Solid phase synthesis of a redox delivery system with the aim of targeting peptides into the brain

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A solid phase approach for the preparation of peptides attached to a redox chemical delivery system derived from stable annulated NADH models is reported. The synthesis starts with the grafting on a Merrifield resin of quinoline **4b**, precursor of the redox carrier. From the resulting quinoline supported resin **4d**, the stepwise SPPS of both octapeptide OP (RPGLLDLK) and octadecaneuropeptide ODN (QATVGDVNTDRPGLLDLK), two neuropeptides exhibiting anorexigenic effects, was successfully achieved by conventional methods. Quaternization of the quinoline moiety prior to cleavage of the modified OP and ODN peptides from the resin, led to the expected quinolinium salt **8a** and **8b** respectively linked to OP or ODN peptides. Finally, the reduction with $NaBH_4$ monitored by UV–vis, provided the desired annulated NADH models as peptides carriers with either the OP (**11a,b**) or ODN (**12a,b**) moiety.

Introduction

Due to their hydrophilic properties, many neuropharmaceutical peptides cannot cross the lipoidal bilayer of the blood–brain barrier (BBB) and consequently exhibit limited access to the central nervous system (CNS)[1] in the absence of a specific transfer system. Moreover, peptides may also be recognized by a variety of neuropeptide-degrading enzymes expressed in the BBB, thus hampering their transport into the CNS.[2] To overcome these limitations, many authors[3,4] have designed a brain-targeted chemical delivery system based on the NADH/NAD+ redox system (Fig. 1). Thus, biologically active compounds covalently linked to a lipophilic dihydropyridine can readily penetrate the BBB. The dihydropyridine which may be considered as a redox "targeter" is then subjected to enzymatic oxidation in the brain. The corresponding water-soluble and lipid-insoluble pyridinium salt thus formed is "locked-in" in the brain promoting retention of the targeted compound in the CNS. Subsequently, cleavage of the pyridinium carrier induced by enzymes leads to the release of the biologically active compound reaching its site of action.

In spite of the interesting pharmacological results described by Bodor *et al.*,[3] this strategy remains limited due to the use of a 1,4-dihydropyridine carrier. Indeed, 1,4-dihydropyridines are rather unstable[5] and are subjected to many side reactions. In particular a hydration reaction may occur on the 5,6-double bond of the dihydropyridine. For several years, our research group has been involved in the synthesis of stable annulated NADH models in the quinoline series.[6,7] In addition, we were also interested in the study of the octadecaneuropeptide (ODN, QATVGDVNTDRPGLLDLK). ODN, which has been characterized as an endogenous ligand of benzodiazepine receptors (BZRs) and proved to be a potent inhibitor of food intake in rodents.[8] However, we have shown that the anorexigenic effect of ODN is not mediated through BZRs[9] and that intravenous administration of 200 times the intracerebroventricular effective dose does not affect food intake demonstrating that ODN exerts its anorexigenic effect centrally and is unable to cross the BBB.[8] We have also demonstrated that ODN increases intracellular calcium concentration in cultured rat astrocytes through activation of the G protein-coupled receptor,[10] and we have found that the C-terminal octapeptide OP (RPGLLDLK) is the shorter isoactive fragment.[11]

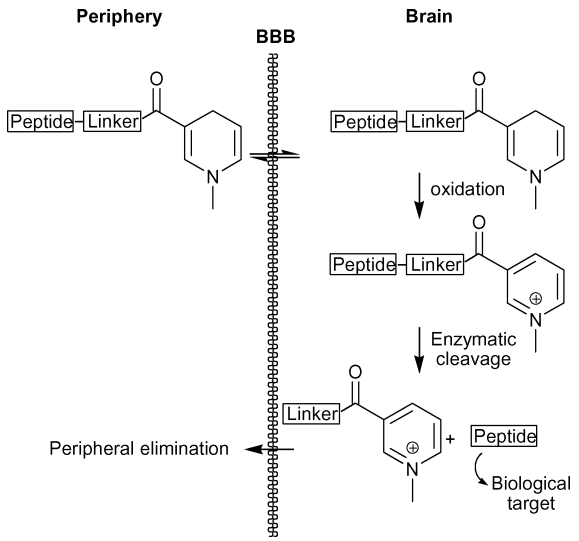

Fig. 1 Brain targeting by means of NADH pyridinium models.

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In this context, we examined the possibility of developing a strategy for targeting these peptides to the brain by means of a redox chemical delivery system derived from a stable dihydroquinoline NADH model. To link the peptide and the carrier, we next speculated that a general solid-phase strategy would be well-suited (Fig. 2). To this end, the grafting of the chemical carrier on a Merrifield resin was planned prior to conventional solid-phase peptide synthesis. Quaternization of the quinoline ring and subsequent cleavage from the resin followed by reduction of the quinolinium salt was expected to provide the desired peptide bearing the dihydroquinoline carrier. This solid-phase strategy offers not only the advantage to be general and applicable to the brain targeting of a large range of drugs, but should also be easily exploitable by biochemists, commonly familiar with solid-phase peptide synthesis.

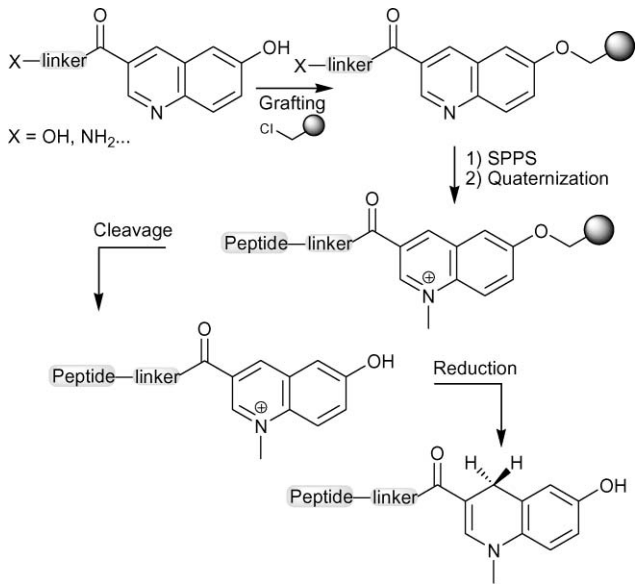


Fig. 2 General solid-phase approach for targeting peptides.

In this paper, we describe the solid-phase synthesis of ODN or OP linked to stable annulated dihydropyridines.

Results and discussion

Design of the peptide carriers

To obtain a stable and lipophilic peptide carrier based on the NADH/NAD+ redox system (Fig. 3), we planned to use annulated NADH models developed by our research group.[6,7] Annulation protects the dihydropyridine moiety against electrophilic attacks on the 5,6-enamine double bond. As a result of this protection, the reducing properties of annulated NADH models are significantly altered. To overcome this side effect, the electron donating alkoxy groups are expected to restore the reducing properties obtained with simple 1,4-dihydropyridine carriers. Besides, alkoxy groups could be advisedly used in the solid phase synthesis to graft the peptide carrier. Finally, phenylalaninol was envisaged as a linker between the chemical delivery system and the active peptide. In the final step, esterases are consequently expected to cleave the peptide from the quinoline carrier.

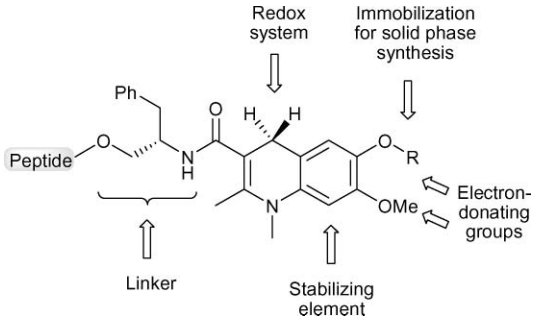
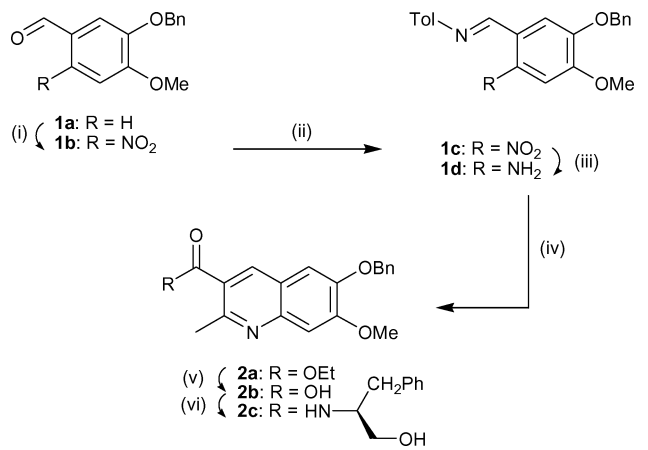


Fig. 3 Design of a redox chemical delivery system by means of stable annulated NADH model.

