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Amino acids as selective acylating agents: regioselective N^1-acylation of imidazolidin-4-one derivatives of the antimalarial drug primaquine☆

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ABSTRACT

The acylation of bioactive primaquine-based imidazolidin-4-ones was studied using N^{α}-Boc-protected glycine as acylating agent. Two synthesis routes, eight different coupling methods and seven distinct solvents were compared. Mild carbodiimide-based couplings on high dielectric constant solvents such as DMF or MeCN increased acylation yields, whereas alcohols inhibited carbodiimide-mediated acylations to take place. Achievement of the synthetic goals was limited by the size of the imidazolidin-4-one ring substituents R^1, R^2 and R^3, but resort to MW-assisted synthesis allowed overcoming such obstacle, though with very modest reaction yields. All reactions involving a Boc-protected amino acid were regioselective, independent of reaction conditions employed. In contrast, regioselective acetylation of the imidazolidin-4-ones could only be achieved by resort to very mild coupling procedures.

1. Introduction

Primaquine (PQ, **1**) is an antimalarial well known for its excellent gametocytocidal activity but also for its toxicity. The main factor for this toxicity is the nature of its major metabolites. Some of the undesired PQ metabolic pathways can be overridden by modification of the drug's structure, e.g., protection of its terminal primary amino group.[1–5]

With this purpose in mind, our research group has been developing imidazolidin-4-one derivatives (**2**) of PQ α-aminoamides (**3**).[6–9] The latter are prepared in a previous step where the N-terminal amine of PQ is acylated with N^{α}-Boc-protected amino acids (BocAAOH), thus further insertion of the imidazolidin-4-one moiety also avoids compound degradation by the action of amino- and endopeptidases.[10,11] Imidazolidin-4-ones **2** have shown antimalarial activity against *Plasmodium berghei* comparable or higher than that of the parent PQ,[7] which prompted us to engage in further modification of these compounds through acylation of the imidazolidin-4-one N-1 with another amino acid, yielding compounds **5**. Given that structures **2** bear two secondary amines able to be acylated, i.e., at quinoline's C-8 and imidazolidin-4-one's N-1, regioselectivity was a relevant issue to the pursuit of our synthetic goals. Previous reports described N^{α}-protected amino acids as selective sulfanilamide acylants, where only monoacylation at the sulfanilamide's N-4 occurred by employing classical carbodiimide-mediated coupling methods, leaving the sulfonamide's N^1 unreacted.[12,13] Thus, it was reasonable to assume that by equally resorting to a BocAAOH as acylating agent and to carbodiimide-mediated coupling, we would achieve our purpose, i.e., regioselective production of compounds **5**. The regioselectivity and efficiency of the coupling reactions were tested using imidazolidin-4-ones **2a** and **2b** (**2** in Scheme 1 with, respectively, R^1=H, R^2=R^3=Me and R^1=H, R^2 and R^3=–$(CH_2)_5$–) as starting materials. Different coupling methods, reaction conditions and solvents were assayed, so we now wish to herein report and discuss the results thus obtained.

Abbreviations: Boc, *tert*-butyloxycarbonyl; BocAAOH, N^{α}-Boc-protected amino acid; DCCI, *N,N′*-dicyclohexylcarbodiimide; DCM, dichloromethane; DIEA, *N*-ethyl-*N,N*-diisopropylamine; DIP or DIPCDI, *N,N′*-diisopropylcarbodiimide; DIU, *N,N′*-diisopropylurea; DME, 1,2-dimethoxyethane; DMF, *N,N*-dimethylformamide; DMSO, dimethyl sulfoxide; ESI-MS, electrospray ionisation mass spectrometry; Gly, glycine residue; HBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate; HCTU, 2-(6-chloro-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; MW, microwave irradiation; NMR, nuclear magnetic resonance; TBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate; TEA, triethylamine; THF, tetrahydrofuran; TFA, trifluoroacetic acid.

☆ Some results from this study were recently supplied as Supplementary data in Ref. 19.

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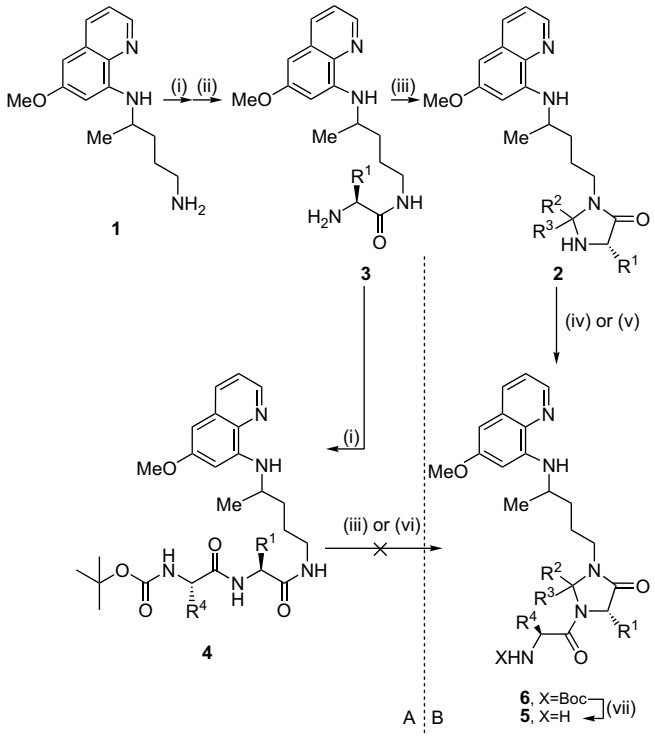


