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TETRAHEDRON

Synthesis of 1,3-diazaazulene derivatives of colchicinoids and isocolchicinoids via *ipso*- or *tele*-substitution-condensation with amidines

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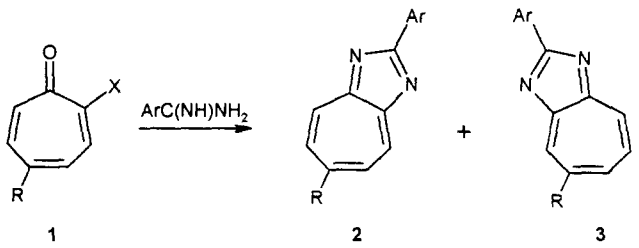
Abstract: Condensation of colchicinoids and isocolchicinoids with amidines in dry benzene affords regioselectively 1,3-diazaazulene derivatives: *ipso*-substitution-condensation products (e.g. **7**) are best obtained from cycloheptatrienone-ring deactivated substrates (e.g. **4**), whereas isomeric *tele*-substitution-condensation products (e.g. **13**), are best accessible from cycloheptatrienone-ring activated substrates (e.g. **10**). Hydroxylic solvents inhibit *tele*-substitution-condensation, arguably by undergoing protonation in preference of the intermediate σ adduct.

Colchicine (10-methoxycolchicide) (**4**), a potently antimitotic natural alkaloid has lost interest as a drug because of its deleterious side effects while emerging as a useful laboratory tool in cancer research.[1] Colchicinoids are also gaining interest as potentially useful agents in the control of pests in agriculture. Therefore, in the search of new such leads, we have provided new methodologies for the re-functionalization of the cycloheptatrienone nucleus of colchicine and isocolchicine by amines, in particular exploiting the nucleophilic *tele*-substitution.[2] Here we report a notable variant on this theme, using amidines to provide regioselectively 1,3-diazaazulene derivatives in both series. This is an extension of methods for troponoids **1**, where *ipso*-substitution followed by condensation to give type-**2** products, or *tele*-substitution to type-**3** products has been reported,[3] Scheme 1). The extension is not straightforward, however, since colchicinoids and isocolchicinoids have special requirements.



1 2 3

Scheme 1

Colchicide series

We examined colchicide (Scheme 2, X = H) derivatives bearing either good (OTs, **5**) or poor nucleofugic groups at C-10 (OMe, **4** and SEt, **6**) towards benzamidine in dry benzene (Scheme 2). In all cases only the *ipso*-condensation product **7** (here and in the following the colchicinoid/isocolchicinoid numbering

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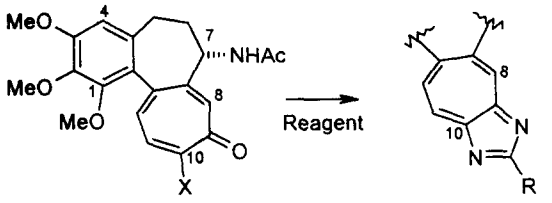
is arbitrarily used) was observed, in higher yield for methoxy than either tosyloxy or ethylthio leaving groups, notwithstanding the intermediate reactivity of the methoxy derivative **4** between the high rate of the tosyloxy derivative **5** and the very low rate of the ethylthio derivative **6**.[4] With guanidine, which required the addition of a hydroxylic solvent to benzene, poorer yields of the *ipso*-condensation product **8** were obtained.



4 X = OMe	$PhC(NH)NH_2$[a]	**7** R = Ph (96%)
"	$HNC(NH_2)_2$[b]	**8** R = NH_2 (55%)
5 X = OTs	$PhC(NH)NH_2$[a]	**7** R = Ph (35%)
6 X = SEt	$PhC(NH)NH_2$[c]	**7** R = Ph (15%)

Scheme 2 [a]excess benzamidine, C_6H_6, r.t., 24 h; [b]excess guanidine, 6:1 C_6H_6-MeOH, r.t., 48 h; [c]excess benzamidine, C_6H_6, reflux, 7h

