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Novel Fluorescent Reactive Dyes as Intermediates for the Preparation of UV and Vis Wavelength Fluorescent Probes

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Summary. To prepare reactive dyes matching the optical properties of 2-(1,1-dicyano-propenyl-2)-6-dimethylaminonaphthalene (*DDNP*), 2-ethylaminoethanol or 4-piperidinemethanol were used in a *Bucherer* reaction with 1-(6-hydroxy-2-naphthyl)-1-ethanone. The two amines were chosen to mimic the dimethylamino group's steric and electronic (inductive) effects and to provide a reactive site for ligand attachment. The intermediate hydroxy derivatives were transformed into fluorescent reactive *p*-toluenesulfonic acid esters which in turn were conjugated to the test ligand spiperone. *Knoevenagel* condensation with malononitrile afforded Vis wavelength fluorescent probes.

Keywords. *Bucherer* reaction; Fluorescent dyes; Fluorescent probes; Spiperone conjugates.

Neue reaktive Fluoreszenzfarbstoffe als Zwischenprodukte zur Herstellung von Fluoreszenzindikatoren im ultravioletten und sichtbaren Bereich

Zusammenfassung. Zur Herstellung reaktiver Farbstoffe mit optischen Eigenschaften, die jenen von 2-(1,1-Dicyanopropenyl-2)-6-dimethylaminonaphthalin (*DDNP*) entsprechen, wurden 2-Ethylaminoethanol bzw. 4-Piperidinmethanol in einer *Bucherer*-Reaktion mit 1-(6-Hydroxy-2-naphthyl)-1-ethanon umgesetzt. Die beiden Amine wurden gewählt, um die sterischen und elektronischen (induktiven) Effekte der Dimethylaminogruppe zu imitieren und zugleich ein reaktives Zentrum für Substituitionsreaktionen bereitzustellen. Die als Zwischenprodukte auftretenden Hydroxyderivate wurden in reaktive fluoreszierende *p*-Toluolsulfonsäureester übergeführt, die ihrerseits mit dem Testliganden Spiperon umgesetzt wurden. Durch *Knoevenagel*-Kondensation mit Malonsäurenitril wurden Fluoreszenzindikatoren für den sichtbaren Bereich erhalten.

Introduction

After the discovery of the novel polarity/viscosity sensitive Vis wavelength fluorescent compound 2-(1,1-dicyanopropenyl-2)-6-dimethylaminonaphthalene (*DDNP*) [1] we set out to modify its structure in order to prepare a reactive dye

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which would inherit *DDNP*'s unique optical properties and would allow for the preparation of new fluorescent probes for the probing of biological systems. In this work we chose 2-ethylaminoethanol and 4-piperidinemethanol as substitutes for dimethylamine in the synthesis of these analogs. Both contain an amine nitrogen, required for the production of the fluorophore, and a hydroxyalkyl functional group which can be effectively activated for nucleophilic substitution by a ligand of choice. To test this approach, spiperone, a highly potent ligand for the dopamine D_2 receptors, was chosen as the ligand.

Results and Discussion

The synthetic approach used in the preparation of 2-acetyl-6-N,N-dimethyl-aminonaphthalene (*ADMAN*) [1], utilizing the corresponding lithium amide and 2-acetyl-6-methoxynaphthalene, could not be employed with 2-ethylaminoethanol and 4-piperidinemethanol. The competing faster reaction of dimethylamine liberated in the decomposition of hexamethylphosphoric amide [2] lead to exclusive formation of *ADMAN*, irrespective of the presence of the intended amine.

This prompted us to resort to the *Bucherer* reaction [3]. Starting from 1-(6-hydroxy-2-naphthyl)-1-ethanone (**1**) and using 2-ethylaminoethanol or 4-piperidine-methanol as substrates in the presence of sodium hydrogensulfate resulted in relatively poor yields of the desired naphthalene derivatives **2** and **3** (Fig. 1).