Scheme 1. Synthetic routes to PQ-derived imidazolidin-4-ones **2** and their N^1-aminoacyl derivatives **5**: (i) 1 equiv BocAAOH, 1.1 equiv DIPCDI, 1.1 equiv HOBt, 1 equiv TEA, DCM, 0 °C/rt; (ii) neat TFA, rt, 30% aq Na_2CO_3, extraction with $CHCl_3$; (iii) $R^2(C{=}O)R^3$, TEA, refluxing MeOH;[6] (iv) 5 equiv BocAAOH, 5 equiv *condensation agent(s)*, 3 equiv TEA, *solvent*, −10 °C→rt, inert atmosphere; (v) 5 equiv BocAAOH, 5 equiv DIPCDI, 5 equiv HOBt, 3 equiv TEA, DMF, MW irradiation (150 W, 20 min, 90 °C); (vi) 2 equiv TEA, 15 equiv propanone or 5 equiv cyclohexanone, MeOH, microwave irradiation (150 W, 10–20 min, 90–120 °C); (vii) TFA/DCM 30%, rt, Na_2CO_3 aq 30%, extraction with $CHCl_3$ or DCM.

2. Results and discussion

2.1. Synthesis route and coupling method

Synthetic route A on Scheme 1 was initially taken to reach the target N^1-acyl-imidazolidin-4-ones **6**. To this purpose, compound **3a** was condensed with BocGlyOH to yield the corresponding N^{α}-Boc-protected dipeptidylprimaquine **4a**. This was then reacted with propanone by a previously described method[6] to insert the imidazolidin-4-one ring between both amides, but **4a** was quantitatively recovered unreacted. This route was assayed using other compounds **3**, BocAAOH and ketones, but formation of structures **6** was never detected (data not shown).

Failure of preparing structures **6** through route A was attributed to steric constraints, as conversion of **4** into **6** would require cis/trans isomerisation of amide bonds followed by condensation of the ketone and concomitant ring closure at the middle of the peptide backbone, i.e., surrounded by amino acid side chains R^1 and R^4 as well as by the Boc protecting group. In an attempt to obviate the putative steric constraints behind the failure of route A, microwave-assisted synthesis was carried out. To that purpose, a commercially available monomode microwave (MW) instrument equipped with a sealed reactor, the CEM Discover® BenchMate™, was used. This reactor has the advantage to focus the microwave irradiation on the vessel in order to avoid dispersion and the ability to maintain the target temperature while subjecting the reaction mixture to continuous irradiation with microwaves by cooling the reaction vessel with a stream of nitrogen. However, not even when resorting to MW-assisted synthesis at temperatures as high as 120 °C and MW power of 150 W was synthetic route A by any means productive.

In view of the above, we stepped forward to assay route B (Scheme 1) in order to reach our synthetic goal. Thus, **3a** was firstly reacted with propanone to yield **2a**, as previously reported.[6] Condensation of **2a** with BocGlyOH was then carried out by seven different coupling methods, starting from 1 mol equiv (1 equiv) of **2a** and 5 equiv of BocGlyOH as reactants and using *N*,*N*-dimethylformamide (DMF) as solvent (Table 1, entries A–G). As expected, the reaction did not take place by simply reacting the amine and acyl components under reflux, in the absence of a condensation agent (entry A). In the remaining cases, the reaction always led to formation of a single product, monoacylated at the imidazolidin-4-one's N-1 (**6a**), as confirmed by nuclear magnetic resonance (NMR). The same result was observed when replacing the BocGlyOH+coupling agents mixture by the BocGlyOH succinimide active ester, BocGlyOSu (entry H). The diisopropylcarbodiimide/1-hydroxybenzotriazole (DIPCDI/HOBt) method[14] was the most efficient (entry C), whereas the worst result was obtained by replacement of HOBt with 4-(*N*,*N*-dimethyl)aminopyridine (DMAP)[15] as auxiliary nucleophile (entry D). Oddly, more recent and potent HOBt-derived amminium salts such as TBTU[16] (entry E) or HCTU[17] (entry G) were amongst the least effective to promote the desired condensation, even when reactions were carried out at room temperature from the start, i.e., without an initial ~3 h phase at −10 °C (data not shown). Remarkably, HBTU-mediated condensation (entry F) led to a substantially higher yield, despite HBTU differs from TBTU only at its counter-ion (hexafluorophosphate instead of tetrafluoroborate, respectively).

Acidolytic removal of Boc[18] from **6a** to yield **5a** was always successfully achieved by using 30% trifluoroacetic acid (TFA) in dichloromethane (DCM), as confirmed by NMR and electrospray-ionisation mass spectrometry (ESI-MS) analyses.