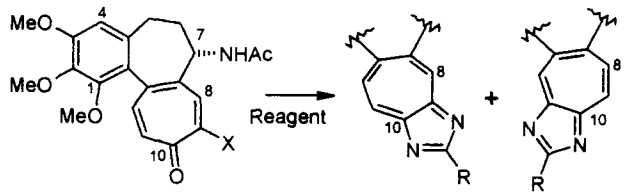
Structures **7** and **8** are fully supported by ^1H and ^{13}C NMR data, including DEPT and one-bond ^1H-^{13}C correlation experiments, which allowed all key resonances to be assigned (Experimental). Atom counting by NMR found nice agreement with high-resolution EI-MS data for either the molecular ions or fragment ions. With most compounds, because of weak signals for the molecular ion, these measurements required great care in ensuring a low background noise.[5]

The results for the methoxy derivative **4** are in line with previous observations for nucleophilic reactions in either the colchicine[2] or the troponoid series:[6] the OMe group deactivates the cycloheptatrienone ring towards nucleophilic attack and therefore only *ipso*-substitution, and thus only *ipso*-condensation, are allowed, albeit at low rate since OMe is also a poor leaving group. Both OTs and EtS are expected to enhance the C-8 electrophilicity[6] but, if nucleophilic attack at this position occurred at all, it failed to lead to products of *tele*-substitution-condensation; possibly this stems from steric interference with the fused system at some stage during the multistep substitution process, which also requires protonation at C-10 followed by 1,3-elimination. In any event, parasitic reactions (cycloheptatrienone ring contractions[6] from circumstantial evidence) come into play in the case of the best leaving group, OTs, determining low yields.

Isolcolchicide series

It is known that a nucleofugic group at C-9 in isocolchicide (Scheme 3, X = H) may trigger substitution by amines not only at the *ipso* position but also at the *tele*-position (C-11).[7] The latter process is likely to occur whenever the C-9 substituent activates the ring for nucleophilic attack at C-11 and protonation at C-9 is possible, such as in the case of protic amines[2,7] or thiolate/thiol mixtures as nucleophiles.[8] It is also known that 1,3-diazaazulene derivatives are formed from amidines and tropones bearing a nucleofugic α-group, Scheme 1.[3] On these basis we have explored the behaviour of benzamidine and, albeit less extensively, of guanidine towards colchicides bearing at C-9 either activating groups of good nucleofugic aptitude[6], like OTs (**10**) or Cl (**11**), or deactivating groups of poor nucleofugic aptitude,[6] like OEt (**9**), or even activating groups of poor nucleofugic aptitude,[6] like SMe (**12**) (Scheme 3).[8] Both *ipso* and *tele* condensation products were observed, not wherever expected,[2,6] however, and with an extremely large

variation in overall yields (Scheme 3). As in the colchicide series, the best yields were obtained with an alkoxide as a leaving group[9] along the *ipso* condensation pathway only (Scheme 3, **9**). The regiospecificity in this case fits general theories of deactivation of the cycloheptatrienone ring by alkoxyl groups,[3,6] whilst the high yield is surprising vis-a-vis the poor yield for leaving groups that, like OTs (**10**) or Cl (**11**), activate the ring. This may be rationalized by admitting that substituents X at C-9 which activate the cycloheptatrienone ring towards nucleophilic attack - by which the useful *tele*-condensation arises - also induce unidentified parasitic reactions, probably ring contractions as in the colchicide series.[6] In any event, the most practical entry to products of *tele*-condensation is offered by the tosylate as a leaving group.[10] This is because the ratio of yields *tele-* / *ipso*-substitution-condensation is lower for OTs (**10**) than for either SMe (**12**) or Cl (**11**) as leaving groups, but the reaction of the methylthio substrate **12** is much slower, and thus subjected to worsening if the temperature is raised, whilst the reaction of the chloro-substituted substrate **11** has lower overall yield. As an additional advantage, the tosylate **10** is more readily accessible than either the methylthio (**12**) or chloro (**11**) substrates.



