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Synthetic studies toward the development of novel minoxidil analogs and conjugates with polyamines

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ABSTRACT

Syntheses of novel polyamine-modified minoxidil analogs (PMMs) and minoxidil–polyamine conjugates (MPCs) are described in an effort to improve the biological activity and selectivity of minoxidil and its poor solubility in water.

Minoxidil (**MNX**, Fig. 1) is an antihypertensive agent and a peripheral vasolidator. The antihypertensive activity of **MNX** is due to the rapid relaxation of vascular smooth muscle by its sulfated metabolite (**MNXS**).[1] Formation of **MNXS** is catalyzed by sulfotransferase enzymes.[2] **MNXS** is one of several chemically unrelated drugs, which cause opening of plasma membrane adenosine triphosphate (ATP)-sensitive potassium channels (K_{ATP} channels) and it is likely that this property explains its relaxant effect on vascular smooth muscle.[3] K_{ATP} channels have been identified in a variety of tissues (muscle cells, pancreatic β-cells, and neurons) and exhibit important physiological roles in cellular signaling processes that regulate heart rate, smooth muscle contraction, insulin secretion, and neurotransmitter release.[4] **MNX** is the drug of choice in the clinical management of male pattern baldness, also known as *androgenic alopecia*. Its use is associated with hirsutism and hypertrichosis.[5] A number of in vitro effects of minoxidil have shown that it is involved in cell proliferation, collagen synthesis, and prostaglandin synthesis. However, neither these activities nor the correlation of **MNXS** with K_{ATP} channels explains how it promotes hair growth.[6]

Naturally occurring polyamines (PAs), or their conjugates with other biomolecules, show interesting biological activities. Furthermore, conjugation of biologically interesting molecules with PAs is a means of either improving the activity of the non-PA moiety or combining the activities of the conjugated molecules.[7] We therefore considered it interesting to examine the possibility of incorpo-

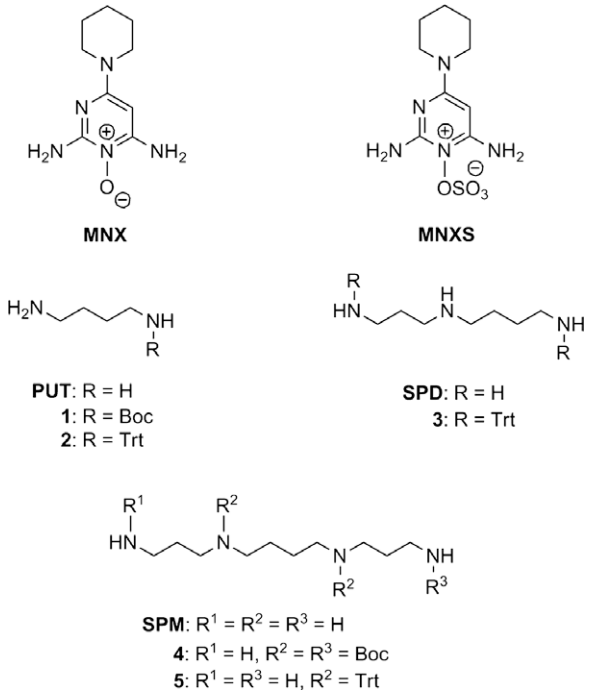

Figure 1. Compounds relevant to this work.