The synthetic route based on DIPCDI/HOBt condensation in DMF (entry C) followed by Boc removal with 30% TFA in DCM has been successfully applied to prepare a set of compounds **5** that differed only at the amino acid attached to N^1 (different R^4).[19]

2.2. Solvent

The best condensation method, according to the previous section, was further assayed in six additional solvents, namely, DCM, tetrahydrofuran (THF), methanol (MeOH), acetonitrile (MeCN), 1,2-dimethoxyethane (DME) and MeOH/DMF 1:1 (v/v), to test for coupling efficiency. The solvent collection originally chosen

Table 1
Acylation of **2a** with BocGlyOH to yield **6a**: comparison of coupling methods

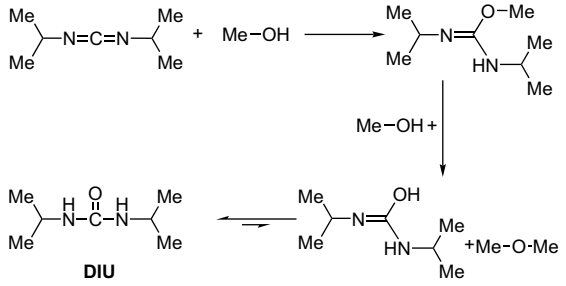
Reaction	Condensation agents	Solvent[c]	Yield/%
A	None (reflux)	DMF (36.7)	0[d]
B	DIPCDI		10
C	DIPCDI/HOBt		87
D	DIPCDI/DMAP		4
E	TBTU/DIEA[a]		21
F	HBTU/DIEA[a]		59
G	HCTU/DIEA[a]		10
H	BocGlyOSu[b]		15
C2	DIPCDI/HOBt	MeCN (37.5)	76
C3		MeOH (32.6)	0[e]
C4		DCM (9.1)	34
C5		THF (7.6)	0[d]
C6		DME (7.2)	48
C7		MeOH/DMF 1:1 (v/v)	0[e]

[a] In a 1:1 proportion, i.e., only 5 equiv of DIEA was added, as 3 equiv TEA was used in the 'base conditions' of all reactions assayed.
[b] Use of the active ester of BocGlyOSu (5 equiv) instead of BocGlyOH+coupling agent.
[c] Solvent dielectric constants at 20 °C, as taken from http://organicdivision.org/organic_solvents.html, are given in parenthesis.
[d] Quantitative recovery of unreacted **2a**.
[e] Quantitative recovery of **2a**+quantitative formation of diisopropylurea (DIU).

included a larger set of both protic and aprotic solvents having a wide range of dielectric constants, but not all of them could be used either due to solubility problems or melting points incompatible with the fact that carbodiimide activation usually requires use of low temperatures (−10 °C).[20]

In all those solvents where the condensation reaction actually took place (see below), regioselective formation of **6a** was consistently observed, though to different extents (Table 1, entries C and C2–C7). Acylations are usually performed in dry inert solvents in the presence of a non-nucleophilic tertiary amine (TEA, DIEA, *N*-methylmorpholine, etc.),[21] with DCM being one of the most popular solvents for peptide couplings.[22] However, in our particular case, the synthesis carried out in DCM had only a modest yield (34%), as shown in Table 1 (entry C4), quite below the yields recorded for the same reaction in DMF (87%, entry C) or MeCN (76%, entry C2). In contrast, reactions in THF, MeOH or DMF/MeOH 1:1 (v/v) did not take place at all, as the starting compound (**2a**) was quantitatively recovered together with the DIPCDI-derived urea, diisopropylurea (DIU).

As inferred from data in Table 1, the synthesis yield cannot be firmly correlated with the solvent's dielectric constant. Even so, the latter seems to have a key role, as higher yields were obtained in solvents with higher dielectric constants, with the exception of reactions carried out in MeOH-containing media. The explanation for this exception most probably lies on the fact that MeOH reacts with DIPCDI to form the corresponding *O*-methyl-isourea (Scheme 2). Reaction of alcohols with carbodiimides has been explored by Aresta and co-workers,[23] who investigated the factors that influence the yield and selectivity of reaction of *N*,*N*′-dicyclohexylcarbodiimide (DCCI) with alcohols and concluded that the alkylation is dependent on the nature and rate of addition of the alcohol to the C=N bond, and that reaction rate generally increases with temperature and the presence of a catalyst. The occurrence of such a reaction in our case simultaneously explains the extensive formation of DIU and the fact that synthesis of **6a** is completely inhibited in a solvent mixture that contains 50% MeOH.

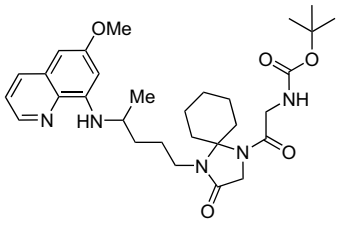


Scheme 2. Reaction of MeOH with DIPCDI to yield dimethyl ether and DIU.

2.3. Substituent groups at the imidazolidin-4-one's C-2 and C-5 positions

Condensation of **2b** with BocGlyOH was never achieved regardless of the solvent or condensation method employed. This was attributed to steric constraints imposed by the cyclohexane ring on the imidazolidin-4-one's C-2 spiro-carbon neighbouring the N-1 nitrogen that should be acylated. This observation was consistent with other failed attempts to obtain structures **6** from precursors **2** bearing large R^1, R^2 and R^3 groups,[19] showing the relevance of steric constraints on positions adjacent to the imidazolidin-4-one's N-1 atom. Similar difficulties were reported for the solid-phase synthesis of Leu-Enkephalin peptidomimetics based on the imidazolidin-4-one scaffold, where resin-bound dipeptide imidazolidin-4-ones containing two methyl groups at C-2 failed to give the desired N^1-acyl derivatives.[24]

MW-assisted synthesis was tried on the acylation of **2b** with BocGlyOH and found to lead to formation of **6b** through a 20-min reaction under MW irradiation at 150 W and 90 °C (all other reaction parameters were kept unchanged respective to the best synthesis of **6a**). Nonetheless, **6b** was obtained in a very modest yield (~13%).