Synthesis of quinolines 2a,c. To prepare quinolines **2a,c** (Scheme 1), we used a Friedlander[12] type condensation with a Borsche modification.[13] In the first step, nitration of commercially available compound **1a** led to **1b** (81%) and subsequent reaction with *p*-toluidine afforded imine **1c** (88%) which was reduced by Na_2S[14] to furnish amine **1d** (85%). The stable amino imine **1d** was condensed with ethyl acetoacetate to produce the expected quinoline **2a** in high yield (89%). The direct conversion of ester **2a** into amide **2c** with (*S*)-phenylalaninol was rather tedious: heating in toluene or in the presence of trimethylaluminium in CH_2Cl_2 always led to low yields (*ca.* 10%). Alternatively, carboxylic acid **2b** was obtained by reacting **2a** with lithium hydroxide (82%) and conversion to the corresponding acid chloride was achieved with oxalyl chloride. Finally, subsequent reaction with (*S*)-phenylalaninol gave the required amide **2c** (74%).



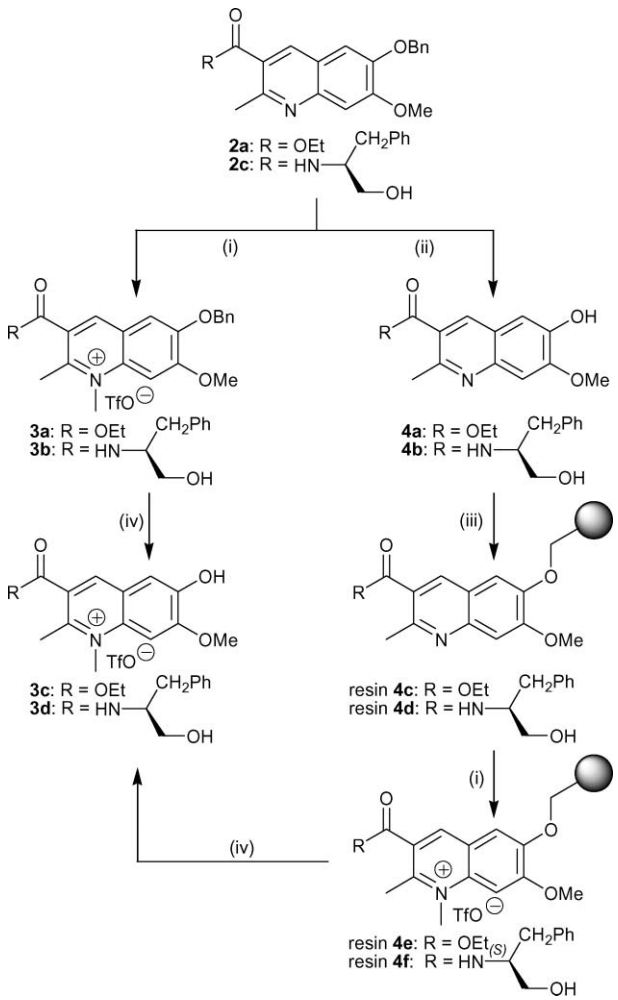
Scheme 1 *Reagents and conditions*: (i) HNO_3, 1 h at 0 °C then 2 h at 20 °C (81%); (ii) *p*-toluidine, EtOH, 2 h, reflux (88%); (iii) Na_2S, EtOH, 15 min at reflux then 4 h at 0 °C (85%); (iv) Ethyl acetoacetate, piperidine, EtOH, 8 h, reflux (89%); (v) LiOH, H_2O, EtOH, 3 h, reflux (82%); (vi) $(COCl)_2$, NEt_3, DMF, CH_2Cl_2, 12 h, 20 °C then (*S*)-phenylalaninol, 30 min at −5 °C then 24 h at 20 °C (74%).

Synthesis of polymer-supported quinolinium salts and optimization of the cleavage step conditions

Polymer-supported NADH models have been previously reported by our laboratory[7,15] and others.[16] Our strategy is based on the reaction of phenolic compounds with Merrifield resins prior to quaternization and reduction steps. However, in the present study it was also necessary to cleave the polymer-supported quinolinium

salts prior to the final reduction step. So, to validate the whole sequence of the solid phase approach, it was first essential to determine the reaction conditions for the cleavage step of the quinolinium salt from the resin.

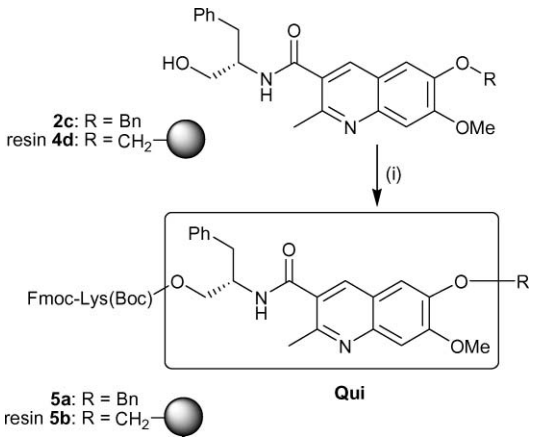
Initially, we envisaged working with ester **2a** and amide **2c** as models (Scheme 2), the benzyl group mimicking in both cases the Merrifield resin. Quaternization of quinolines **2a,c** proceeded smoothly with the highly reactive methylating agent methyl trifluoromethanesulfonate affording quinolinium salt **3a,b** in 72% and 90% yield respectively. In the literature, TMSI and TMSOTf were successfully used to deprotect benzyl ether groups and proved to be compatible with a pyridinium salt.[17] Thus, the quinolinium salts **3a,b** were conveniently deprotected with TMSOTf to furnish the desired compounds **3c,d** in 90% yield. It was then necessary to validate these deprotection conditions on a Merrifield resin. To this end, benzyl ether cleavage of **2a,c** was accomplished by hydrogenation to provide **4a,b** in 64% and 100% yield respectively. Phenolic derivatives **4a,b** were reacted with Merrifield resin (1% DVB, $f_0 = 1.2$ mmol g^{-1}) in DMF for 4 days at 20 °C mediated by NaH to yield the functionalized resins **4c,d** with a loading of 0.93 mmol g^{-1} and 0.90 mmol g^{-1} respectively (estimated by nitrogen microanalysis). Resins **4c,d** were treated following the same reaction sequence previously developed with **2a** and **2c** (Scheme 2). Thus, quaternization reaction gave resins **4e,f** (loading 0.81 mmol g^{-1} and 0.63 mmol g^{-1} respectively). The final cleavage step with TMSOTf was accomplished affording pure compounds **3c,d** in rather fair yields. This sequence of reactions was validated with compounds **2c** and resin **4d**, both bearing the phenylalaninol linker.



Scheme 2 *Reagents and conditions*: (i) MeOTf, CH_2Cl_2, 1 h, 20 °C from **2a** (72%), 4 h from resins **4c,d** or $CHCl_3$, 6 h from **2c** (90%); (ii) H_2, Pd/C (10%), MeOH, 2 h, 20 °C from **2a** (64%) and 24 h from **2c** (100%); (iii) Merrifield resin (1% DVB; 1.2 mmol g^{-1}), NaH, DMF, 4 days, 20 °C; (iv) TMSOTf, CH_2Cl_2, 2 h, 20 °C from resin **4e** and 4 h from **3a,b** and resin **4f**.