9 X = OEt	$PhC(NH)NH_2$[a]	**7** R = Ph (82%)	-
10 X = OTs	$PhC(NH)NH_2$[a]	**7** R = Ph (6.4%)	**13** R = Ph(25.6%)
"	$PhC(NH)NH_2$[b]	**7** R = Ph (18%)	-
"	$HNC(NH_2)_2$[c]	**8** R = NH_2(13%)	-
11 X = Cl	$PhC(NH)NH_2$[d]	**7** R = Ph (1.4%)	**13** R = Ph(20%)
12 X = SMe	$PhC(NH)NH_2$[a]	**7** R = Ph	**13** R = Ph[e]

Scheme 3 [a]excess benzamidine, C_6H_6, r.t., 24 h; [b]excess benzamidine, 6:1 C_6H_6-MeOH, r.t., 24 h; [c]excess guanidine, 6:1 C_6H_6-MeOH, r.t., 24 h; [d]excess benzamidine, C_6H_6, r.t., 1 h; [e]because of very low substrate conversion, only the ratio of yields of products (3/7) can be given.

All this relates to benzene as solvent. Things change if a hydroxylic solvent, such as methanol, is added to the reaction mixture. Thus, the tosylate **10** with the same reagent, benzamidine, gave only *ipso* condensation and the same was observed for guanidine, which forcefully required the addition of a hydroxylic solvent. Possibly, this departure from the *tele* condensation pathway is due to a levelling off of the acidity by the hydroxylic solvent, which disfavours protonation at the carbon bearing the leaving group, which is needed in this process.[6]

EXPERIMENTAL

General. All evaporations were carried out under reduced pressure and yields are given based on reacted substrate. DMSO was freshly distilled from CaH_2 in flamed glassware and was stored on 4 Å molecular sieves in Schlenk tubes under N_2. Benzene was distilled from, and stored on, 4 Å molecular sieves in Schlenk tubes under N_2. Colchicine was purchased from Aldrich and used as such. TLC was performed on Merck Kieselgel $60PF_{254}$ and reversed-phase HPLC on Perisorb RP18 4.4 x 250 mm with 1:1 MeCN-H_2O, 1mL min^{-1}. UV spectra were taken with a Perkin-Elmer Hitachi 200 spectrophotometer. NMR spectra were recorded with a Varian Gemini BB200 (^1H 199.975 MHz and ^{13}C 50.289 MHz) in $CDCl_3$

δ values were obtained with reference to internal residual-solvent signal and are reported with respect to $SiMe_4$ ($\delta = 0$). J values are in Hz. Carbon assignments are from DEPT and one-bond ^1H-^{13}C correlation experiments. Mass spectra (EI) were taken with a Kratos MS80 spectrometer with home-built computerized acquisition system.

