Very Short and Efficient Syntheses of the Spermine Alkaloid Kukoamine A and Analogs Using Isolable Succinimidyl Cinnamates

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Direct selective acylation of the primary amino functions of spermine and spermidine with a variety of isolable succinimidyl cinnamates, followed by catalytic hydrogenation, gave high yields of the spermine alkaloid kukoamine A and analogs suitable for structure-activity relationship studies. Suitable succinimidyl cinnamates were readily obtained through Wittig reaction of aromatic aldehydes with the ylides $Ph_3P{=}CRCO_2Me$, followed by saponification and activation with *N*-hydroxysuccinimide in the presence of *N*,*N*′-dicyclohexylcarbodiimide.

Kukoamine A (KukA, **1a**), a spermine alkaloid isolated from the dried root bark of Lycium chinense, is a clinically effective hypotensive agent[1] and a potent and selective inhibitor of trypanothione reductase (TryR),[2] a crucial enzyme for the survival of pathogenic trypanosomatid parasites.[3] KukA therefore constitutes an interesting lead for the development of effective antiparasitic agents. Several multi-step synthetic protocols, in liquid[4,5] or on solid phase,[6] for the preparation of KukA have been published, along with the preparation and *anti*-TryR activities of a series of mono- and disubstituted spermine (SPM) and spermidine (SPD) analogs of KukA, such as the SPD analog **2a** (SkukA) and the SPM analogs **1k** and **3a** (Figure 1).[2] Many other spermine or spermidine derivatives bearing arylalkyl or arylacyl units on their primary or secondary amino functions respectively have been prepared based on KukA[7,8] and have also been shown to exhibit potent *anti*-TryR activity. Preparation of such compounds also typically requires several steps of sequential orthogonal protection/deprotection of the primary and secondary amino functions. We have recently shown that all four possible regioisomers of KukA, namely KukA-D, and all three possible SPD regioisomers (SkukA-C) can be readily obtained, albeit in multi-step syntheses, by using suitably protected SPM and SPD derivatives and *O*,*O*′-bisbenzylcaffeoyl chloride to introduce the required dihydrocaffeoyl unit into the polyamine skeleton.[9] However, in the course of a different study we have observed that the corresponding isolable, crystalline, succinimidyl ester **8b** is a more stable alternative to the aforementioned chloride, and very selective towards the primary amino functions of polyamines.[10] Although the selective reactivity of certain succinimidyl[11] and alkylphenyl[12] carbonates has been previously applied in the preparation of selectively protected polyamine derivatives, the use of succinimidyl esters for the direct synthesis of polyamine conjugates appears to have received little attention. We now wish to report on a very short, simple, and efficient synthetic protocol which exploits this selectivity and provides easy access to KukA, SkukA, and a series of KukA analogs (Figure 1) suitable for structure-activity relationship studies.

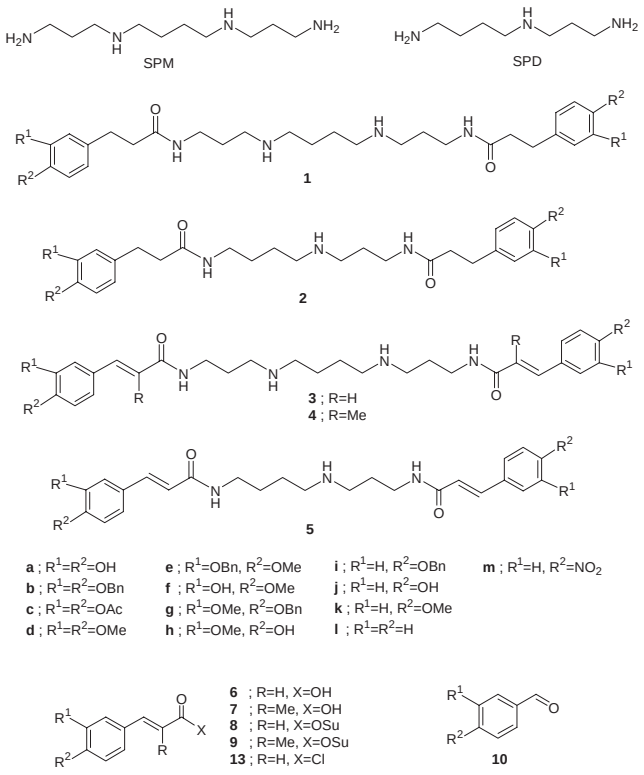
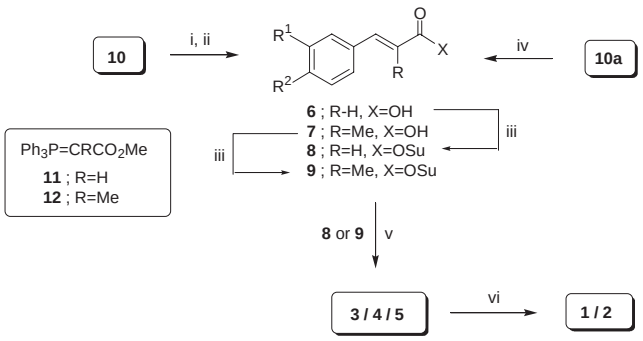


Figure 1. Structures of compounds encountered in this work.