Synthesis of ODN and OP on polymer-supported quinoline carrier

The next step of the synthesis was the introduction of the first N^{α}-protected L-amino acid. This was achieved by the coupling reaction either with **2c** or resin **4d** in a customary manner by using Fmoc-L-Lys(Boc)–OH and PyBop/DIEA activation (Scheme 3) giving compound **5a** in moderate yield (33%) and resin **5b** in good yield (loading 0.53 mmol g^{-1}). Surprisingly, several attempts (*i.e.* FEP/DIEA or MSNT/MeIm) to improve the yield of compound **5a** failed.

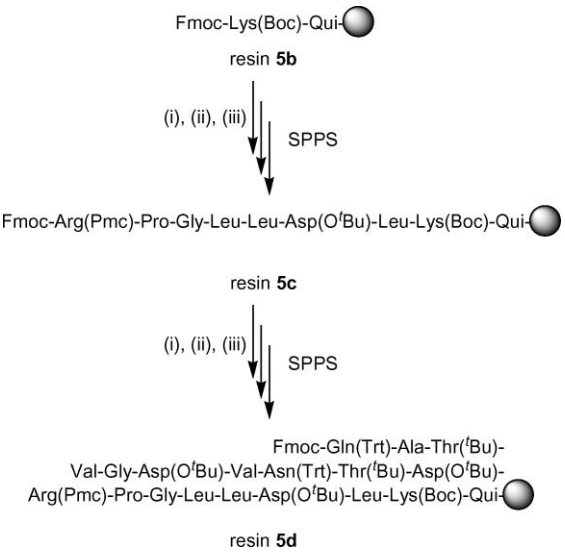


Scheme 3 *Reagents and conditions*: (i) Fmoc-L-Lys(Boc)–OH, PyBop, DIEA, DMF, 20 °C, 3 h from **2c** (33%) and 2 h from resin **4d**.

Both peptides, OP and ODN were synthesized from resin **5b** using a solid phase peptide synthesis (SPPS) with the standard Fmoc strategy as previously described[8] (Scheme 4). All the corresponding protected (O^{t}Bu for Asp, Pmc for Arg, Trt for Asn and Gln, ^{t}Bu for Thr) Fmoc-L-amino acids were sequentially coupled by *in situ* activation with HBTU/HOBt and DIEA in NMP. Acetic anhydride was used in the capping procedure and the *N*-terminal Fmoc group was successively removed by treatment with piperidine in NMP. According to this procedure, we obtained the functionalised resins **5c,d** with the protected peptides OP and ODN respectively.

Quaternization reaction and cleavage of resins 5c,d

We must point out that Yajima *et al.*[18] have reported for the chemical synthesis of proteins, an efficient deprotecting procedure with TMSOTf. Since this reactant could be used with proteins, we first attempted the cleavage step on resins **5c,d** with TMSOTf associated with trifluoroacetic acid in the presence of appropriate scavengers (Scheme 5) from 0 °C to 20 °C for 2 h. Following this procedure, the expected compounds **6a,b** were obtained after



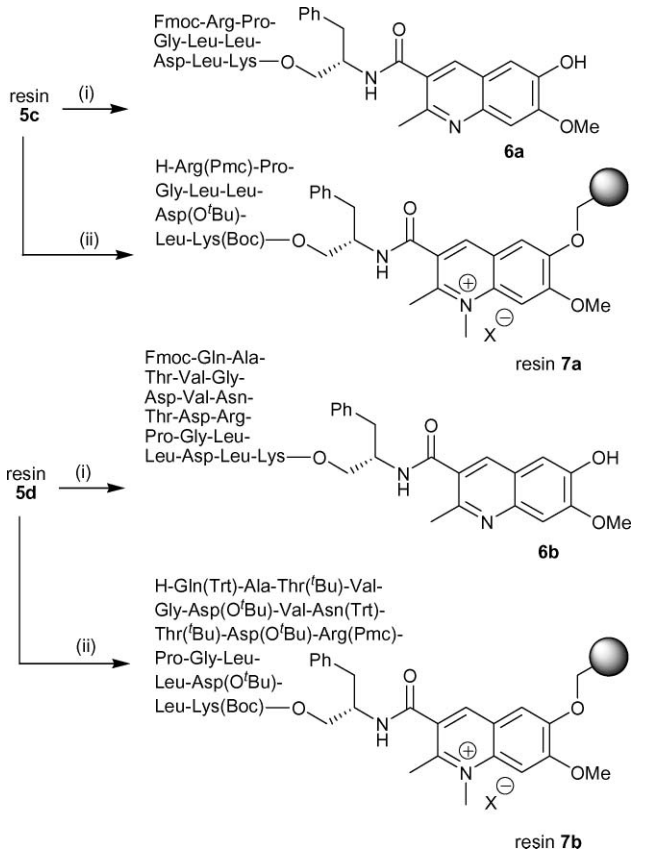
Scheme 4 *Reagents and conditions*: (i) piperidine 20%; (ii) N^{α}-Fmoc corresponding L-amino acid (4 equiv.), HBTU, HOBt, DIEA, NMP; (iii) Ac_2O, HOBt, DIEA.

side-chain deprotection and cleavage of the quinoline moiety from the resin. MALDI-TOF analysis revealed the occurrence of **6a,b** as the major products. However, we notice that, in addition to the presence of **6b**, we also observed two other products corresponding respectively to the loss of one and two molecules of water. In the literature,[19] a dehydratation reaction of the carboxamide part of pyroglutamide to give a nitrile group was described in the presence of $TMSCl/ZnCl_2$. Consequently, the use of TMSOTf in the presence of TFA could also promote such a side reaction from Gln and Asp residues of ODN since both contain a carboxamide group.

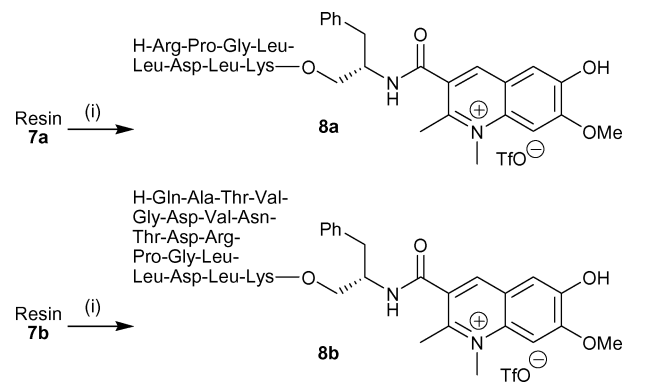
Finally, we undertook the quaternization reaction of the quinoline moiety of resins **5c,d** (Scheme 5) with MeOTf. Subsequent removal of the Fmoc group was accomplished by treatment with 20% piperidine in DMF for 20 minutes to afford the expected resins **7a,b**. Cleavage of resins **7a,b** was carried out with TMSOTf/TFA in the presence of scavengers and conducted at 0 °C for 2 h to lead to compounds **8a,b** (Scheme 6). MALDI-TOF analysis showed that **8a,b** were the major products in the crude mixture. In contrast to **6b**, we observed in the case of **8b** only a small peak on the mass-spectrum corresponding to the loss of one molecule of water. This is probably due to the fact that the cleavage step was carried out at 0 °C with resins **7a,b** instead of from 0 °C to 20 °C as previously done with resins **5c,d**. Furthermore, MALDI-TOF analysis suggests that permethylation also occured with MeOTf in addition to the quaternization reaction of quinoline. With peptide OP, only monomethylation took place while mono, di and trimethylation were observed with peptide ODN. Before the final reduction step of the quinolinium salt, compounds **8a,b** were purified by using a preparative HPLC.