6b

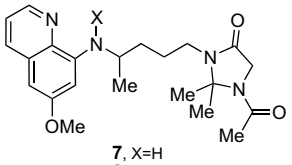
Other N-acylations of imidazolidin-4-ones **2** with Boc-amino acids were carried out both with and without MW irradiation, in all cases for 20 min at 90 °C so that MW power would be the only variable (data not shown). This allowed to observe that (i) all successful acylations were regioselective at the imidazolidin-4-one's N-1 even at 90 °C; (ii) acylations that occurred in the absence of MW were equally efficient (i.e., similar yields) by MW-assisted synthesis as long as the same temperature was used; (iii) MW irradiation enabled some sterically demanding N^1-acylations to take place, though in modest yields.

In view of the above, it appears that MW-assisted N^1-acylation of imidazolidin-4-ones **2** is not necessary except for specific cases where steric constraints disable the reaction to take place by non-MW approaches. Though reactions were substantially faster at high temperatures (promoted or not by MW-irradiation), these should be avoided when using chiral amino acids to prevent them to undergo base (TEA)-catalysed racemisation.

2.4. Regioselectivity

Reaction regioselectivity was a relevant issue, as the two secondary amino groups in **2a** could attack the acylating agent to form tertiary amides. It is true that electron delocalisation of the nitrogen lone pair of the 8-aminoquinoline moiety to the quinoline ring (**2**, Scheme 1) can account for a lower nucleophilicity of the 8-amino group as compared to the imidazolidin-4-one N-1 amine. However, the complete absence of N-acylation at the 8-amino group was noteworthy and made us wonder if Boc-amino acids were acting as selective acylants, as previously observed in similar N^4-acylations of sulfanilamides.[12,13]

To further investigate whether the quinoline's 8-amino substituent was completely inert towards acylation, we worked on the acetylation of **2a** by two different methods. Acetyl was chosen as acyl component to differ from the amino acid acylants and to rule out, or not, the putative influence of the latter on regioselectivity. Thus, **2a** was reacted in DMF with acetic anhydride, instead of BocGlyOH, as above described. Again, only the N^1-monoacylated product **7** was formed in this case (ca. 83% yield), as confirmed by ESI-MS and NMR. However, when **2a** was dissolved in neat acetic anhydride (20 equiv) under reflux, as described for the synthesis of 5-chloroarylidene derivatives of imidazoline-4-ones,[25] only the *N*,*N*′-diacetyl derivative **8** was produced (94% yield).

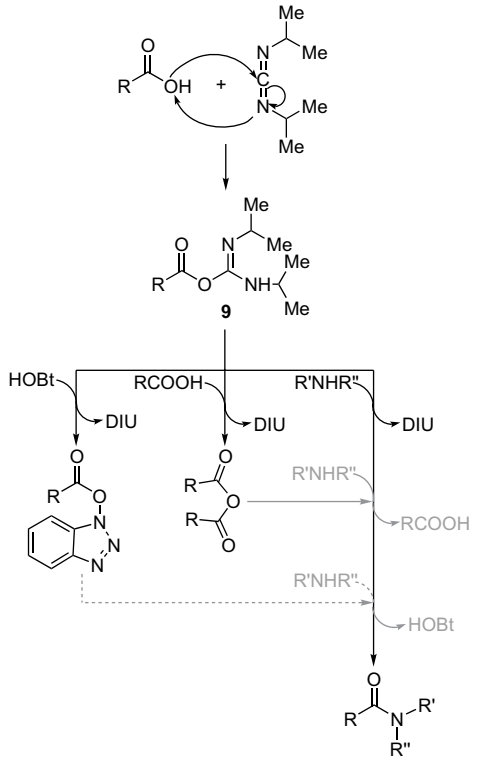


7, X=H
8, X=COMe

Altogether, these findings show that imidazolidin-4-ones like **2a** are regioselectively acylated at N-1 by Boc-amino acids even under stronger conditions (high temperature, 150 W MW irradiation), whereas the use of a general acylant as acetic anhydride would require very mild coupling conditions in order to keep such regioselectivity.

It could be argued that formation of **8** by refluxing **2a** in acetic anhydride might be due to the large excess of the acylating agent used; however, if this was entirely true, reactions with BocGlyOH, carried out with a fivefold molar excess of the acylating species, should have given at least a small amount of the diacyl derivative.