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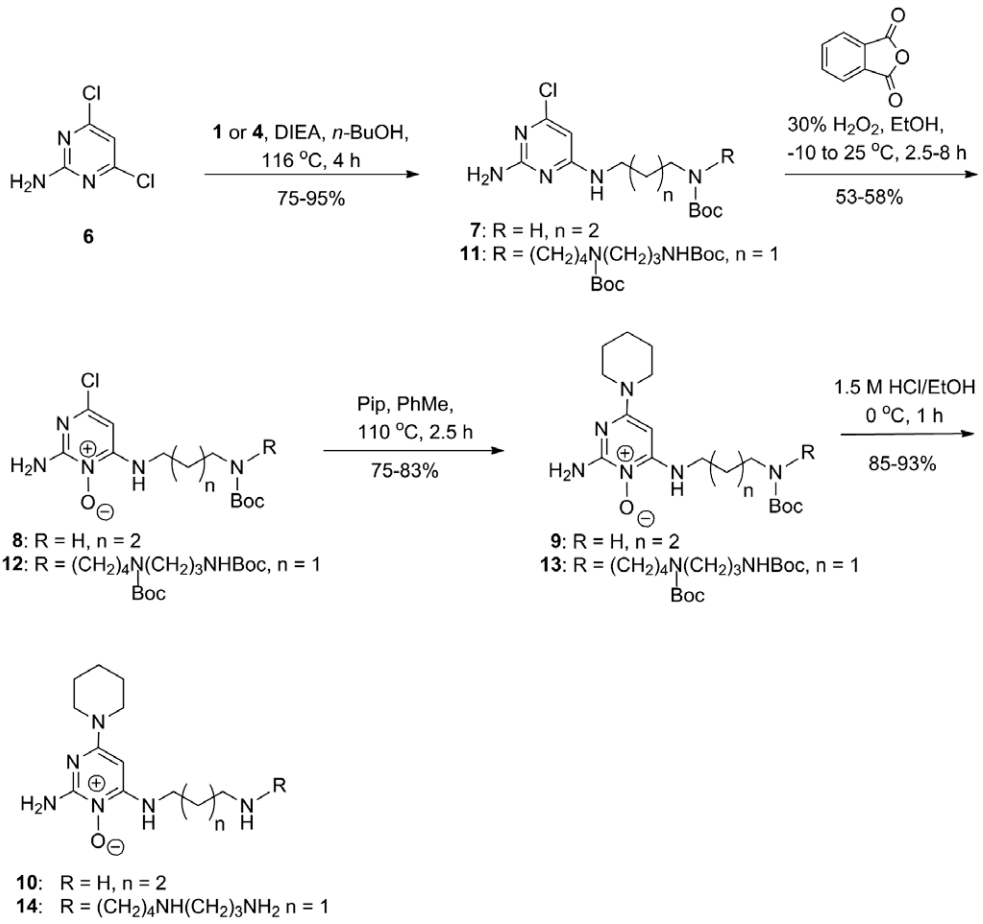


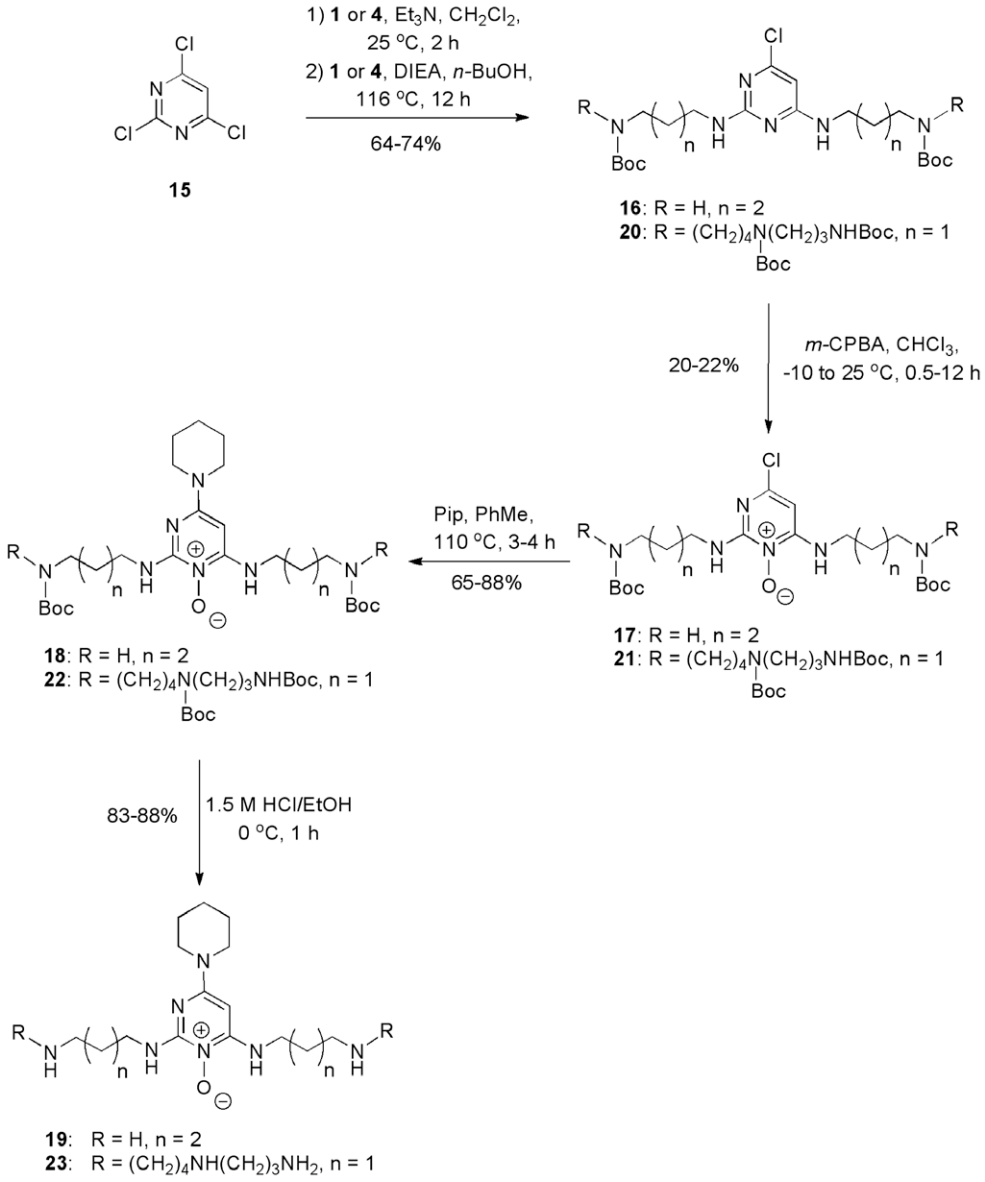
Scheme 1. Syntheses of PMMs **10** and **14**.