(S)-N-*(5,6,7,8a-tetrahydro-1,2,3-trimethoxy-10-phenylbenzo[6,7]heptaleno[2,3-*d*]imidazol-7-yl)acetamide* (**7**) *(a) From colchicine* (**4**). To a solution of colchicine (58 mg, 0.145 mmol) in dry benzene (5 mL) was added benzamidine[3] (100 mg, 0.83 mmol). The mixture was briefly warmed to dissolve all benzamidine and it was then left aside for one day at r.t. The solvent was evaporated and the residue was subjected to preparative TLC with 1:1 $CHCl_3$/acetone; a R_f 0.4 yellow band was collected to give **7** (65 mg, 0.14 mmol, 95%); λ_{max}(EtOH)/nm/(log ε) 393 (4.41), 274 (4.56), 258 (4.56); δ_H 10.15 (br., N*H*), 9.35 (s, 8-H), 8.74 (d, J = 10.9, 12-H or 11-H), 8.60 (m, *ortho* Ph protons), 8.30 (d, J = 10.9, 11-H or 12-H), 7.57 (m, *meta* and *para* Ph protons), 5.00 (m, 7-H), 6.56 (s, 4-H), 3.99, 3.92 and 3.63 (three s, C*H*$_3$O's), 2.4-2.0 (two m, 5-H_2 and 6-H_2), 2.21 (s, COC*H*$_3$); δ_C 139.2 (d, C-12 or C-11), 131.9 (d, C-11 or C-12), 131.6 (d for *meta* Ph carbons), 129.2 (d for *para* Ph carbons), 128.9 (d, *ortho* Ph carbons), 128.6 (d, C-8), 107.4 (d, C-4), 61.9, 61.6 and 56.2 (q's, *C*H$_3$O's), 53.9 (d, C-7), 37.7 (t, C-5), 29.7 (t, C-6), 22.8 (q, COC*H*$_3$), besides 175.6, 170.9, 161.9, 161.8, 154.6, 150.9, 148.9, 147.1, 142.0, 134.7 and 133.0 s's for the non-protonated carbons; *m/z* (EI): 469.2 (M^+, 5%), 352 ([M - $PhCN_2$]$^+$, 1), 117 ($PhCN_2$, 0.5), 28 (100); [Found (EI): M^+, 469.20015±0.00033. $C_{28}H_{27}N_3O_4$ requires 469.20015].
(b) From 10-tosyloxycolchide (**5**).[9] The reaction was run as in (*a*) above, obtaining **7** in 35% yield.
(c) From 10-ethylthiocolchicide (**12**).[2] To a solution of 10-ethylthiocolchicide (**12**) (12 mg, 0.028 mmol) in dry benzene (3 mL) was added benzamidine[3] (131 mg, 1.1 mmol). The mixture was briefly warmed to dissolve all benzamidine and it was then left aside for one day at r.t. , after which no sign of reaction could be noticed. The solution was then heated at reflux for 7 h. The solvent was evaporated and the residue was subjected to reverse-phase HPLC determining **7** in 15% yield for an 85% conversion of **6**.

(S)-N-*(5,6,7,8a-tetrahydro-1,2,3-trimethoxy-10-aminobenzo[6,7]heptaleno[2,3-*d*]imidazol-7-yl) acetamide* (**8**) To a solution of colchicine (48 mg, 0.12 mmol) in dry benzene (3 mL) was added a 1.2 M methanolic solution of guanidine (0.5 mL, 0.61 mmol) obtained by adding NaOMe (197 mg) to guanidine hydrochloride (393 mg) in MeOH (3 mL) . The mixture was left aside for two days at r.t. and the solvent was then evaporated and the residue was subjected to preparative TLC with 9:1 $CHCl_3$/MeOH, collecting the R_f 0.3 pale-yellow band that gave **8** (27 mg, 0.06 mmol, 55%); λ_{max}(EtOH)/nm/(log ε) 388 (4.52), 372 (4.52), 300 (sh), 266 (4.80); δ_H 9.35 (d, J = 6.6, N*H*), 8.55 (s, 8-H), 8.08 (d, J = 11.0, 12-H or 11-H), 7.99 (d, J = 11.0, 11-H or 12-H), 6.58 (s, 4-H), 6.3 (br., NH_2), 4.88 (dt, J = 11.5, 6.6, 7-H), 3.96 3.91 and 3.59 (three s, C*H*$_3$O's), 2.5-2.0 (two m, 5-H_2 and 6-H_2), 2.05 (s, COC*H*$_3$); δ_C 139.5 (d, C-12 or C-11), 124.7 (d, C-11 or C-12),121.6 (d, C-8), 107.3 (d, C-4), 61.7, 61.5 and 56.2 (q's, *C*H$_3$O's), 53.3 (d, C-7), 37.8 (t, C-5), 30.0 (t, C-6), 22.8 (q, COC*H*$_3$), besides 173.9, 170.4, 163.5, 163.2, 153.9, 150.9, 149.1, 141.8 and 140.3 s's for the non-protonated carbons; *m/z* (EI): 408.1 (M^+, 3%), 28 (100); [Found (EI): M^+, 408.17946±0.00029. $C_{22}H_{24}N_4O_4$ requires 408.17975].