It could also be claimed that the nature of the acylating species is different in reactions with acetic anhydride, as this is by itself, as a symmetrical anhydride, the active acylating species; nevertheless, the majority of condensation methods assayed with BocGlyOH had DIPCDI as coupling agent, thus leading to an *O*-acyl-isourea intermediate **9** that is readily attacked by either an auxiliary nucleophile such as HOBt, the amine component to be acylated, or another molecule of BocGlyOH, in which case an active symmetrical anhydride will also be formed (Scheme 3). So, BocGlyOH activation via a symmetrical anhydride surely also happened in most reactions tested and such did not affect reaction regioselectivity. Moreover, just to ensure that it was not the 'anhydride nature' that dictated the non-regioselective formation of the diacyl derivative **8**, we have used acetic anhydride, instead of acetic acid, in the reaction that was carried out under exactly the same conditions as those used with BocGlyOH (DIPCDI/HOBt in DMF, entry C in Table 1). As above referred, such reaction led to regioselective acylation of the N-1 nitrogen in **2a**, yielding compound **7**.



Scheme 3. Parallel pathways in carbodiimide/HOBt-mediated acylation of a secondary amine.

So, overall, it seems that the regioselectivity consistently observed in the acylations of imidazolidin-4-ones **2** with Boc-amino acids depends on the relative nucleophilicity of the two secondary amine nitrogens and, mainly, on the nature of the acylating agent. This is consistent with our previous findings that regioselective acylation of sulfanilamides with N^{α}-protected amino acids is highly dependent on the acidity of the sulfonamido's nitrogen (N-1).[12,13] In the present case, the preferential acylation of the imidazolidin-4-one's N-1 nitrogen atom may be ascribed to its higher basicity ($pK_a \sim 5$)[8,26] when compared to that of the quinoline's nitrogen atom ($pK_a \sim 3$).[27]

3. Concluding remarks

N^1-Acyl-imidazolidin-4-ones **5** can be successfully prepared through regioselective N^1-acylation of the imidazolidin-4-one precursors **2** by means of mild peptide condensation methods. High dielectric constant solvents such as DMF or MeCN increase acylation yields, whereas alcohols should be avoided when activation of the acyl component is promoted by carbodiimides. Gathering the present with previous[19] findings led to conclude that the success of the coupling reactions was limited by the size of the imidazolidin-4-one ring substituents R^1, R^2 and R^3, but resort to MW-assisted synthesis is a way to overcome such obstacle, enabling the successful acylation of bulkier imidazolidin-4-ones, though with modest yields.

Boc-amino acids seem to act as selective acylating agents for imidazolidin-4-ones **2**, in a way parallel to that previously found on similar reactions involving N^4-acylation of sulfanilamides. Ongoing computational studies to establish the molecular basis for the regioselectivity herein described will be timely reported.

4. Experimental section

4.1. Chemicals and instrumentation

N^{α}-*tert*-Butyloxycarbonylglycine (BocGlyOH), HBTU and HCTU were purchased to NovaBiochem (VWR International, Portugal). TBTU was from Bachem (Switzerland). All solvents (p.a. quality) were from Merck (VWR International, Portugal) and dried over activated 4 Å molecular sieves prior to usage. All remaining chemicals were from Sigma–Aldrich (Portugal). Silica gel for column chromatography (ref. 60A, 35–70 μm; 550 m^2/g) was from SDS (France).

Microwave reactions were carried out in Discover BenchMateTM (CEM) microwave instruments equipped with 10 mL vessels. NMR spectra were recorded on a Bruker AMX 300 spectrometer and ESI-MS spectra were acquired on a Finnigan Surveyor LCQ DECA XP Max spectrometer.

4.2. Synthesis

Synthesis and spectroscopic data are only given in detail for those compounds relevant for the present study. A larger set of N^1-acyl-imidazolidin-4-ones **5** have been prepared as described for **5a** to be evaluated as potential antimalarials, as reported elsewhere.[19] NMR data are abbreviated as follows: br s, broad singlet; d, doublet; dd, double doublet; dq, double quartet; dt, double triplet; δ, chemical shift (in ppm); m, unresolved multiplet; q, quartet; Q, hydrogen on quinoline ring; Q*C*, carbon on quinoline ring; s, singlet; t, triplet.

*4.2.1. Acylation of **2a** with Boc-glycine—synthesis of **6a** (entries B–C7, Table 1)*

Compound **2a** (1 mmol) was suspended in solvent (20 mL), TEA (3 equiv) and the mixture was stirred in an ice-water/acetone/NaCl bath (−10 °C) for 20 min, under inert atmosphere. After addition of either BocGlyOSu (5 equiv) or BocGlyOH (5 equiv) plus the coupling agent(s) (5 equiv), the mixture was kept at −10 °C for further 4 h, under stirring. The temperature was then slowly increased to

+10 °C and thus maintained till the end of reaction (24 h). The solid phase formed in DIPCDI-mediated reactions was removed by suction filtration and identified as DIU. The liquid phase was evaporated at 90 °C in vacuo to dryness and the resulting residue was dissolved in 40 mL of DCM. This solution was washed three times with 15 mL portions of 10% aq $NaHCO_3$ and the organic layer dried over anhydrous $MgSO_4$ and evaporated to dryness. The residue was submitted to column chromatography on silica using DCM/acetone as eluent. The chromatographically homogeneous product was isolated as yellow-orange oil and identified as **6a**.

