9.

ESI-MS: *m*/*z* (%) = 809.9 ([M + H]$^+$, 21%).

Anal. Calcd for $C_{48}H_{84}N_6O_4$: C, 71.24; H, 10.46; N, 10.39. Found: C, 71.25; H, 10.47; N, 10.39.

Methylamide Derivative 2-(1-(2-Aminoethyl)-3-(*tert*-butyl)ureido)-*N*^{1},*N*^{1}-didecyl-*N*4-methylsuccinamide (13)

Compound **12b** (100 mg, 0.12 mmol) was dissolved in an 8 M ethanolic solution of $MeNH_2$ (2 mL). After 30 min, the solution was diluted with EtOAc and washed with aq 1 N HCl. The organic layer was dried (Na_2SO_4), filtered and the solvent evaporated under pressure. The crude was purified by flash chromatography. The obtained *N*-Boc methyl amide was treated with a 10% solution of TFA in DCM (2 mL) for 30 min. The solvents were evaporated under pressure and co-evaporated with toluene (2 ×); yield: 83 mg (94%).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.42 (br d, *J* = 5.7 Hz, 1 H), 6.69 (s, 1 H), 6.54 (br s, 2 H), 5.09 (dd, *J* = 9.6, 4.3 Hz, 1 H), 3.56–3.40 (m, 1 H), 3.40–3.08 (m, 5 H), 2.94–2.67 (m, 5 H), 2.58 (dd, *J* = 15.7, 4.1 Hz, 1 H), 1.55 (t, *J* = 7.3 Hz, 4 H), 1.36–1.11 (m, 41 H), 0.94–0.78 (m, 6 H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 171.6, 169.2, 158.5, 162.0 (q, *J* = 34.7 Hz), 116.7 (q, *J* = 294.1 Hz), 77.2, 53.2, 51.0, 47.7, 46.7, 42.8, 40.8, 37.3, 31.8, 29.5, 29.5, 29.4, 29.3, 29.2, 29.0, 27.3, 27.1, 27.0, 26.2, 22.6, 13.9.

ESI-MS: *m*/*z* (%) = 590.6 ([M + Na]$^+$, 3); 568.5 ([M + H]$^+$, 100).

Anal. Calcd for $C_{32}H_{65}N_5O_3$: C, 67.68; H, 11.54; N, 12.33. Found: C, 67.70; H, 11.54; N, 12.32.

Guanidine Dihydroorotic Acid-Based Amphiphilic Compounds 14a–c; General Procedure

To a solution of compound **11** (■■?■■ mmol) in DCM (0.1 M solution) were added TEA (1.5 equiv per TFA) followed by solid *N*,*N*′-bis-boc-1-guanylpyrazole (1.2 equiv per NH_2) at rt and the solution was stirred overnight. The solution was diluted with DCM and washed with aq 1 N HCl and brine. The organic layer was dried (Na_2SO_4), filtered and the solvent evaporated under reduced pressure. The crude was purified by flash chromatography. The obtained *N*-Boc guanidino derivatives were treated with a 20% solution of TFA in DCM (0.1 M solution) for 2 h. The solvents were evaporated under pressure and co-evaporated with toluene (2 ×) providing pure guanidine dihydroorotic acid-based amphiphilic derivatives **14a–c** as TFA salts.

(*Z*)-1-(*tert*-Butyl)-3-(2-guanidinoethyl)-*N*-(heptadec-8-en-1-yl)-2,6-dioxohexahydropyrimidine-4-carboxamide x TFA (14a)

Yield: 83%.

^{1}H NMR (CD_3OD, 400 MHz): δ =5.42–5.19 (m, 2 H), 4.14 (dd, *J* = 5.7, 4.1 Hz, 1 H), 3.70–3.52 (m, 1 H), 3.41–3.19 (m, 5 H), 3.11 (t, *J* = 7.0 Hz, 2 H), 2.75 (dd, *J* = 15.9, 4.2 Hz, 1 H), 2.61 (dd, *J* = 15.9, 5.8 Hz, 1 H), 2.05–1.84 (m, 3 H), 1.53 (s, 9 H), 1.49–1.36 (m, 3 H), 1.36–1.09 (m, 24 H), 0.84 (t, *J* = 6.5 Hz, 3 H).

^{13}C NMR (CD_3OD, 101 MHz): δ = 174.8, 171.0, 159.6, 158.9, 130.8, 130.8, 59.0, 57.9, 41.5, 40.7, 40.6, 36.6, 33.5, 33.0, 30.8, 30.7, 30.6, 30.6, 30.5, 30.4, 30.34, 30.26, 28.93, 28.89, 28.1, 28.0, 23.6, 14.3.

ESI-MS: *m*/*z* (%) = 549.3 ([M + H]$^+$, 100).

Anal. Calcd for $C_{30}H_{56}N_6O_3$: C, 65.66; H, 10.29; N, 15.31. Found: C, 65.67; H, 10.30; N, 15.30.

1-(*tert*-Butyl)-3-(2-((2-guanidinoethyl)amino)ethyl)-*N*-octadecyl-2,6-dioxohexahydropyrimidine-4-carboxamide x 2TFA (14b)

Yield: 79%.