rating **MNX** and PA molecules in a single compound. We also anticipated that these conjugates would improve the solubility of **MNX** in water[8] and thus lead to an efficient liquid-vehicle delivery to the hair follicle. It has been recently reported that **MNX** topical foam, free of the irritant solvent propylene glycol traditionally used for its solubilization, is more advantageous over the corresponding **MNX** topical solution.[9]

Although several analogs of **MNX** have been synthesized,[10] none incorporating PAs have been so far reported. We envisaged that such modification could be accomplished through the following two molecular entities: a polyamine-modified minoxidil (PMM) and a minoxidil–polyamine conjugate (MPC).[11] In the present study, we chose to use putrescine (PUT), spermidine (SPD), and spermine (SPM) as PAs for the preparation of the PMMs and MPCs. These PAs were to be introduced into the projected molecules through their derivatives **1**,[12] **2**,[13] **3**,[13a] **4**,[14] and **5**.[15] **MNX** analogs **10**, **14**, **19**, and **23** (Schemes 2 and 3) were envisaged as examples of PMMs. Furthermore, mono-substituted **MNX**s such as **26**, **28**, and **30** and the symmetric conjugate **32** are considered examples of MPCs (Scheme 3).

We anticipated that the synthesis of PMMs **10**, **14**, **19**, and **23** could be realized through the sequential amination[16] of the commercially available functionalized pyrimidines, such as 2-amino-4,6-dichloropyrimidine (**6**), (Scheme 1) and 2,4,6-trichloropyrimidine (**15**) (Scheme 2). Initially, **6** was reacted with the selectively protected linear PAs N^1-*tert*-butoxycarbonylputrescine (**1**) and N^1,N^5,N^9-tris(*tert*-butoxycarbonyl)spermine (**4**) in *n*-BuOH, in the presence of *N*,*N*-diisopropylethylamine (DIEA), at 116 °C for 4 h to give the anticipated mono-substituted derivatives **7** and **11**, respectively, in 75–95% yield (Scheme 1). The latter were *N*-oxidized[17] using phthalic anhydride/H_2O_2[17d] at −10 °C to ambient temperature for 2.5–8 h to afford the desired pyrimidine *N*-oxides **8** and **12**, respectively, in 53–58% yield. This system gave the highest yields of *N*-oxides compared to other methods involving H_2O_2 or *m*-CPBA as the oxidant. The obtained *N*-oxides were treated with anhydrous piperidine (Pip) in PhMe at 110 °C for 2.5–5 h to afford the corresponding compounds **9** and **13**, respectively, in 75–83% yield. Finally, routine deprotection with 1.5 M HCl in EtOH afforded the PMMs **10** and **14**, as the hydrochloride salts, in 85–93% yield.