Reduction of quinolinium salts 3b,d and 8a,b

We have previously reported the quantitative and regioselective reduction of quinolinium salt derivatives[7] by addition of a large excess of sodium dithionite and sodium carbonate giving rise to 1,4-dihydroquinolines. In the literature,[20] an alternative route using

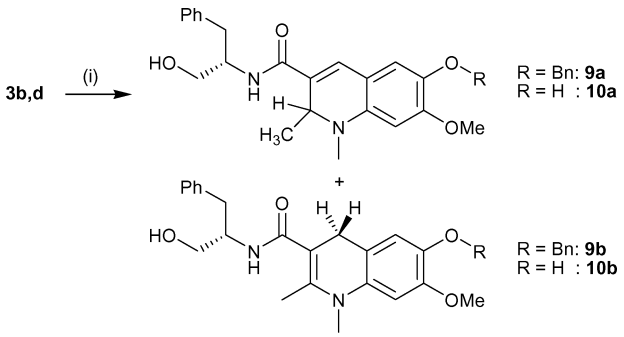


Scheme 5 *Reagents and conditions*: (i) TMSOTf, TFA, thioanisole, ethanedithiol, *m*-cresol, 0 °C then 20 °C, 2 h; (ii) MeOTf, 0 °C, 4 h then piperidine (20%), DMF then AcOH (5%), CH_2Cl_2.



Scheme 6 *Reagents and conditions*: (i) TMSOTf, TFA, thioanisole, ethanedithiol, *m*-cresol, 0 °C, 2 h.

BNAH (1-benzyl-1,4-dihydronicotinamide) also provided regioselective reduction of quinolinium salts to 1,4-dihydroquinolines derivatives. But, despite our efforts, reduction of compound **3b** either with sodium dithionite or BNAH failed. Speculating that both 1,2- and 1,4-dihydroquinoline may undergo enzymatic oxidation in the brain to give the corresponding quinolinium salt, we turned our attention to a non-regioselective reduction using $NaBH_4$ (Scheme 7). Thus, reduction of quinolinium salt **3b** gave the expected compounds **9a,b**. The analysis of the ^{1}H-NMR spectra assigned by NOE effects showed that a 11 : 9 mixture of **9a** and **9b** respectively was obtained. We were mainly interested in



Scheme 7 *Reagents and conditions*: (i) $NaBH_4$, EtOH, 20 °C, 2 h.

the reduction of compounds **8a,b**. However, it was first necessary for **8a,b**, which are available only on a small scale, to find a non-destructive and efficient analytical tool to monitor this reduction step. Pyridinium salts are characterised by a strong UV-absorption at 270 nm that decreases when reduced to the corresponding dihydropyridine. Besides, a second band at 360 nm characteristic of the pyridinium salt should be observed.[21] Then, we first attempted to investigate the reduction reaction by using UV–vis spectrometry with **3b**. The reduction of **3b** was recorded after 2, 5, 10 and 20 min of reaction (Fig. 4). It was obvious that both bands (255 nm and 365 nm) from the quinolinium salt **3b** decreased rapidly to afford dihydroquinolines **9a,b** after 20 min. Reduction was also undertaken with **3d** to obtain dihydropyridine derivatives **10a,b**. The so-obtained UV spectra were very close to those obtained with **3b**. The study being successful, the next step was to perform the same experiment with **8a,b** (Scheme 8). In both cases, the evolution of UV–vis spectrum is similar to that observed with **3b,d** (Fig. 5 for **8a**). Thus, the expected dihydropyridine derivatives **11a,b** and **12a,b** were obtained according to the disappearance of the two characteristic bands (255 nm and 365 nm) of the quinolinium salt **8a,b** and confirmed by a MALDI-TOF analysis.

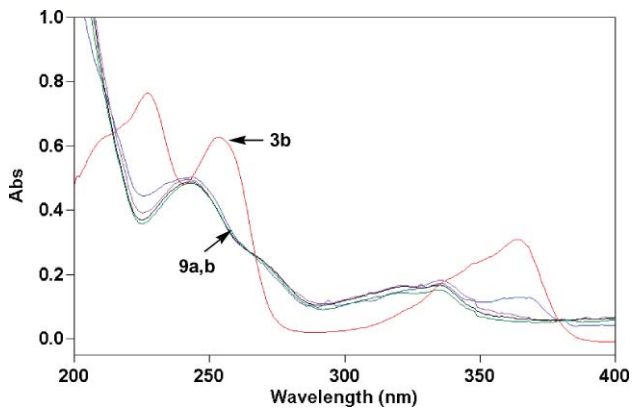
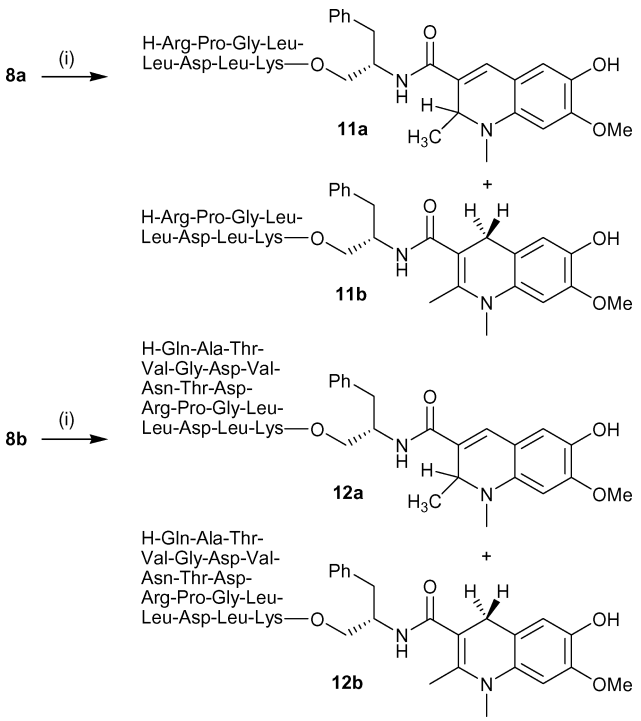


Fig. 4 UV–vis spectra of quinolium salt **3b** (red) and dihydropyridine derivatives **9a,b** formed by addition of $NaBH_4$ on **3b** after 2 min (blue), 5 min (violet), 15 min (black) and 20 min (green).

Conclusion

The fairly good stability of annulated NADH models makes this class of potential peptide carriers of particular interest. The



Scheme 8 *Reagents and conditions*: (i) $NaBH_4$, EtOH, 20 °C, 2 h.

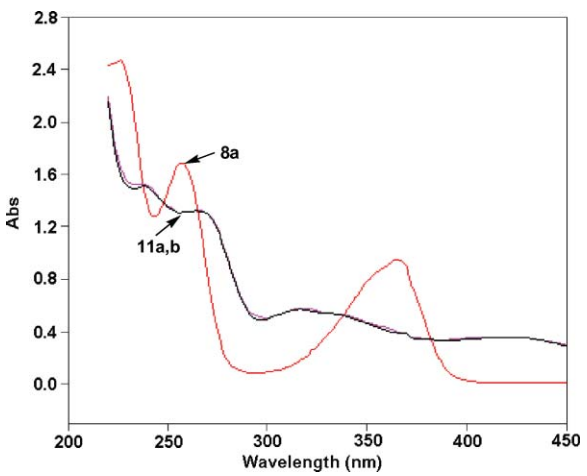


Fig. 5 UV–vis spectra of quinolium salt **8a** (red) and dihydropyridine derivatives **11a,b** formed by addition of $NaBH_4$ on **8a** after 15 min (violet) and 20 min (black).

rational design of new peptide carriers led us to prepare polymer-supported NAD+ annulated models. To this end, the grafting of quinolines to a Merrifield resin and subsequent stepwise SPPS of sophisticated neuropeptides such as OP and ODN was successfully achieved. Then, quaternization reaction and subsequent cleavage from the Merrifield resin of the so-obtained quinolinium salt derivatives followed by a final reduction afforded the expected compounds **11a,b** and **12a,b** as a mixture of 1,2- and 1,4-dihydropyridine derivatives.