9-Ethoxyisocolchicide (**9**). To a solution of 9-tosyloxyisocolchicide (**5**)[9] (94.5 mg, 0.174 mmol) in abs EtOH (2.5 mL) was added $Ti(OEt)_4$ (Fluka) (198 mg, 0.87 mmol); the mixture was heated at reflux for 3 h and then evaporated and the residue was subjected to preparative TLC with 6:4 $CHCl_3$/acetone. The UV-fluorescent band at R_f 0.26 gave 9-ethoxyisocolchicide (**9**) (33 mg, 43%); δ_H 7.80 (d, J = 7.0, N*H*), 7.40 (d, J = 13.0, 12-H), 7.25 (s, 8-H), 7.09 (d, J = 13.0, 11-H), 6.56 (s, 4-H), 4.59 (dt, J = 12.4, 7.0, 7-H), 4.30 (q, J = 6.6. OCH_2CH_3), 3.90 3.87 and 3.63 (three s, CH_3O's), 2.4-2.0 (two m, 5-H_2 and 6-H_2), 2.02 (s, $COCH_3$), 1.45 (t, J = 6.6, OCH_2CH_3); δ_C 141.5 (d, C-12), 134.9 (d, C-11),111.5 (d, C-8), 107.5 (d, C-4), 64.70 (t, OCH_2CH_3), 61.5 (q's, two CH_3O's), 56.2 (q, remaining CH_3O), 52.9 (d, C-7), 38.1 (t, C-5), 30.0 (t, C-6), 22.9 (q, $COCH_3$), 14.5 (q, OCH_2CH_3), besides 179.5, 170.2, 163.3, 153.6, 150.9, 145.1, 134.7, 133.6 and 125.9 s's for the non-protonated carbons; (Found: C, 66.9; H, 6.7; N, 3.3. $C_{23}H_{27}NO_6$ requires C, 66.8, H, 6.6, N, 3.4).

Reaction of 9-ethoxyisocolchicide (**9**) *with benzamidine*. The reaction was run as with colchicine (**4**) above isolating **7** in 82% yield.

Reaction of 9-tosyloxyisocolchicide (**10**) *with benzamidine. (a) In benzene*. To a solution of **10** (40 mg, 0.074 mmol) in dry benzene (3 mL) was added benzamidine[3] (95 mg, 0.8 mmol). The mixture was briefly warmed to dissolve all benzamidine and than it was then left aside for one day at r.t. The solvent was evaporated and the residue was subjected to preparative TLC with 1:1 $CHCl_3$/acetone. Two yellow bands at R_f 0.4 and 0.23 were collected to give **7** (2.2 mg, 6.4%) and (*S*)-*N*-(5,6,7,9a-tetrahydro-1,2,3-trimethoxy-11-phenylbenzo[9,10]heptaleno[2,3-*d*]imidazol-7-yl) acetamide (**13**) (8.9 mg, 25.6%), respectively. Data of **13**: λ_{max}(EtOH)/nm/(log ε) 432 (3.50), 406 (3.69), 361 (4.32), 343 (4.35), 3.25 (sh), 270 (4.32), 256 (4.33); δ_H 8.99 (s, 12-H), 8.75 (d, J = 10.9, 9-H or 8-H), 8.63 (m, *ortho* Ph protons), 8.17 (d, J = 10.9, 8-H or 9-H), 7.54 (m, *meta* and *para* Ph protons), 6.58 (s, 4-H), 4.90 (dt, J = 12.0, 6.3 7-H), 3.97, 3.92 and 3.59 (three s, CH_3O's), 2.6-2.0 (two m, 5-H_2 and 6-H_2), 2.08 (s, $COCH_3$); δ_C 139.0 (d, C-9 or C-8), 132.6 (d, C-8 or C-9), 131.4 (d for *meta* Ph carbons), 129.3 (d for *para* Ph carbons), 128.8 (d, *ortho* Ph carbons), 128.7 (d, C-12), 107.3 (d, C-4), 61.5, 61.4 and 56.2 (q's, CH_3O's), 53.9 (d, C-7), 29.7 (t, C-5), 38.9 (t, C-6), 23.1 (q, $COCH_3$), besides 176.1, 169.7, 162.2, 161.2, 154.2, 151.3, 142.4, 134.2, 133.1, 126.1 s's for the non-protonated carbons (one of which could not be detected or was superimposed); *m/z* (EI): 469 (24%), 352 ([M - $PhCN_2$]$^+$, 3), 117 ($PhCN_2$, 1), 28 (100); [Found (EI): M^+, 469.20015±0.00012. $C_{28}H_{27}N_3O_4$ requires 469.20015].
(b) In benzene/methanol 6:1. To a solution of **10** (51.8 mg, 0.096 mmol) in dry benzene (3 mL) was added a 1.15 M methanolic solution of benzamidine[3] (0.5 mL, 0.57 mmol). The mixture was left standing overnight at r.t., then evaporated and subjected to preparative TLC with 1:1 $CHCl_3$/acetone to give **7** (R_f 0.4, 8.1 mg, 18%) besides unidentified products as a pale-yellow band at R_f 0.3).