Next, **15** was reacted with the PA derivative **1** or **4** in CH_2Cl_2, in the presence of triethylamine, at ambient temperature for 2 h to give a mixture of the two anticipated regioisomers in the ratio 55:45 in the case of **1** (see Supplementary data) which could be separated by flash column chromatography, if so desired. However, for the purpose of the present study, the mixture of regioisomers was used as such in the next experiment, after evaporation of the solvent and flash column chromatography to eliminate baseline impurities. Treatment of the above-mentioned regioisomeric mixture with further **1** or **4**, respectively, in *n*-BuOH, in the presence of DIEA, at 116 °C for 12 h, finally gave the disubstituted compounds **16** and **20**, respectively, in 64–74% overall yield. These were N-oxidized using *m*-CPBA in $CHCl_3$ at −10 °C to ambient temperature, for 0.5–12 h to give the desired pyrimidine *N*-oxides **17** and **21**, respectively, in 20–22% yield. In these cases, *m*-CPBA provided better yields, compared to the phthalic anhydride/H_2O_2 system. The obtained *N*-oxides were then treated with an anhydrous piperidine in PhMe at 110 °C for 3–4 h, to afford compounds **18**

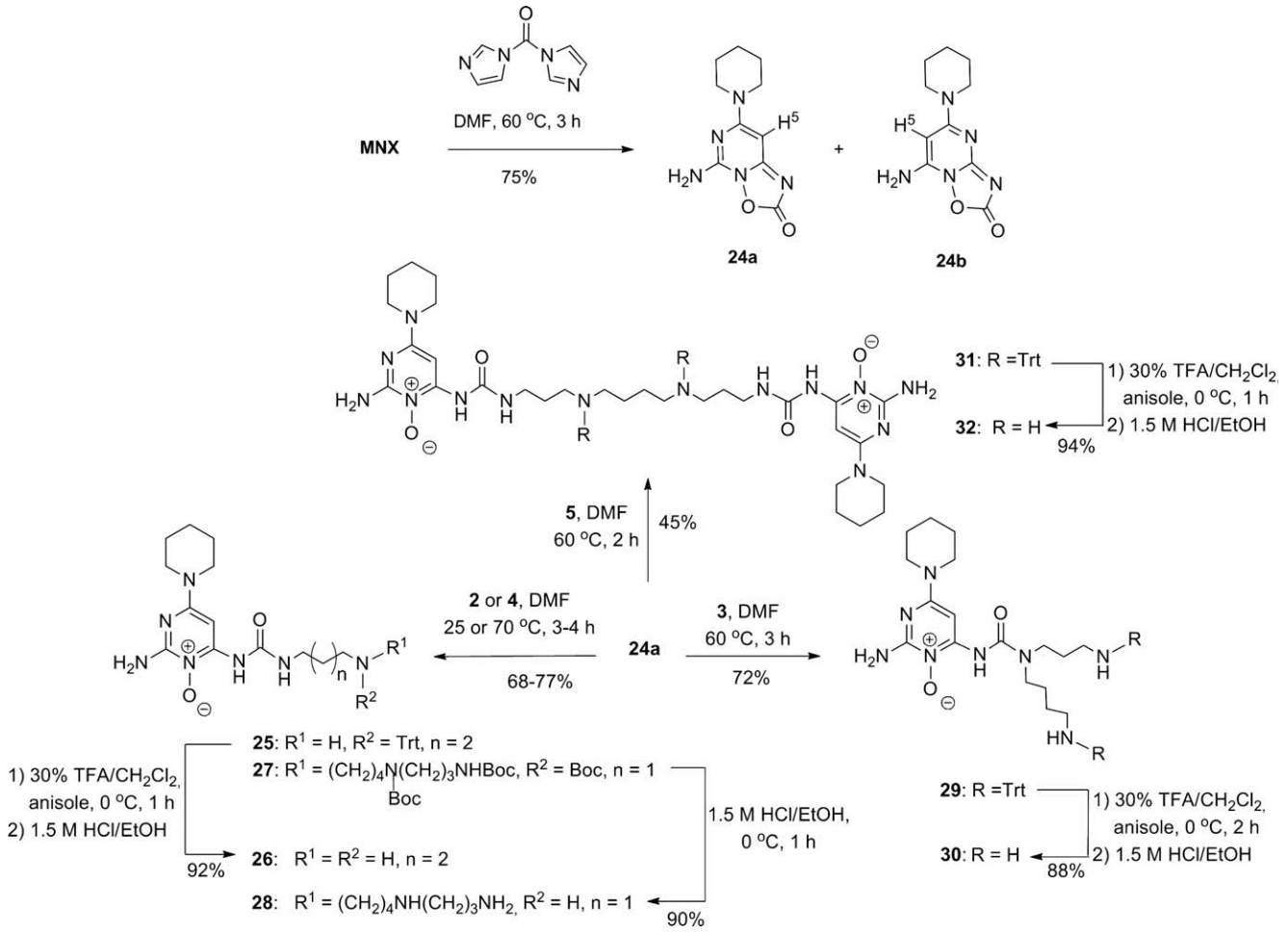


Scheme 2. Syntheses of PMMs **19** and **23**.

and **22**, in 65–88% yield. Finally, deprotection with 1.5 M HCl in EtOH gave the PMMs **19** and **23**, as the hydrochloride salts, in 83–88% yield.

For the attachment of PA molecules on **MNX** we reasoned, based on the literature findings[18] that an intermediate such as **24** (Scheme 3) would serve the purpose of activating **MNX** toward amine nucleophiles. This kind of intermediate had been previously obtained through pyrolysis of suitable carbamates, which in turn were obtained by alkylchloroformate treatment of *o*-amino-substituted pyridine or pyrimidine *N*-oxides.[19] We envisaged that *N*,*N*′-carbonyldiimidazole (CDI) would activate **MNX** under mild reaction conditions. Indeed, when **MNX** was reacted with CDI at 60 °C in an anhydrous DMF for 3 h the activated intermediate **24** was obtained in 75% yield, as a mixture of the two expected isomers in the ratio **24a**:**24b** = 80:20 (LC–MS and ^{1}H NMR). We based our identification of the isomers on the chemical shift of H-5[19] which for **24a** (proton is *ortho* to the carbamate function) was at δ 5.73 ppm and that for **24b** (proton is *ortho* to the free amino function) at δ 5.32 ppm. The main isomer **24a** could be obtained pure after crystallization from MeCN. The formation of both isomers with the observed preference for isomer **24a**, under the present reaction conditions, might be explained by initial acylation of the negatively charged O atom of **MNX** followed by an internal nucleophilic attack on the activated carbonyl function by the more nucleophilic C-6 amino group.