Experimental

Infrared spectra were recorded on a Beckmann IR 4250 spectrometer. ^1H and ^{13}C-NMR spectra were recorded on a

200 MHz or 300 MHz Bruker apparatus and calibrated with the residual undeuterated solvent unless specified. Spectra were recorded in deuteriochloroform. The UV–vis spectroscopic measurements were carried out with a Varian Cary 100 biospectrophotometer. All amino-acid residues, *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU), 1-hydroxybenzotriazole (HOBt), piperidine and *N*,*N*-diisopropylethylamine (DIEA) were purchased from Applied Biosystems (St Quentin en Yvelines, France). Trifluoroacetic acid, trichloroacetic acid, phenol, thioanisol, ethanedithiol, *N*-methylpyrrolidin-2-one (NMP), *N*-methylmorpholine (NMM), *N*,*N*-dimethylformamide (DMF) were from Sigma-Aldrich Chimie. Merrifield resin (f = 1.2 meq g^{-1}, 1% DVB, 200–400 mesh) was from Novabiochem. Resin **4c** was prepared by using a Quest® 210 parallell synthesizer (Argonaut Technologies A.G.). Flash chromatography was performed with silica gel 60 (70–230 mesh from Merck) and monitored by thin layer chromatography (TLC) with silica plates (Merck, Kieselgel 60 F254). Peptides **8**, **11**, **12a,b** were purified by reversed-phase HPLC on a semipreparative Vydac C_{18} column (1 × 25 cm, Touzart and Matignon, Courtaboeuf, France) using a linear gradient (10–50% over 40 min) of CH_3CN/TFA (99.9 : 0.1, v/v) at a flow rate of 5 mL min^{-1}. Analytical RP-HPLC (1 mL min^{-1}) was performed on a Vydac C_{18} column (0.45 × 25 cm) using a linear gradient (10–40% over 30 min) of CH_3CN/TFA (99.9 : 0.1, v/v) at a flow rate of 1 mL min^{-1}. The purified peptides **8**, **11**, **12a,b** were characterized by FAB-MS on a conventional EB geometry mass spectrometer JEOL model AX-500 equipped with a DEC data system (JEOL-Europe SA, Croissy-sur-Seine, France). Compounds **6a,b** and **8a,b** were characterized by using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) MS (Tofspec E, Micromass, Manchester, UK) in the reflectron mode with α-cyano-4-hydroxycinnamic acid as a matrix.

5-Benzyloxy-4-methoxy-2-nitrobenzaldehyde 1b. Nitric acid (10 mL, 68% in water) was introduced in a round bottomed flask fitted with a mechanical stirrer and cooled with an ice bath at 0 °C. Thereafter, 3 benzyloxy-4-methoxybenzaldehyde (2 g, 14 mmol) was slowly added. The reaction mixture was stirred for 30 min at 0 °C and 1 h at 20 °C. Then, the reaction mixture was poured on ice-water to lead to a yellow solid which was filtered and washed with water. The yellow solid was dried under high vacuum to afford 5-benzyloxy-4-methoxy-2-nitrobenzaldehyde **1b** (1.93 g, 81%). Mp 130 °C (from H_2O); (Found: C, 62.93; H 4.55; N 4.72. Calc. for $C_{15}H_{13}NO_5$: C 62.72; H 4.56; N 4.88%); ν_{max} cm^{-1} 1683, 1577, 1512, 1338, 1280 1216, 1061; δ_H(200 MHz, $CDCl_3$, TMS) 4.03 (3 H, s), 5.27 (2 H, s), 7.44 (6 H, m), 7.63 (1 H, s), 10.42 (1 H, s); δ_C(50 MHz, $CDCl_3$) 56.7, 71.3, 107.3, 111.3, 125.35, 127.5 (×2), 128.6, 128.8 (×2), 135.0, 143.9, 152.3, 152.8, 187.6.

(5-Benzyloxy-4-methoxy-2-nitro-benzylidene)-*p*-tolylamine 1c. In a flask were introduced 5-benzyloxy-4-methoxy-2-nitrobenzaldehyde (4.31 g, 15 mmol), *p*-toluidine (1.93 g, 18 mmol) and absolute ethanol (300 mL). The mixture was refluxed for 2 h and, after cooling at 0 °C, a solid was filtered. Drying under high vacuum gave compound **1c** as a yellow solid (4.97 g, 88%). Mp 150 °C (from EtOH); (Found: C 70.27; H 5.34; N 7.56. Calc for $C_{22}H_{20}N_2O_4$: C 70.18; H 5.35; N 7.44%); ν_{max}/cm^{-1} 1567, 1511, 1382, 1322 1274, 1221, 1062, 986; δ_H(200 MHz, $CDCl_3$, TMS) 2.40 (3 H, s), 4.00 (3 H, s), 5.32 (2 H, s), 7.23 (4 H, s), 7.39–7.53 (5 H, m), 7.64 (1 H, s), 7.90 (1 H, s), 9.04 (1 H, s); δ_C(50 MHz, $CDCl_3$) 21.0, 56.4, 71.2, 107.4, 111.3, 121.1 (×2), 125.7, 127.7 (×2), 128.4, 128.6 (×2), 129.8 (×2), 135.4, 136.6, 142.5, 148.5, 150.8, 152.2, 155.0.

6-(*p*-Tolylaminomethylidene)-4-benzyloxy-3-methoxyaniline 1d. A solution of compound **1c** (3.76 g, 10 mmol) in EtOH (250 mL) was heated to reflux and sodium sulfite nonahydrate (5.28 g, 22 mmol) was added. After a few minutes, a vigorous reaction occurred and the heating was maintained for 10 min. After cooling at 0 °C for 4 h, the precipitate was filtered. The remaining solution was concentrated under reduced pressure. Water (100 mL) was added to the residue and a new crop of precipitate was obtained. Compound **1d** was obtained as a yellow solid (2.94 g, 85%). Mp 142 °C (from H_2O); (Found: C 76.31; H 6.39; N 8.13. Calc for $C_{22}H_{22}N_2O_2$: C 76.28; H 6.40; N 8.09%); ν_{max}/cm^{-1} 3383, 1629, 1599, 1548, 1506, 1455, 1245, 1208, 1140, 1006; δ_H(200 MHz, $CDCl_3$, TMS) 2.37 (3 H, s), 3.90 (3 H, s), 5.07 (2 H, s), 6.24 (1 H, s), 6.46 (2 H, br s), 6.85 (1 H, s), 7.06–7.20 (4 H, m), 7.31–7.47 (5 H, m), 8.34 (1 H, s); δ_C(50 MHz, $CDCl_3$) 20.9, 55.7, 72.6, 99.1, 110.0, 115.2, 120.7 (×2), 127.5 (×2), 127.8, 128.4 (×2), 129.6 (×2), 134.8, 137.4, 139.4, 145.6, 149.5, 153.9, 161.1; m/z (EI) 346.

Ethyl 6-benzyloxy-7-methoxy-2-methylquinoline-3-carboxylate 2a. To a solution of compound **1d** (3.46 g, 10 mmol) and ethylacetoacetate (1.52 g, 12 mmol) in EtOH (150 mL) were added a few drops of piperidine. The resulting solution was refluxed for 10 h. After cooling at 0 °C, the precipitate was filtered, rinsed with petroleum ether (75 mL) and dried to furnish compound **2a** as a beige powder (3.12 g, 89%). Mp 151 °C (from EtOH); (Found: C 71.38; H 5.98; N 4.04. Calc: for $C_{21}H_{21}NO_4$: C 71.78; H 6.02; N 3.99%); ν_{max}/cm^{-1} 2968, 1702, 1496, 1270, 1214, 1188, 1164, 1069; δ_H(300 MHz, $CDCl_3$, TMS) 1.37 (3 H, t, J 7.1), 2.90 (3 H, s), 3.95 (3 H, s), 4.34 (2 H, q, J 7.1), 5.16 (2 H, s), 7.01 (1 H, s), 7.25–7.44 (6 H, m), 8.45 (1 H, s); δ_C(75 MHz, $CDCl_3$) 14.1, 25.3, 55.9, 60.8, 70.5, 107.2, 120.7, 121.3, 127.0 (×2), 127.9, 128.4 (×2), 135.9, 137.8, 145.9, 148.5, 154.4, 156.4, 166.4.

