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Solid-phase synthesis of head and tail bis-acridinylated peptides

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Abstract—Amino-acridine conjugates play an important role as biochemical probes and/or drugs. Solid-phase synthesis of such compounds suitable for library construction and biological screening is described.

Many types of drugs are known to bind to DNA through intercalation between consecutive nucleotides in the DNA strand.[1,2] Among them, acridine and its derivatives have long been a well-established class of DNA and RNA binding compounds.[3] The mode of binding of acridine molecules involves intercalation of the acridine tricyclic ring between adjacent base pairs in the DNA duplex.[4,5] The binding of acridine molecules interferes with normal DNA function by blocking the DNA starter required by polymerases to synthesize RNA and DNA and hence inhibits protein synthesis.[6] Some time ago, it was shown that bis-intercalators including acridine nuclei, exhibited both higher affinity[7] and selectivity[8,9] in binding. Recently, acridine-based compounds were characterized as a novel class of compounds with anti-prion activity. Prions have been implicated as causative agents in many neurodegenerative diseases such as Creutzfeld–Jacobs disease and BSE. Moreover, the covalent dimers of amino-acridine, due to increased local concentration of the active moiety, were found to be the most potent inhibitors of prion association resulting from the propensity of prion protein to assemble into multimers. A potent bis-acridine inhibitor of prion aggregation in ScN2a cells is depicted in Figure 1, possessing a potentially acceptable therapeutic index.[10,11]

We propose that solid-phase synthesis of on-bead combinatorial libraries of head and tail bis-9-amino-acridine peptides could lead to compounds with variable spacer length (approximately 2–4 Å per amino acid) and charged, polar or hydrophobic residues at desired

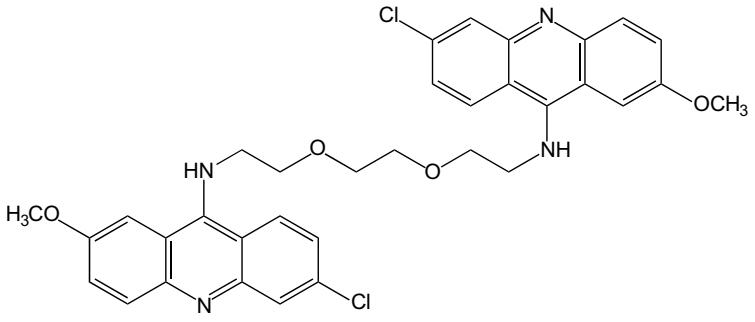

Figure 1. The most powerful in vivo synthetic inhibitor of prion aggregation.

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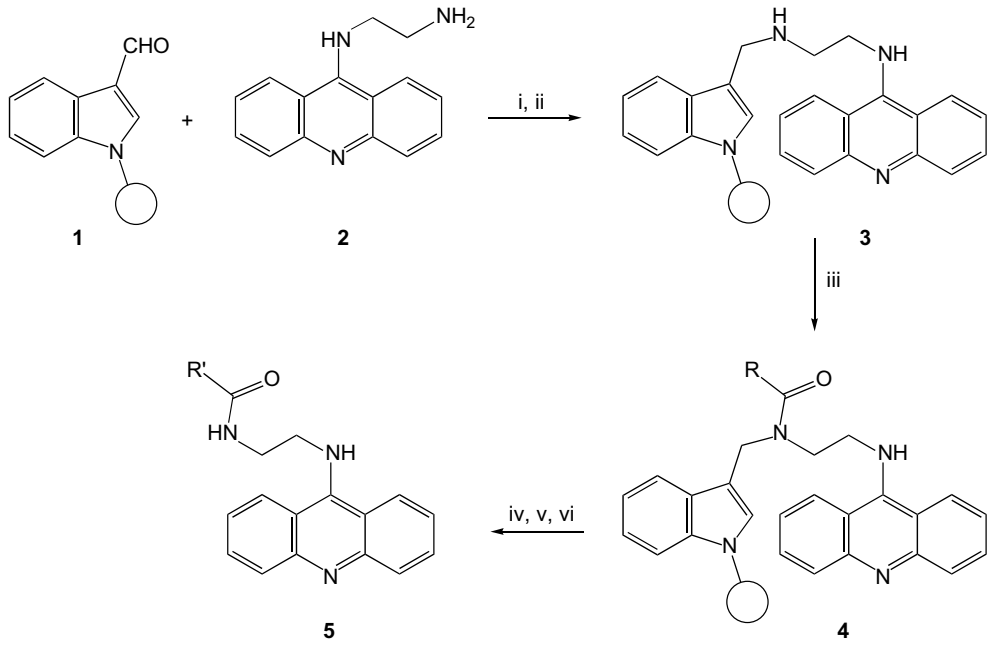

Figure 2. Synthesis of peptides with 9-amino-acridine at the C-terminus. (i) Stirring 24 h, (ii) $NaBH_4$, THF/EtOH, 4–20 h, (iii) Fmoc-Gly-OH/DIC/HOBt, (iv) 25% piperidine/DMF, (v) repetition of coupling and deprotection steps until the desired structure was obtained, (vi) TFA/anisole/DCM (2:1:20) and then TFA/triisopropylsilane/H_2O (95:2.5:2.5).