*4.2.1.1. 1-[(3-tert-Butyloxycarbonyl)-3-aza-1-oxopropyl]-3-{4-[(6-methoxyquinolin-8-yl)-amino]pentyl}-2,2-dimethylimidazolidin-4-one (**6a**).* δ_H ($CDCl_3$, 300 MHz) 8.51 (dd, *J*=4.20, 1.38 Hz, 1H, Q2), 7.92 (dd, *J*=8.26, 1.42 Hz, 1H, Q4), 7.30 (dd, *J*=8.28, 4.20 Hz, 1H, Q3), 6.34 (d, *J*=2.45 Hz, 1H, Q5), 6.28 (d, *J*=2.43 Hz, 1H, Q7), 6.01 (d, *J*=8.22 Hz, 1H, –N*H*–CH(CH_3)–CH_2–), 5.35 (br s, 1H, –N*H*–CO–O–), 3.95 (s, 2H, –CO–CH_2–N–), 3.89 (s, 3H, CH_3–O–), 3.81 (d, *J*=4.31 Hz, 2H, –CO–CH_2–NH–), 3.69–3.63 (m, 1H, –NH–C*H*(CH_3)–CH_2–), 3.30–3.18 (m, 2H, –(CH_2)$_2$–CH_2–N–), 1.84–1.72 (m, 4H, –CH_2–CH_2–CH_2–), 1.60(4) and 1.59(9) (s+s, 6H, –N–C(CH_3)$_2$–N–), 1.44 (s, 9H, –O–C(CH_3)$_3$), 1.31 (d, *J*=6.30 Hz, 3H, –NH–CH(CH_3)–). δ_C ($CDCl_3$, 75.4 MHz) 166.03 (–*C*O–CH_2–N–), 165.36 (–*C*O–CH_2–NH–), 159.50 (Q*C*6), 155.84 (–*C*O–O–), 145.03 (Q*C*2), 144.43 (Q*C*8), 135.42 (Q*C*4), 134.89 (Q*C*10), 129.99 (Q*C*9), 121.99 (Q*C*3), 96.90 (Q*C*7), 91.88 (Q*C*5), 80.91 (–O–*C*(CH_3)$_3$), 79.99 (–N–*C*(CH_3)$_2$–N–), 55.33 (*C*H_3–O–), 47.92 (–NH–*C*H(CH_3)–CH_2–), 47.16 (–CO–*C*H_2–N–), 43.80 (–*C*H_2–NH–CO–), 39.91 (–(CH_2)$_2$–*C*H_2–), 34.13 (–*C*H_2–(CH_2)$_2$–), 28.42 (–O–C(*C*H_3)$_3$), 25.84 (–N–C(*C*H_3)(CH_3)–N–), 24.60 (–N–C(CH_3)(*C*H_3)–N–), 24.52 (–CH_2–*C*H_2–CH_2–), 20.81 (–NH–CH(*C*H_3)–CH_2–). $C_{27}H_{39}N_5O_5$ (513.30 g mol^{-1}): found *m*/*z*=514.21([M+H]$^+$).

*4.2.2. Acylation of **2b** with Boc-glycine—synthesis of **6b***

To **2b** (100 μmol) suspended in DMF (6 mL), TEA (3 equiv), were added BocGlyOH (5 equiv), HOBt (5 equiv) and DIPCI (5 equiv). The mixture was kept under stirring on an appropriate reaction vessel and MW irradiated (150 W) for 20 min at a constant temperature of 90 °C. The solvent was removed at 90 °C under reduced pressure and the residue was retaken in 20 mL of DCM. This solution was washed three times with 8 mL portions of 10% aq $NaHCO_3$ and the organic layer dried over anhydrous $MgSO_4$ and evaporated to dryness. The residue was submitted to column chromatography on silica using DCM/acetone as eluent. The chromatographically homogeneous product was isolated as yellow oil and identified as **6b**.

*4.2.2.1. 1-[(3-tert-Butyloxycarbonyl)-3-aza-1-oxopropyl]-3-{4-[(6-methoxyquinolin-8-yl)-amino]pentyl}-1,4-diazaspiro[4.5]decan-2-one (**6b**).* δ_H ($CDCl_3$, 300 MHz) 8.52 (dd, *J*=6.08, 3.63 Hz, 1H, Q2), 7.89 (dd, *J*=6.12, 3.12 Hz, 1H, Q4), 7.47 (dd, *J*=6.32, 3.06 Hz, 1H, Q3), 6.34 (d, *J*=2.18 Hz, 1H, Q5), 6.28 (d, *J*=2.49 Hz, 1H, Q7), 5.56 (d, *J*=4.22 Hz, 1H, –N*H*–CH(CH_3)–CH_2–), 3.96 (d, *J*=4.20 Hz, 2H, –CO–CH_2–NH–), 3.92–3.89 (m, 1H, –C*H*(CH_3)–CH_2–), 3.76 and 3.74 (br s+br s, 1H, –N*H*–CO–O–), 3.43–3.38 (m, 1H, –(CH_2)$_2$–C*H*H–), 3.28–3.23 (m, 1H, –(CH_2)$_2$–CH*H*–), 2.99 (s, 2H, –CO–CH_2–N(–)–), 2.97 (s, 3H, CH_3–O–), 1.84–1.52 (m, 10H, –(CH_2)$_5$–), 1.48–1.46 (m, 4H, –CH_2–CH_2–CH_2–), 1.45 (s, 9H, –O–C(CH_3)$_3$), 1.21 and 1.17 (d+d, *J*=6.54 and 6.43 Hz, 3H, –NH–CH(CH_3)–). $C_{30}H_{43}N_5O_5$ (553.33 g mol^{-1}): found *m*/*z*=554.47 ([M+H]$^+$).