6-Benzyloxy-7-methoxy-2-methyl-quinoline-3-carboxylic acid 2b. A solution of lithium hydroxide monohydrated (252 mg, 6 mmol) in absolute ethanol (15 mL) was refluxed and ester **2a** (702 mg, 2 mmol) was added. The reflux was continued for 3 h. Thereafter, the hot solution was filtered and cooled to 20 °C. The mixture was acidified with a solution of hydrochloric acid (2N). The acid which precipitated was filtered and dried under vacuum to furnish compound **2b** as a white solid (529 mg, 82%). (Found: C 70.71; H 5.09; N 4.63. Calc for $C_{19}H_{17}NO_4$: C 70.58; H 5.30; N 4.33%); δ_H(300 MHz, DMSO-d_6) 2.85 (3 H, s), 4.05 (3 H, s), 5.30 (2 H, s), 7.40–7.75 (7 H, m), 8.70 (1 H, s); δ_C(75 MHz, DMSO-d_6) 25.4, 56.2, 70.3, 107.4, 107.9, 121.1, 122.2, 128.6 (×3), 128.8 (×2), 136.7, 138.0, 145,8, 148.6, 154.4, 155.9, 168.2.

***N*-(1-Hydroxymethyl-2-phenylethyl)-6-benzyloxy-7-methoxy-2-methyl-quinoline-3-carboxamide 2c.** In a flask, flushed with nitrogen, was introduced compound **2b** (400 mg, 1.24 mmol) and 20 mL of CH_2Cl_2. Oxalyl chloride (0.44 mL, 5 mmol) and a few drops of DMF were added and the reaction mixture was stirred overnight. After evaporation of the solvent under reduced pressure, the residue was placed under a nitrogen atmosphere. In a tricol flask flushed with nitrogen, were introduced

S-phenylalaninol (755 mg, 5 mmol), NEt_3 (0.7 mL, 5 mmol) and dichloromethane (20 mL). The reaction mixture was cooled at −10 °C. Dichloromethane (20 mL) was added to the intermediate acid chloride and the suspension was added dropwise to the tricol flask in order to keep the temperature below 0 °C. At the end of addition, the reaction medium was kept at −5 °C for 1 h and stirred at 20 °C for 18 h. The solution was concentrated under reduced pressure and the residue was purified by column chromatography on silica (CH_2Cl_2/EtOH: gradient from 19 : 1 to 9 : 1). Compound **2c** was obtained as a white-yellow solid (418 mg, 74%). (Found: C 74.01; H 6.05; N 6.04. Calc for $C_{28}H_{28}N_2O_4$: C 73.66; H 6.18; N 6.14%); ν_{max}/cm^{-1} 3277, 3028, 2925, 1639, 1495, 1252, 1028, 739, 698; δ_H(300 MHz, $CDCl_3$, TMS) 2.49 (3 H, s), 2.73–2.84 (1 H, dd, J_3 8, J_4 13), 2.91–2.98 (1 H, dd, J_1 7 $J_2 = 13$), 3.38–3.54 (2 H, ddd, J_1 7 J_3 4 J_2 11), 3.95 (3 H, s), 4.32 (1 H, m), 5.19 (2 H, s), 7.24 (1 H, s), 7.21–7.42 (12 H, m), 7.71 (1 H, s); δ_C(50 MHz, $CDCl_3$, TMS) 22.6, 37.1, 53,2, 56.3, 63.5, 71.1, 106.7, 107.6, 127.5 (×2), 128.4, 128.7 (×3), 128.9 (×3), 129.5 (×2), 134.0, 136.4, 138.3, 145.1, 149.0, 154.2, 170.1.

6-Benzyloxy-3-ethoxycarbonyl-7-methoxy-1,2-dimethyl-quinolinium trifluoromethanesulfonate 3a. In a flask flushed with nitrogen, methyl trifluoromethanesulfonate (136 μL, 1.2 mmol) was added to a solution of ester **2a** (351 mg, 1 mmol) in CH_2Cl_2 (20 mL). The solution was stirred for 1 h and then concentrated in vacuum. Dry diethyl ether (50 mL) was added and the precipitate collected by filtration, rinsed with 10 mL of dry diethyl ether, and dried under vacuum. Compound **3a** (371 mg, 72%) was obtained as a a beige solid. (Found: C 53.37; H 4.79; N 3.02; S 6.35. Calc for $C_{23}H_{24}F_3NO_7S$: C 53.59; H 4.69; N 2.72; S 6.22%); ν_{max}/cm^{-1} 3072, 3002, 1723, 1625, 1428, 1321, 1262, 1033; δ_H(300 MHz, $CDCl_3$, TMS) 1.46 (3 H, t, $J = 7.1$), 3.17 (3 H, s), 4.21 (3 H, s), 4.46 (2 H, q, $J = 7.1$), 4.51 (3 H, s), 5.25 (2 H, s), 7.37–7.50 (6 H, m), 7.65 (1 H, s), 9.06 (1 H, s); δ_C(75 MHz, $CDCl_3$, TMS) 14.1, 19.8, 41.0, 58.2, 63.1, 71.4, 99.4, 109.0, 120.6 (J_{C-F} 319 Hz), 123.3, 123.8, 127.4 (×2), 128.7, 128.9 (×2), 134.7, 139.4, 144.1, 151.1, 155.7, 160.1, 163.8; δ_F(282 MHz, $CDCl_3$) −78.7.

6-Benzyloxy-3-(1-hydroxymethyl-2-phenyl-ethylcarbamoyl)-7-methoxy-1,2-dimethyl-quinolinium trifluoromethanesulfonate 3b was obtained as a white solid in 90% yield (56 mg), as described for **3a**, from compound **2c** (50 mg, 0.1 mmol) in $CHCl_3$ (20 mL), methyl trifluoromethanesulfonate (15 μL, 0,12 mmol) and stirring for 6 h. (Found C 57.86, H 4.98, N 4.52, S 5.06. Calc for $C_{30}H_{31}F_3N_2O_7S$ C 58.06, H 5.03, N 4.51, S 5.17%); δ_H(300 MHz, $CDCl_3$, TMS) 2.60 (3 H, s), 2.80–3.00 (2 H, m), 3.70 (3 H, m), 4.00 (3 H, s), 4.20 (3 H, s), 4.32 (1 H, m), 5.15 (2 H, s), 7.05–7.42 (12 H, m), 7.85 (1 H, d, J 12), 8.25 (1 H, s); δ_C(50 MHz, $CDCl_3$, TMS) 19.7, 37.2, 39.9, 54.1, 57.4, 64.0, 71.6, 98.3, 109.3, 120.5 (J_{C-F} 319 Hz), 124.1, 126.8, 127.8, 128.7 (×2), 128.9 (×2), 129.1 (×2), 129.5, 129.7, 131.2, 135.2, 138.1, 138.3, 141.3, 151.0, 153.6, 158.7, 166.2; δ_F(282 MHz, $CDCl_3$) −78.9; *m*/*z* (FAB-MS) 471 (M^+–OTf).

3-Ethoxycarbonyl-6-hydroxy-7-methoxy-1,2-dimethyl-quinolinium trifluoromethanesulfonate 3c. To a solution of compound **3a** (500 mg, 0.97 mmol) in CH_2Cl_2 (10 mL) under a nitrogen stream, trimethylsilyl trifluoromethanesulfonate (0.57 mL, 2.91 mmol) was added dropwise. The solution became red and was stirred for 3 h. A solution of MeOH (3 mL) saturated with HCl gas was added and the solution concentrated to a volume of 2 mL. Dry diethyl ether (50 mL) was added and the precipitate was collected by filtration, rinsed with dry diethyl ether, and dried under vacuum to give compound **3c** as a beige solid (371 mg, 90%). ν_{max}/cm^{-1} 3460br, 1731, 1408, 1321, 1247, 1024; δ_H(300 MHz, $(CD_3)_2CO$) 1.44 (3 H, t, J 7.1), 3.31 (3 H, s), 4.29 (3 H, s), 4.50 (2 H, q, J 7.1), 4.69 (3 H, s), 7.77 (1 H, s), 7.98 (1 H, s), 9.29 (1 H, s); δ_C(75 MHz, $(CD_3)_2CO$) 14.8, 20.4, 41.5, 58.4, 63.8, 100.5, 112.7, 123.5 (J_{C-F} 337 Hz), 125.2, 125.8, 139.6, 145.3, 151.0, 157.4, 159.4, 165.6; δ_F(282 MHz, $(CD_3)_2CO$) −78.7.