Table 1. The acridine–peptide conjugates synthesized and the dimeric by-products

Entry	Compounds[a,b]	HPLC-RT[c] (min)	Yield (%)
1	**Fmoc-Gly**-$NHCH_2CH_2NH$-Acr	23.5	65
2	(Fmoc-Gly-$NHCH_2)_2$	29.8	34
3	**Acr-Gly-Gly-Gly**-$NHCH_2CH_2NH$-Acr	11.9	50
4	(Acr-Gly-Gly-Gly-$NHCH_2)_2$	14.3	48
5	**Acr-Gly-Gly-Tyr-Gly**-$NHCH_2CH_2NH$-Acr	15.7	40
6	(Acr-Gly-Gly-Tyr-Gly-$NHCH_2)_2$	16.2	40

[a] Acr stands for acridin-9-yl.
[b] R′-CO- from Figure 2 is emphasized with bold font.
[c] HPLC RP C-18 LiChroCart column, gradient MeCN/H_2O, 0–100%, 40 min.

positions, which can increase binding affinity, conformation stability and/or biological activity.[12–15] Approaches based on difunctionalized amino-acridines have already been reported in the literature.[16–18] They require introduction of another reactive group (e.g., carboxyl) to either the C-terminal amino acid (Asp, Glu) side-chain or in the acridine moiety. This group serves to anchor the molecule to the resin, however, after cleavage it remains as a part of the target molecule. Acridinylation on resin has been described, although it required high temperatures, which affected resin stability.[19,20] Here, we describe the synthesis of bis-acridine-containing peptides assembled on polymer support, which can be used for solution and in some cases (demanding acridines suitably positioned on the peptide chain) on-bead assays. We have found that C-terminal *N*-alkyl-9-amino-acridines cannot be prepared by nucleophilic displacement on Kenner or Kaiser resins because of unwanted cleavage of the acridine from the N-terminus.

As acidic cleavage is required for C-terminal *N*-alkyl-9-amino-acridines, a Merrifield resin with an indole-3-carboxaldehyde amide-backbone-acid-labile linker **1**[21] was chosen. The resin was loaded with *N*-(acridin-9-yl)ethylene diamine **2** freshly released from its dihydrobromide salt and prepared following a synthesis of its propylene diamine analogue.[22,23] Subsequent reduction of the aldimine with $NaBH_4$ (Fig. 2) was monitored by IR and/or Raman spectroscopy as the aldimine peak at 1635 cm^{-1} disappears. First, an amino acid was loaded using DIC/HOBt in DMF and an on-bead acridinylated derivative at the C-terminus was obtained **4**. Conventional Fmoc/*tert*-butyl peptide synthesis was carried out to build up an appropriate derivative. A second acridine was introduced at the end, with in situ generated *N*-(acridin-9-yl)glycine activated ester, to the N-terminus. Bis-acridine peptides were cleaved from the resin with TFA/anisole/DCM (2:1:20) and side-chain protection was removed by subsequent treatment with TFA/triisopropylsilane/water (95:2.5:2.5). All the peptides prepared were accompanied by dimer by-products in amounts depending on the duration of borohydride treatment. Acridine-containing peptides were purified by RP-HPLC and characterized by RP18-HPLC, FAB-

MS, amino acid analysis and some of them by ^{1}H NMR spectroscopy. Table 1 shows a set of synthesized peptide–acridine conjugates and the dimeric by-products.

In summary, solid-phase synthesis of N- and C-terminal acridinylated peptides was performed. The synthetic route reported herein seems to be suitable for the synthesis of a combinatorial library of anti-prion compounds, which is now under study in our laboratory.

Acknowledgements

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23. Loading of Acr-NHCH$_2$CH$_2$NH$_2$ **2** to 3-formyl-indole resin **1**: Freshly released **2** (7.76 g, 32.7 mmol) from its HBr salt was dissolved in 50 mL THF/DMF (2:3) which was followed by the addition of 3-formylindole polystyrene resin (**1**, 5.3 g; 5.46 mmol). The slurry obtained was stirred for 20 h at rt and then extensively washed with THF and dried in vacuo overnight. The course of reaction was monitored by comparison of the reference band at 1601 cm^{-1} with the disappearing and increasing bands at 1660 cm^{-1} and 1634 cm^{-1}, respectively. IR: *v* (C=N–) 1634 cm^{-1}, *v* (polystyrene) 1601 cm^{-1}. Raman *v* (C=N–) 1640 cm^{-1}, *v* (polystyrene) 1602 cm^{-1}. The prepared resin was treated with $NaBH_4$ (2 g, 52.8 mmol) in 120 mL THF/EtOH (1:1) for approximately 12–24 h until the band at 1634 cm^{-1} vanished. Yield: 6.28 g of loaded (0.5 mmol g^{-1} content of N) resin. IR: *v* (polystyrene) 1601 cm^{-1}.