The key-step in the present synthetic protocol is the direct condensation of succinimidyl esters **8** or **9** (suitably protected, where necessary), with the commercially available polyamines SPM and SPD. Most of the cinnamic acids required were readily obtained through a two-step sequence involving Wittig reaction with an aromatic aldehyde **10**, followed by saponification. Where necessary, (**6b**, **6e**, **6f**, **6i**) these were appropriately *O*-protected with the hydrogenolytically cleavable benzyl (Bn) group or the hydrolytically cleavable acetyl (Ac) group, if retention of the cinnamoyl double bond was required. Cinnamic acid **6c** was obtained through a Perkin reaction of the aldehyde **10a** with excess Ac_2O which resulted in simultaneous bisacetylation (Scheme 1). From the thus obtained cinnamic acids **6** or **7d,** the corresponding crystalline active esters **8** and **9d**, respectively, were readily obtained through reaction with *N*-hydroxysuccinimide (HOSu) in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) (Table 1).



Scheme 1. Synthesis of KukA and analogs. *Reagents and conditions*: (i) $Ph_3P{=}CHCO_2Me$ or $Ph_3P{=}C(Me)CO_2Me$, MeCN, reflux 10 h, 70–97%; (ii) 4N NaOH, DMSO/MeOH (2:1), 0 °C, 30 min then 25 °C, 12 h, 85–96%; (iii) HOSu/DCC, THF/DMF (3:1), 0 °C, 1 h then 25 °C, 12 h, 72–88%; (iv) Ac_2O, Et_3N, reflux 12 h, 72%; (v) SPM or SPD/$^i Pr_2NEt$, CH_2Cl_2, 0 °C, 30 min to 2 h; (vi) H_2 (1 atm)/20% Pd–C, MeOH/AcOH/H_2O (4:1:0.1), 25 °C, 2–10 h, 73–89%.

Table 1. Selected data for succinimidyl esters (SUEs), the corresponding polyamine conjugates (PACs) and the fully deprotected KukA analogs[a]

Entry	SUEs[b]	Yield /%	PACs	Yield /%	KukA analogs	Yield /%
1	**8b**	83	**3b**	85	**1a**	89
			5b	70	**2a**	81
2	**8c**[c]	72	**3c**[d]	—	**3a**[d]	—
3	**8d**	75	**3d**	77	**1d**	84
			5d	75	**2d**	86
4	**8e**	88	**3e**	82	**1f**	82
5	**8g**[c]	72	**3g**	70	**1h**	73
6	**8i**	84	**3i**	72	**1j**	78
7	**8k**	82	**3k**	83	**1k**	83
8	**8l**	80	**3l**	80	**1l**	81
9	**8m**	86	**3m**	89		
10	**9d**	87	**4d**	86		

[a]The structures of new compounds described in this communication were determined by a combination of spectroscopic techniques (UV, IR, ESI-MS, NMR) and microanalysis. Final products, i.e. KukA analogs, were characterized by MALDI-TOF/TOF HR-MS. [b]SUEs showed characteristic C=O IR bands at 1768 and 1738 cm^{-1} and the following typical characteristic (compound **8b**) ^1H NMR (400 MHz, $CDCl_3$) data: δ 7.79 (1H, d, J 16 Hz), 7.14 (1H, d, J 2 Hz), 7,12 (1H, dd, J 8.2 and 2 Hz), 6.93 (1H, d, J 8.2 Hz), 6.36 (1H, d, 16 J Hz), 2.86 (4H, br. s). [c]Isolated yield following FCC purification using as eluant PhMe/EtOAc (1:1) and (7:3), respectively. [d]The reaction of active ester **8c** with SPM leads directly to KukA analog **3a** together with the corresponding mono- and di-*N*-acetylated derivative and succinimidyl acetate as shown by HPLC, ESI–MS, and ^1H NMR.

Treatment of a solution of 1.7 mmol of SPM in 25 mL CH_2Cl_2 with 5 mmol of esters **8** or **9d** for 1 h at 0 °C lead to precipitation of the conjugates **3** or **4d** as the corresponding bishydroxysuccinimidate salts, which were obtained pure by simple filtration and washing with ice-cold CH_2Cl_2, following overnight refrigeration. The free bases **3** or **4d** were obtained in excellent (70–89%) yields through partitioning of these salts between $CHCl_3$ and a 5% aq $NaHCO_3$ solution, followed by washings with H_2O, drying and evaporation (Table 1). In the case of the SPD conjugates **5**, 2.5 mmol of $^i Pr_2NEt$ was also included to neutralize HOSu and the reaction mixture was directly processed to aqueous work-up as for conjugates **3** to give free bases **5** in 70–75% yields, following trituration with Et_2O (Table 1). Final catalytic hydrogenation of the double bonds and hydrogenolysis of the Bn groups, where necessary, was effected as follows. Fully protected conjugates (1 mmol), e.g. **3b**, **3e**, **3g**, **3i** and **5b**, in MeOH (16 mL)/AcOH (4 mL)/H_2O (0.4 mL) were hydrogenolyzed over 0.1 g of 20% Pd on C under an atmosphere of H_2 for 2–10 h at ambient temperature. Filtration through celite, washings with MeOH/AcOH (4:1) and finally evaporation of the filtrates under reduced pressure left an oily residue. This was treated with a ca. 2 M solution of HCl in anhydrous MeOH, triturated with dry Et_2O and refrigerated to give the corresponding bishydrochloride salts of compounds **1a**(KukA), **1f**, **1h**, **1j** and **2a** (SkukA) in 73–89% yields.

The present protocol constitutes a significantly more direct and cost-effective approach to such SPM and SPD conjugates than other methodologies reported to date, avoiding cumbersome protection/deprotections of the polyamine moiety and importantly, without the need for chromatographic purifications.

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