6-Hydroxy-3-(1-hydroxymethyl-2-phenyl-ethylcarbamoyl)-7-methoxy-1,2-dimethyl-quinolinium trifluoromethanesulfonate 3d was obtained as described for **3c**, from compound **3b** (170 mg, 0.28 mmol) in CH_2Cl_2 (15 mL), trimethylsilyl trifluoromethanesulfonate (0.22 mL, 1 mmol) and stirring for 4 h. The solution was centrifuged at 1200 rpm after addition of diethyl ether to lead to compound **3d** in a complete conversion and as a very hygroscopic solid. (Found: C 52.21; H 4.95; N 4.99; S 5.85. Calc for $C_{23}H_{25}F_3N_2O_7S$: C 52.07; H 4.75; N 5.28; S 6.04%); δ_H(300 MHz, $(CD_3)_2CO$) 2.60 (3 H, s), 2.60–2.70 (2 H, m), 3.60 (2 H, m), 4.00 (3 H, s), 4.30 (4 H, m), 7.00–7.20 (5 H, m), 7.30 (1 H, s), 7.50 (1 H, s), 7.95 (1 H, d, J 12), 8.30 (1 H, s); δ_C(75 MHz, $(CD_3)_2CO$) 20.4, 38.1, 41.2, 55.3, 58.1, 63.5, 100.0, 112.0, 125.2, 127.1, 129.6 (×2), 130.7 (×2), 132.2, 139.9, 141.7, 150.7, 155.1, 158.1, 170.1.

6-Hydroxy-7-methoxy-2-methyl-quinoline-3-carboxylic acid ethyl ester 4a. In a flask purged with nitrogen were placed compound **2a** (351 mg, 1 mmol), absolute ethanol (100 mL) and Pd/C (10%, 125 mg, 0.12 mmol). Nitrogen was replaced by hydrogen and the reaction mixture was stirred at 20 °C for 2 h. After a filtration over celite, the solution was evaporated under reduced pressure and left for 2 h at 20 °C to afford compound **4a** (167 mg, 64%) as beige powder. Mp 196 °C; (Found: C 64.04; H 6.05; N 5.35. Calc. for $C_{14}H_{15}NO_4$: C 64.36; H 5.79; N 5.36); ν_{max}/cm^{-1} 3390, 1714, 1495, 1303, 1207, 1077; δ_H(300 MHz, $CDCl_3$, TMS) 1.45 (3 H, t, J 7.1), 2.95 (3 H, s), 4.06 (3 H, s), 4.42 (2 H, q, J 7.1), 6.33 (1 H, br s), 7.24 (1 H, s), 7.39 (1 H, s), 8.57 (1 H, s); δ_C(75 MHz, $CDCl_3$, TMS) 14.3, 25.2, 56.3, 61.2, 106.5, 108.9, 121.7, 122.1, 138.2, 145.4, 146.2, 152.2, 156.2, 166.7.

***N*-(1-Hydroxymethyl-2-phenylethyl)-6-hydroxy-7-methoxy-2-methyl-quinoline-3-carboxamide 4b** was obtained as a yellow solid (310 mg, 97%) as described for **4a**, from compound **2c** (400 mg, 0.87 mmol) in MeOH (50 mL), Pd/C (10%, 211 mg, 0.2 mmol) and stirring for 24 h. δ_H(300 MHz, CD_3OD) 2.30 (3 H, s), 2.65 (1 H, dd, J 9 and 13), 2.95 (1 H, dd, J 9 and 13), 3.60 (2 H, d_{app}, J 11), 3.85 (3 H, s), 4.35 (1 H, m), 6.90 (1 H, s), 7.00–7.20 (6 H, m), 7.65 (1 H, s); δ_C(75 MHz, CD_3OD) 22.9, 38.7, 55.1, 56.9, 65.2, 106.9, 110.3, 123.5, 127.9, 129.9 (×2), 130.3, 130.7 (×2), 135.0, 140.4, 145.3, 149.6, 154.2, 155.0, 171.8; *m*/*z* (IC^+) 367 $[MH]^{+\bullet}$.

Polymer-supported compound 4a (resin 4c). Reactions were carried on a Quest® 210. In a round-bottomed flask flushed with nitrogen were introduced a Merrifield resin (400 mg, $f = 1.2$ meq g^{-1}), compound **4a**, sodium hydride (19 mg of a dispersion in oil at 60%, 0.47 mmol), and DMF (10 mL). The reaction mixture was mechanically stirred for 4 days at 20 °C. The resin was removed by filtration and washed successively with CH_2Cl_2 (3 × 50 mL), water/THF (1 : 1, 3 × 50 mL), MeOH (3 × 50 mL), CH_2Cl_2 (3 × 50 mL) and placed in a drying oven under reduced pressure. A

beige resin was obtained (Found: N 1.30 corresponding to f = 0.93 mmol g^{-1}); ν_{max}/cm^{-1} 3059–2849, 1718, 1491, 1200, 1062.

Polymer-supported compound 4b (resin 4d) was obtained as a beige resin as described for resin **4c**, from compound **4b** (400 mg, 1.1 mmol), Merrifield resin (500 mg, f = 1.2 meq g^{-1}), sodium hydride (42 mg of a dispersion at 60% in oil, 1.05 mmol) and DMF (5 mL). Drying was achieved with a nitrogen stream. (Found: N 2.53 corresponding to f = 0.90 mmol g^{-1}); ν_{max}/cm^{-1} 3398, 1656, 1600, 1243, 1154, 1026, 739, 695.

Polymer-supported compound 3c (resin 4e). In a flask flushed with nitrogen were placed resin **4c** (400 mg, f = 0.93 meq g^{-1}) and CH_2Cl_2 (10 mL). Methyl triflate (110 μL, 0.97 mmol) was added dropwise and the reaction mixture was mechanically stirred for 4 h. The resin was filtered and washed three times with CH_2Cl_2, twice with MeOH, and then three times with CH_2Cl_2. The resin was placed in a drying oven under reduced pressure to furnish a beige resin (Found: N 1.13 corresponding to f = 0.81 mmol g^{-1}); δ_F(282 MHz, $CDCl_3$) −78.5; ν_{max}/cm^{-1} 3024, 2919, 1728, 1621, 1505, 1253, 1151, 1029.

Polymer-supported compound 3d (resin 4f) was obtained as described for resin **4e**, from resin **4d** (354 mg, f = 0.90 mmol g^{-1}), CH_2Cl_2 (5 mL) and methyl triflate (96 μL, 0.85 mmol) after drying with a nitrogen stream. (Found: N 1.76 corresponding to f = 0.63 mmol g^{-1}); δ_F(282 MHz, $CDCl_3$) −79.

Cleavage of resin 4e. In a dry round-bottomed flask flushed with nitrogen, resin **4e** (300 mg, f = 0.81 mmol g^{-1}) and CH_2Cl_2 (10 mL) were introduced. Thereafter, trimethylsilyle trifluoromethanesulfonate (210 μL, 1 mmol) was added dropwise and the reaction mixture was mechanically stirred for 2 h. A few drops of a solution of MeOH saturated with HCl gas was added until the solution went colourless. The solution was filtered and the resin was washed with CH_2Cl_2, then with dry acetone. The solution was concentrated under reduced pressure and a few drops of dichloromethane and dry diethyl ether were added. The precipitate was filtered to afford compound **3c** (71 mg, 69%). The analyses were in agreement with those described previously in this paper for **3c**.