*4.2.3. Acidolytic removal of Boc—synthesis of **5a***

Compound **6a** was dissolved in TFA at 30% in DCM (8 mL) and the reaction allowed to proceed for 2 h at room temperature. Excess TFA was neutralised by drop wise addition of 30% aq Na_2CO_3 until pH 9; the supernatant oily layer formed was extracted six times with 10 mL portions of chloroform and the organic layers pooled, dried over anhydrous $MgSO_4$ and evaporated to dryness. Chromatographically homogeneous yellow-orange oil was obtained and identified as **5a**.

*4.2.3.1. 1-(2-Amino-1-oxoethyl)-3-{4-[(6-methoxyquinolin-8-yl)amino]-pentyl}-2,2-dimethylimidazolidin-4-one (**5a**).* δ_H ($CDCl_3$, 300 MHz) 8.51 (dd, *J*=4.16, 1.45 Hz, 1H, Q2), 7.91 (dd, *J*=8.22, 1.39 Hz, 1H, Q4), 7.29 (dd, *J*=8.22, 4.21 Hz, 1H, Q3), 6.33 (d, *J*=2.34 Hz, 1H, Q5), 6.27 (d, *J*=2.29 Hz, 1H, Q7), 6.00 (d, *J*=8.46 Hz, 1H, –N*H*–CH(CH_3)–CH_2–), 3.91 (s, 2H, –CO–CH_2–N(CO–)–), 3.88 (s, 3H, CH_3–O–), 3.69–3.60 (m, 1H, –NH–C*H*(CH_3)–CH_2–), 3.30 (s, 2H, –CO–CH_2–NH_2), 3.27–3.20 (m, 2H, –(CH_2)$_2$–CH_2–), 1.83–1.66 (m, 6H, –CH_2–CH_2–CH_2–, –CO–CH_2–NH_2), 1.61 and 1.60 (s+s, 6H, –N–C(CH_3)$_2$–N–), 1.30 (d, *J*=6.34 Hz, 3H, –NH–CH(CH_3)–CH_2–). δ_C ($CDCl_3$, 75.4 MHz) 170.54 (–*C*O–CH_2–N–), 166.07 (–*C*O–CH_2–NH_2), 159.82 (Q*C*6), 145.36 (Q*C*2), 144.77 (Q*C*8), 135.75 (Q*C*4), 135.22 (Q*C*10), 130.32 (Q*C*9), 122.32 (Q*C*3), 97.22 (Q*C*7), 92.20 (Q*C*5), 81.14 (–N–*C*(CH_3)$_2$–N–), 55.66 (*C*H_3–O–), 48.26 (–NH–*C*H(CH_3)–CH_2–), 47.54 (–CO–CH_2–N–), 45.54 (–CO–*C*H_2–NH_2), 40.21 (–(CH_2)$_2$–*C*H_2–), 34.50 (–*C*H_2–(CH_2)$_2$–), 26.20 (–CH_2–*C*H_2–CH_2–), 24.95 (–N–C(*C*H_3)(CH_3)–N–), 24.90 (–N–C(CH_3)(*C*H_3)–N–), 21.13 (–NH–CH(*C*H_3)–CH_2–). $C_{22}H_{31}N_5O_3$ (413.24 g mol^{-1}): found *m*/*z*=414.47 ([M+H]$^+$).

*4.2.4. N^1-Monoacetylation of **2a**—synthesis of **7***

Compound **2a** (1 mmol) was suspended in DMF (20 mL), TEA (3 equiv) and the mixture was stirred in an ice-water bath with NaCl and acetone (−10 °C) for 20 min, under inert atmosphere. Acetic anhydride (5 equiv), HOBt (5 equiv) and DIPCDI (5 equiv) were added to the mixture, which was kept at −10 °C for further 4 h. The reaction was allowed to proceed as described in Section 4.2. Work-up for product isolation was also similar to that described in Section 4.2, allowing to isolate chromatographically homogeneous yellow oil that was identified as compound **7**.