Treatment of intermediate **24a** with suitably protected linear PAs (Scheme 3), such as N^1-tritylputrescine (**2**), **4**, N^1,N^8-bistritylspermidine (**3**), and N^4,N^9-bistritylspermine (**5**), in DMF at temperatures varying from ambient to 70 °C provided the anticipated protected MPCs **25**, **27**, **29**, and **31**, respectively, in 45–77% yield. Finally, deprotection afforded conjugates **26**, **28**, **30**, and **32**, as the corresponding hydrochloride salts, in 88–94% yield. It should be noted that attempts to activate, for example, compound **25** with CDI, in order to attach a second same or different PA molecule, were unsuccessful leading only to the recovery of the starting material. In comparison with the parent molecule **MNX**, the new compounds exhibited, as expected, significantly improved solubilities in H_2O (see Supplementary data).



Scheme 3. Syntheses of **MNX**-intermediate **24** and MPCs **26**, **28**, **30**, and **32**.

In conclusion, MPCs were readily obtained through CDI-mediated activation of **MNX**, followed by coupling with suitably protected linear PAs, and finally N-deprotection. On the other hand, PMMs were assembled through sequential replacement of the chlorine atoms of suitable, commercially available, chloropyrimidines with selectively protected linear PAs, followed by N-oxidation, piperidine introduction and finally N-deprotection. All the compounds showed significantly improved solubilities in water compared to **MNX**. Applications of the above-described protocols to the preparation of other MPCs and PMMs, suitable for structure–activity relationship studies, as well as the biological evaluation of all new compounds to various cellular targets are currently in progress. It should be noted that the incorporation of PAs can also increase the toxicity (SPM to a greater extent than SPD or PUT).[20]

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Supplementary data

Supplementary data (experimental procedures, physical, analytical and spectroscopic data and selected copies of ^{1}H and ^{13}C NMR spectra) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.02.037.

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