Cleavage of resin 4f was obtained as described for the cleavage of resin **4e**, from resin **4f** (150 mg, f = 0.63 mmol g^{-1}), CH_2Cl_2 (5 mL) and trimethylsilyl trifluoromethanesulfonate (246 μL, 1.17 mmol) to afford compound **3d** (26 mg, 52%). The analyses were in agreement with those described previously in this paper for **3d**.

6-*tert*-Butyloxycarbonylamino-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-hexanoic acid 2-[(6-benzyloxy-7-methoxy-2-methyl-quinoline-3-carbonyl)-amino]-3-phenyl-propyl ester 5a. A solution of compound **2c** (200 mg, 0.44 mmol), PyBop (230 mg, 0.44 mmol), CH_2Cl_2 (20 mL), *N*-α-Fmoc-*N*-ε-Boc-L-lysine (205 mg, 0.44 mol) and DIEA (160 μL, 0.87 mmol) was stirred for 3 h at 20 °C. The solvent was removed under reduced pressure and the residue was purified by chromatography on silica (from EtOAc/cyclohexane to EtOAc/EtOH) to afford compound **5a** as a white solid (132 mg, 33%). δ_C(75 MHz, $CDCl_3$) 22.5, 23.6, 28.8 (×3), 30.0 (×2), 37.7, 39.8, 47.2, 50.7, 54.6, 56.5, 65.8, 67.1, 71.1, 79.7, 107.5, 107.8, 120.3, 121.1, 125.2, 127.4 (×2), 127.6 (×4), 128.0 (×2), 128.4 (×2), 128.6, 129.1 (×4), 129.6 (×2), 133.7, 136.7, 137.6, 141.5, 143.7, 144.0, 145.3, 149.0, 154.0, 154.5, 156.7, 169.2, 173.2; m/z (FAB-MS) 907 $[M]^{+\bullet}$.

Fmoc-Lys(Boc)–Qui–P (resin 5b) was obtained as described for compound **5a**, from resin **4d** (438 mg, f = 0.90 mmol g^{-1}), PyBop (547 mg, 1.05 mmol), and *N*-α-Fmoc-*N*-ε-Boc-L-lysine (492 mg, 1.0 mmol), DMF (5 mL) and DIEA (581 μL, 3.15 mmol) for 2 h. The resin was washed successively with DMF (3 × 5 mL), CH_2Cl_2 (3 × 5 mL), MeOH (3 × 5 mL), CH_2Cl_2 (3 × 5 mL) and dried with a nitrogen stream to lead to resin **5b** (Found N 2.95, f = 0.53 mmol g^{-1}); ν_{max}/cm^{-1} 3422, 1702, 1491, 1238, 1152, 736, 670.

Peptide synthesis (resins 5c,d). Peptide synthesis was carried out on resin **5b** on a 433A peptide synthesizer (Applied Biosystems, Saint-Quentin-en-Yvelines, France) using the standard manufacturer's procedures as previously described.[11] All Fmoc-L-aminoacids (4 equiv.) were coupled by in situ activation with HBTU/HOBt (3.6 equiv., 1 : 1, mol/mol) and DIEA (8 equiv.) in NMP. Reactive side chains were protected as followed: Gln and Asn, tritylamide (Trt), Thr, *tert*-butyl ether (*t*-Bu), Asp, *tert*-butyl ester (O*t*-Bu), Arg, pentamethylchromansulfonylamide (Pmc), and Lys, *tert*-butyloxycarbonyl (Boc). After completion of chain assembly, N^{α}-acetylation of peptides was performed on the resin by addition of a mixture of acetic anhydride/DIEA/HOBt (4 equiv., 1 : 1 : 1, mol/mol/mol) in NMP for 5 min. Reactions were monitored by the Kaiser test.[22] Micro cleavages were carried out on **5c** and **5d** to give respectively **6a** m/z (MALDI-TOF) 1482.5 (MH^+) and **6b** m/z (MALDI-TOF) 2483 (MH^+).

Compounds 6a,b. To the corresponding resin **5c,d** was added a mixture of TMSOTf/TFA/thioanisole/*m*-cresol/ethanedithiol (10 mL, 1.94 mmol, 6.89 mmol, 1.2 mmol, 0.2 mmol, 0.6 mmol) for 2 h at 0 °C. The solution was filtered and compounds **6a,b** were precipitated by addition of TBME, centrifuged (4500 rpm), washed twice with TBME and lyophilised. **6a** m/z (MALDI-TOF) 1482.5 (MH^+) and **6b** m/z (MALDI-TOF) 2483 (MH^+).

Quaternarization reactions of polymer-supported quinolines (resins 7a,b). To the corresponding resin **5c,d** under an atmosphere of nitrogen were added CH_2Cl_2 (4 mL) and methyl trifluoromethanesulfonate (2 equiv.). The reaction mixture was stirred for 2 h at 20 °C. The resin was washed three times with CH_2Cl_2, twice with MeOH, three times with CH_2Cl_2 and twice with DMF. A solution of piperidine (20% in DMF) was introduced. The resin became red and was stirred for 20 min. The resin was filtered, and washed twice with DMF, twice with CH_2Cl_2 and twice with a solution of acetic acid (5% in CH_2Cl_2). The resin lost its colour and was finally washed twice with CH_2Cl_2. The resin was dried overnight in a dessicator under vacuum. Micro cleavages were done according to the procedure described for **6a,b** with resin **7a** and resin **7b** to afford respectively compounds **8a** m/z (MALDI-TOF) 1273.5 (MH^+) and **8b** m/z (MALDI-TOF) 2274 (MH^+), 2289 (MH^+ + 15), 2304(MH^+ + 30).

Compounds 8a,b were obtained according to the procedure described for **6a,b** with resin **7a** and resin **7b** to afford respectively compounds **8a** m/z (MALDI-TOF) 1273.5 (M^+) and **8b** m/z (MALDI-TOF) 2274 (M^+), 2289 (M^+ + 15), 2304(M^+ + 30).

Dihydroquinolines 9a,b. To compound **3b** (50 mg, 0.08 mmol) in absolute ethanol (1 mL) was added $NaBH_4$ (4.6 mg, 0.12 mmol). The reaction mixture was stirred for 2 h at 20 °C and 1 mL of degassed water was added. The solution was extracted with degassed dichloromethane (3 × 5 mL). The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure to afford **9a,b** (29 mg, 76%) as a yellow oil. λ_{max}(EtOH) 245 and 335 nm; **9a**: δ_H(300 MHz, $CDCl_3$, TMS) 1.00 and 1.25 (3 H, m), 2.7–3.3 (6 H, m), 3.6–3.8 (2 H, m), 3.90 (3 H, s), 4.21 (2 H, m), 5.02 and 5.24 (2 H, 2xs), 6.08 and 6.4–6.6 (2 H, m), 7.15–7.5 (10 H, m); **9b**: 1.72 and 2.20 (3 H, 2xs), 2.7–3.3 (6 H, m), 3.6–3.8 (2 H, m), 3.87 (3 H, s), 4.34 (2 H, m), 5.07 and 5.29 (2 H, 2xs), 6.08 and 6.4–6.6 (2 H, m), 7.15–7.5 (10 H, m).

Dihydroquinolines 10a,b were obtained as a yellow oil (174 mg, 81%) as described for dihydroquinolines **9a,b**, from **3d** (300 mg, 0.56 mmol), $NaBH_4$ (42 mg, 1.12 mmol) and absolute ethanol (1 mL). λ_{max}(EtOH) 245 and 335 nm.

Dihydroquinolines 11a,b and 12a,b. To the corresponding quinolinium salt **8a,b** dissolved in the minimum of absolute ethanol, was added $NaBH_4$ (2 equiv.). The reaction mixture was stirred for 2 h at 20 °C and the solvent was removed under reduced pressure. The residue was dissolved in acetonitrile, and purified on silica grafted C_{18} with a gradient water/acetonitrile (water 100% to acetonitrile 100%). The collected fractions were analysed by HPLC and those containing the dihydropyridines were lyophilised and stocked at −4 °C. **11a,b** and **12a,b**: λ_{max}(EtOH) 240, 265, 315 nm.

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