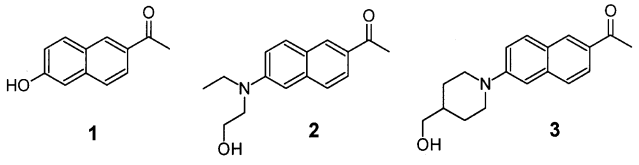
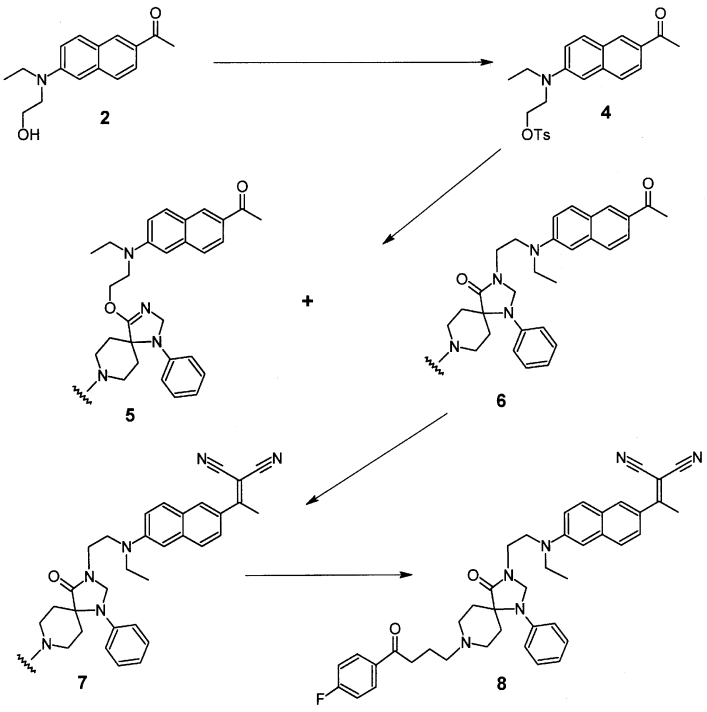


Fig. 1.

In order to conjugate spiperone to the naphthalene ring *via* a dialkylamino spacer, we first activated the hydroxy group in **2** for nucleophilic displacement by transforming it into the tosylate **4**, a reactive dye which can be excited in the UV range (Scheme 1). The latter was then subjected to reaction with spiperone ketal under phase transfer reaction conditions. We isolated two products (**5** and **6**) in a ratio of approximately 1:2, exhibiting the same molecular mass but different NMR spectra. One of the characteristic differences that led to the assignment of the respective structures was the subspectrum resulting from the ethylene group between the naphthalene and spiperone moieties. In **5**, we found two triplets at 4.52 and 3.85 ppm, corresponding to a OCH_2CH_2N structural element. On the other hand, in **6** analogous signals were found overlapping at 3.71–3.81 ppm, consistent with a NCH_2CH_2N moiety [4]. The structures were further supported by chemical shift changes of a well separated singlet, corresponding to the protons at the spiperone C-3 position. In spiperone or its N-2 alkylated derivatives such signals appear at 4.68–4.75 ppm [5]. In **6** the corresponding signal appeared in the expected range (4.68 ppm), whereas it was shifted downfield to 4.99 ppm in **5**,

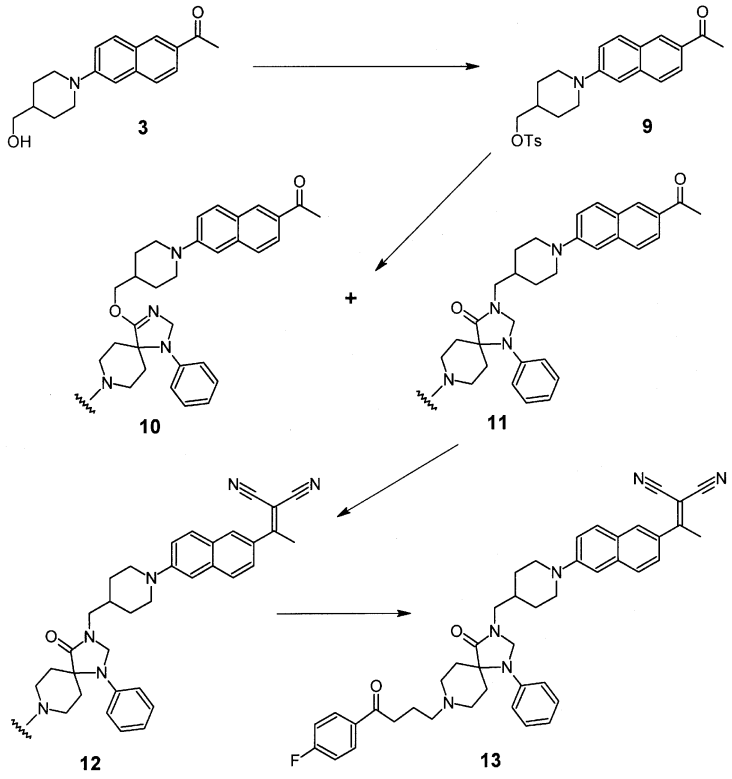


Scheme 1

consistent with imide (**5**) and amide (**6**) structures. Using the *Knoevenagel* reaction with malononitrile, we transformed compound **6** into **7** which upon acid catalyzed deprotection of the spiperone moiety gave the new Vis wavelength fluorescent probe **8**.