Reaction of 9-tosyloxyisocolchicide (**10**) *with guanidine*. To a solution of **10** (51.7 mg, 0.095 mmol) in dry benzene (3 mL) was added a 1.15 M methanolic solution of benzamidine[3] (0.5 mL, 0.57 mmol). The mixture was left standing overnight at r.t., then evaporated and subjected to preparative TLC with 1:1

$CHCl_3$/acetone to give **8** (R_f 0.3, 5.2 mg, 13%).

Reaction of 9-chloroisocolchicide (**11**) *with benzamidine*. To a solution of **11**[11] (10 mg, 0.025 mmol) in dry benzene (3 mL) was added benzamidine[3] (74.2 mg, 0.062 mmol). The mixture was briefly warmed to dissolve all benzamidine and it was then left aside for 2 h at r.t. The solvent was evaporated and the residue was subjected to either HPLC analysis revealing **7** (t_R = 7 min, 1.4%) or preparative TLC with 1:1 $CHCl_3$/acetone providing **13** (R_f 0.23, 2.4 mg, 20%), whilst **11** had disappeared.

Reaction of 9-methylthioisocolchicide (**12**) *with benzamidine*. To a solution of **12**[2] (51 mg, 0.12 mmol) in dry benzene (3 mL) was added benzamidine[3] (90 mg, 0.75 mmol). The mixture was briefly warmed to dissolve all benzamidine and it was then left aside for 24 h. The solvent was evaporated and the residue was subjected to TLC with 1:1 $CHCl_3$/acetone revealing two yellow spots at R_f 0.23 and 0.4 for **13** and **4**, respectively. Reverse-phase HPLC showed two peaks at t_R 6.5 and 7.0 min in 7:3 ratio of areas for **13** and **7**, respectively. Thioether **12** was recovered nearly quantitatively from the reaction mixture

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4 Reaction rates were only roughly estimated from scattered yield vs. time data but no reaction orders could be measured for heterogeneous systems due to the low solubility of the amidines.
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9 9-Alkoxyisocolchicides, like isocolchicine (Scheme 3, X = OMe), can be prepared by diazoalkane alkylation of colchiceine. However, this requires tedious chromatographic separation of 9-alkoxycolchicides from the 10-isomers that are also formed. Therefore, we have devised here a more direct route to 9-alkoxyisocolchicides from the easily available 9-tosyloxyisocolchicide **10** (Staretz, M.E.; Hastie, S.B. *J. Org. Chem.* **1991**, *56*, 428-432) via nucleophilic substitution by titanium tetraalkoxides. This reagent has been used to bring about the exchange the alkoxy groups at C-10 in 10-alkoxycolchicides (Kouroupis, P.; Kessler, J.; Hansen, H.-J. *Helv. Chim. Acta* **1996**, *79*, 208-212).
10 It was previously reported that piperidine in DMSO gives clean *ipso* substitution with tosylate **10**.[2] To compare with present condensations, the above reaction has now been run in dry benzene at room temperature, with the same result.
11 Cavazza, M.; Pietra, F. *Synth. Commun.* **1997**, *27*, 3405-3413.