^{1}H NMR (CD_3OD, 400 MHz): δ = 4.24–4.13 (m, 1 H), 3.84–3.57 (m, 4 H), 3.45–3.24 (m, 4 H), 3.24–3.07 (m, 2 H), 2.88–2.67 (m, 2 H), 1.57 (s, 9 H), 1.54–1.40 (m, 2 H), 1.29 (s, 28 H), 0.90 (t, *J* = 6.7 Hz, 3 H).

^{13}C NMR (CD_3OD, 101 MHz): δ = 174.4, 171.1, 163.0, 162.3, 162.0, 162.18 (q, *J* = 42.3 Hz), 159.0, 121.27 (q, *J* = 298.4 Hz), 86.0, 59.2, 58.8, 47.9, 47.3, 40.7, 40.0, 39.0, 36.0, 33.0, 30.7, 30.6, 30.4, 30.3, 29.0, 28.9, 28.2, 28.0, 23.7, 14.4.

ESI-MS: *m*/*z* (%) = 594.5 ($[M + H]^+$ (100).

Anal. Calcd for $C_{32}H_{63}N_7O_3$: C, 64.72; H, 10.69; N, 16.51. Found: C, 64.75; H, 10.71; N, 16.48.

1-(*tert*-Butyl)-3-(6-((*S*)-2,6-diguanidinohexanamido)hexyl)-*N*-((3*R*,10*R*,13*R*,17*R*)-10,13-dimethyl-17-((*S*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl)-2,6-dioxohexahydropyrimidine-4-carboxamide x 2TFA (14c)

Yield: 85%.

^{1}H NMR (CD_3OD, 400 MHz): δ = 5.37 (s, 1 H), 4.16–4.10 (m, 2 H), 3.77–3.74 (m, 1 H), 3.55–3.45 (m, 3 H), 3.22–3.18 (m, 5 H), 3.10–3.05 (m, 1 H), 2.74 (dd, *J* = 16.0, 4.8 Hz, 1 H), 2.65 (dd, *J* = 16.0, 5.6 Hz, 1 H), 2.21–2.17 (m, 2 H), 2.08–0.96 (m, 52 H), 0.90 (d, *J* = 1.6 Hz, 3 H), 0.89 (d, *J* = 1.6 Hz, 3 H), 0.74 (s, 3 H).

^{13}C NMR (CD_3OD, 101 MHz): δ = 173.9, 170.5, 168.9, 157.2, 140.4, 121.3, 65.4, 57.4, 56.8, 56.2, 55.1, 50.3, 42.1, 40.8, 39.3, 36.4, 31.9, 28.1, 27.7, 27.6, 27.5, 26.6, 23.5, 22.2, 21.8, 21.5, 18.4, 17.8, 10.9.

ESI-MS: *m*/*z* (%) = 894.0 ($[M + H]^+$, 100).

Anal. Calcd for $C_{50}H_{88}N_{10}O_4$: C, 67.23; H, 9.93; N, 15.68. Found: C, 67.22; H, 9.95; N, 15.67.

Conflict of Interest

The authors declare no conflict of interest.

Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/a-1913-3105.

References

(1) Santos, D. K. F.; Rufino, R. D.; Luna, J. M.; Santos, V. A.; Sarubbo, L. A. *Int. J. Mol. Sci.* **2016**, *17*, 401.

(2) (a) Felgner, P. L. G.; Gadek, T. R.; Holm, M.; Roman, R.; Chan, H. W.; Wenz, M.; Northrop, J. P.; Ringold, M. G.; Danielsen, M. *Proc. Natl. Acad. Sci. U. S. A.* **1987**, *84*, 7413. (b) Behr, J. P.; Demeneix, B.; Loeffer, J. P.; Perez-Mutul, J. *Proc. Natl. Acad. Sci. U. S. A.* **1989**, *86*, 6982. (c) Wu, G. Y.; Wu, C. H. *J. Biol. Chem.* **1987**, *262*, 4429.

(3) (a) Zabner, J. *Adv. Drug Delivery Rev.* **1997**, *27*, 17. (b) Zhi, D.; Zhang, S.; Cui, S.; Zhao, Y.; Wang, Y.; Zhao, D. *Bioconjugate Chem.* **2013**, *24*, 487. (c) Zhang, S.; Zhao, B.; Jiang, H.; Wang, B.; Ma, B. *J. Control. Release* **2007**, *123*, 1. (d) Bellucci, M. C.; Volonterio, A. *Antibiotics* **2020**, *9*, 504. (e) Zhou, M.; Zheng, M.; Cai, J. *ACS Appl. Mater. Interfaces* **2020**, *12*, 21292. (f) Zhang, N.; Ma, S. *Eur. J. Med. Chem.* **2019**, *184*, 111743.

(4) (a) Shai, Y. *Biopolymers* **2002**, *66*, 236. (b) Brogen, K. A. *Nat. Rev. Microbiol.* **2005**, *3*, 238. (c) Anderson, N.; Borlak, J. *FEBS Lett.* **2006**, *23*, 5533.