*4.2.4.1. 1-(1-Oxoethyl)-3-{4-[(6-methoxyquinolin-8-yl)amino]pentyl}-2,2-dimethylimidazolidin-4-one (**7**).* δ_H ($CDCl_3$, 300 MHz), 8.50 (dd, *J*=4.25, 1.59 Hz, 1H, Q2), 7.92 (dd, *J*=8.27, 1.56 Hz, 1H, Q4), 7.29 (dd, *J*=8.25, 4.24 Hz, 1H, Q3), 6.33 (d, *J*=2.46 Hz, 1H, Q5), 6.28 (d, *J*=2.46 Hz, 1H, Q7), 6.06 (br s, 1H, –N*H*–CH(CH_3)–), 3.99 (s, 2H, –CO–CH_2–N(CO–)–), 3.87 (s, 3H, CH_3–O–), 3.68–3.58 (m, 1H, –C*H*(CH_3)–CH_2–), 3.29–3.12 (m, 1H, –((CH_2)$_2$–C*H*$_2$)–), 2.01 (s, 3H, CH_3–CO–N(CH_2–)–), 1.82–1.64 (m, 4H, –CH_2–CH_2–CH_2–), 1.58(1) and 1.57(6) (s+s, 6H, –N–C(CH_3)(CH_3)–), 1.29 (d, *J*=6.36 Hz, 3H, –CH(CH_3)–CH_2–). δ_C ($CDCl_3$, 75.4 MHz) 168.66 (–N–*C*O–CH_3), 166.39 (–*C*O–CH_2–), 159.87 (Q*C*6), 145.21 (Q*C*2), 144.62 (Q*C*8), 135.55 (Q*C*4), 135.46 (Q*C*10), 130.43 (Q*C*9), 122.29 (Q*C*3), 97.48 (Q*C*7), 92.30 (Q*C*5), 81.06 (–N–*C*(CH_3)$_2$–), 56.67 (C*H*$_3$–O–), 49.81 (–CO–*C*H_2–), 48.30 (–*C*H(CH_3)–CH_2–), 40.26 (–(CH_2)$_2$–*C*H_2), 34.46 (–CH_2–(CH_2)$_2$–), 26.15 (*C*H_3–CO–N(CH–)–), 24.83 and 24.78 (–C(*C*H_3)(*C*H_3)–), 24.42 (–CH_2–*C*H_2–CH_2–), 21.06 (–CH(*C*H_3)–CH_2–). $C_{22}H_{30}N_4O_3$ (398.23 g mol^{-1}): found *m*/*z*=422.07 ([M+Na]$^+$).

*4.2.5. N,N′-Diacetylation of **2a**—synthesis of **8***

Compound **2a** (1 mmol) was refluxed in acetic anhydride (20 equiv) for 1 h, after which the reaction mixture was evaporated to dryness. The residue was submitted to column chromatography on silica using DCM/MeOH as eluent. The product was isolated as yellow oil and identified as compound **8**.

*4.2.5.1. 1-(1-Oxoethyl)-3-{4-[(6-methoxyquinolin-8-yl)oxoethylamino]-pentyl}-2,2-dimethylimidazolidin-4-one (**8**).* δ_H ($CDCl_3$, 300 MHz) 8.75 (dd, *J*=4.16, 1.44 Hz, 1H, Q2), 8.05 (dd, *J*=8.29, 1.41 Hz, 1H, Q4), 7.36 and 7.33 (dd+dd, *J*=8.17, 4.10 and 8.25, 4.19 Hz, 1H, Q3), 7.17 and 7.15 (d+d, *J*=2.69 and 2.68 Hz, 1H, Q5), 7.09 (d, *J*=2.68 Hz, 1H, Q7), 5.01–4.86 (m, 1H, –C*H*(CH_3)–CH_2–), 4.01 (s, 1H, –CO–CH*H*–N(CO–)–), 3.94 (s, 1H, –CO–C*H*H–N(CO–)–), 3.91 and 3.90 (s, 3H, CH_3–O–), 3.29–3.24 (m, 1H, –(CH_2)$_2$–CH*H*–), 3.02–2.97 (m, 1H,

–$(CH_2)_2$–C*H*H–), 2.00 and 1.97 (s+s, 3H, C*H*$_3$–CO–N(CH_2–)–), 1.98 (s, 3H, C*H*$_3$–CO–N(CH–)–), 1.61 and 1.60 (s+s, 3H, –N–C(C*H*$_3$)(CH_3)–), 1.56 and 1.53 (s+s, 3H, –N–C(CH_3)(C*H*$_3$)–), 1.20 and 0.73 (d+d, J=6.76 and 6.88 Hz, 3H, –CH(C*H*$_3$)–CH_2–). δ_C ($CDCl_3$, 75.4 MHz) 172.26 and 172.00 (CH_3–*C*O–N(CH–)–), 168.78 and 168.75 (–N–*C*O–CH_3), 166.59 and 166.37 (–*C*O–CH_2–), 157.44 and 157.34 (Q*C*6), 148.78 and 148.75 (Q*C*2), 142.15 and 142.12 (Q*C*8), 138.88 and 138.60 (Q*C*10), 135.70 and 135.66 (Q*C*4), 130.51 (Q*C*9), 123.89 and 123.43 (Q*C*7), 122.57 (Q*C*3), 106.42 and 106.39 (Q*C*5), 81.16 and 81.05 (–N–*C*(CH_3)$_2$–), 56.16 and 56.13 (C*H*$_3$–O–), 51.95 and 51.28 (–*C*H(CH_3)–CH_2–), 49.82 and 49.74 (–CO–*C*H_2–), 40.32 and 40.24 (–$(CH_2)_2$–CH_2), 33.89 (–*C*H_2–$(CH_2)_2$–), 24.87 and 24.74 (–C(*C*H_3)(CH_3)–), 24.33 and 24.31 (–C(CH_3)(*C*H_3)–), 23.40 and 23.34 (–CH_2–*C*H_2–CH_2–), 21.44 (*C*H_3–CO–N(CH–)–), 20.16 (–N–CO–*C*H_3), 17.89 (–CH(*C*H_3)–CH_2–).$C_{24}H_{32}N_4O_4$ (440.24 g mol^{-1}): found m/z= 463.87 ([M+Na]$^+$).

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