(5) (a) Miller, A. D. *Angew. Chem. Int. Ed.* **1998**, *37*, 1768. (b) Molla, M. R.; Chakraborty, S.; Munoz-Sagredo, L.; Drechsler, M.; Orian-Rousseau, V.; Levkin, P. A. *Bioconjugate Chem.* **2020**, *31*, 852.

(6) (a) D?rmling, A.; Ugi, I. *Angew. Chem. Int. Ed.* **2000**, *39*, 3168. (b) D?rmling, A. *Chem. Rev.* **2006**, *106*, 17. (c) D?rmling, A.; Wang, W.; Wang, K. *Chem. Rev.* **2012**, *112*, 3083.

(7) (a) Zhang, Z.; You, Y. Z.; Hong, C. Y. *Macromol. Rapid Commun.* **2018**, *39*, 1800362. (b) Llevot, A.; Boukis, A. C.; Oelmann, S.; Wetzel, K.; Meier, M. A. R. *Top. Curr. Chem.* **2017**, *375*, 66. (c) Rivera, D. G.; Ricardo, M. G.; Vasco, A. V.; Wessjohann, L. A.; Van der Eycken, E. V. *Nat. Protoc.* **2021**, *16*, 561. (d) Reguera, L.; Mendez, Y.; Humpierre, A. R.; Valdes, O.; Rivera, D. G. *Acc. Chem. Res.* **2018**, *51*, 1475.

(8) Malinakova, H. C. *Rep. Org. Chem.* **2015**, *5*, 75.

(9) (a) Molla, M. R.; B?ser, A.; Rana, A.; Schwarz, K.; Levkin, P. A. *Bioconjugate Chem.* **2018**, *29*, 992. (b) Molla, M. R.; Chakraborty, S.; Munoz-Sagredo, L.; Drechsler, M.; Orian-Rousseau, V.; Levkin, P. A. *Bioconjugate Chem.* **2020**, *31*, 852.

(10) (a) Bellucci, M. C.; Terraneo, G.; Volonterio, A. *Org. Biomol. Chem.* **2013**, *11*, 2421. (b) Bellucci, M. C.; Volonterio, A. *Eur. J. Org. Chem.* **2014**, *11*, 2386. (c) Bellucci, M. C.; Sani, M.; Sganappa, A.; Volonterio, A. *ACS Comb. Sci.* **2014**, *16*, 711.

(11) Sganappa, A.; Bellucci, M. C.; Nizet, V.; Tor, Y.; Volonterio, A. *Synthesis* **2016**, *48*, 4443.

(12) Bellucci, M. C.; Frigerio, M.; Castellano, C.; Meneghetti, F.; Sacchetti, A.; Volonterio, A. *Org. Biomol. Chem.* **2018**, *16*, 521.

(13) Bellucci, M. C.; Sacchetti, A.; Volonterio, A. *ACS Comb. Sci.* **2019**, *21*, 705.

(14) (a) Majeti, B. K.; Singh, R. S.; Yadav, S. Q.; Bathula, S. R.; Ramakrishna, S.; Diwan, P. V.; Madhavendra, S. S.; Chaudhuri, A. *Chem. Biol.* **2004**, *11*, 427. (b) Chen, H.; Zhang, H.; McCallum, C. M.; Szoka, F. C.; Guo, X. *J. Med. Chem.* **2007**, *50*, 4269. (c) Jubeli, E.; Maginty, A. B.; Khalique, N. A.; Raju, L.; Abdulhai, M.; Nicholson, D. G.; Larsen, H.; Pungente, M. D.; Goldring, W. P. D. *Bioorg. Med. Chem.* **2015**, *23*, 6364.

(15) Shyam, R.; Charbonnel, N.; Job, A.; Blavignac, C.; Forestier, C.; Taillefumier, C.; Faure, S. *ChemMedChem* **2018**, *13*, 1513.

(16) Allam, A.; Dupont, L.; Behr, J-B.; Plantier-Royon, R. *Eur. J. Org. Chem.* **2012**, 817.

(17) Prepared by reacting N^2,N^6-diBoc-lysine and 6-azido-1-hexanamine in the presence of coupling reagent BOP.

(18) Neshchadin, D.; Palumbo, F.; Sinicropi, M. S.; Andreu, I.; Gescheidt, G.; Miranda, M. A. *Chem. Sci.* **2013**, *4*, 1608.

(19) (a) Pennetta, C.; Bono, N.; Ponti, F.; Bellucci, M. C.; Viani, F.; Candiani, G.; Volonterio, A. *Bioconjugate Chem.* **2021**, *32*, 690. (b) Wexselblatt, E.; Esko, J. D.; Tor, Y. *J. Org. Chem.* **2014**, *79*, 6766.

(20) Sugawara, A.; Maita, N.; Gouda, H.; Yamamoto, T.; Hirose, T.; Kimura, S.; Saito, Y.; Nakano, H.; Kasai, T.; Nakano, H.; Shiomi, K.; Hirono, S.; Watanabe, T.; Taniguchi, H.; Omura, S.; Sunazuka, T. *J. Med. Chem* **2015**, *12*, 4984.