In a similar synthetic sequence we attached the spiperone moiety to the fluorophore *via* a 4-methylpiperidine spacer. First, the 4-hydroxymethyl group in **3** was activated by conversion into the tosylate **9**, followed by the reaction with spiperone under phase transfer conditions at room temperature. Two products (**10** and **11**) exhibiting the same molecular mass were isolated in an approximately 1:4 ratio (Scheme 2). Based on characteristic differences in NMR chemical shifts of the signals for the C-3 protons in the spiperone subspectra (singlet, 4.97 and 4.71 ppm, resp.) and the exocyclic methylene group protons in the piperidylmethyl group subspectra (doublet, 4.19 and 3.35 ppm, resp.) [6], we assigned structures **10** and **11** to the two products. Intermediate **11** was subjected to a *Knoevenagel* reaction with malononitrile to yield the Vis fluorescent intermediate **12** which upon acid catalyzed deprotection of the spiperone moiety yielded the Vis fluorescent probe **13**. The proposed structures of compounds **2** through **13** were also confirmed by elemental analyses or HRMS.

The optical properties of compounds **6**, **7**, **11**, and **12** are presented and compared with those of *DNNP* and *ADMAN* in Table 1. Chloroform and methanol were chosen as the solvents to detect solvent polarity dependent differences in absorption, fluorescence excitation, and emission (Fig. 2). The results support our



Scheme 2

Table 1. Absorption (λ_{max} (nm)), fluorescence excitation (λ_{ex} (nm)) and emission maxima (λ_{em} (nm))

Solvent		*ADMAN* [1]	**6**	**11**	*DDNP* [1]	**7**	**12**
	λ_{max}	358	374	370	444	437	433
$CHCl_3$	λ_{ex}	360	359	354	450	436	418, 443
	λ_{em}	436	430	443	548	525	538
	λ_{max}	366	371	371	426	430	419
MeOH	λ_{ex}	365	362, 379	357	428	438	395, 440
	λ_{em}	506	487	494	610	558, 565	563

assumption that formal replacement of the dimethyl amino group in *ADMAN* and *DDNP* by a substituted diethylamino- or 4-methylpiperidino group should not dramatically change the optical properties of the fluorophore. Both *ADMAN* (compounds **6** and **11**) and *DDNP* derivatives (compounds **7** and **12**) match the properties of the parent compounds well. However, the excitation and emission maxima are somewhat blueshifted. This indicates that both amines used disturb the

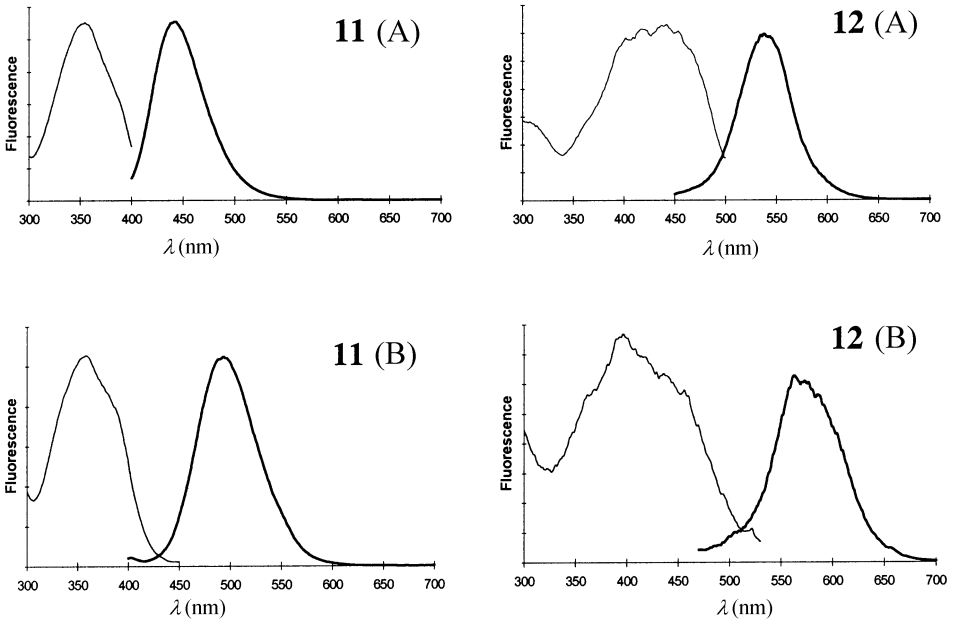


Fig. 2. Fluorescence excitation (—) and emission (**—**) spectra; (A): in $CHCl_3$, (B): in MeOH

electronic density distribution of the parent fluorophores, resulting in fluorescence changes.

Conclusions

We have shown that ligands possessing at least one nucleophilic center where alkylation does not result in significant degradation of binding properties (*e.g.* spiperone) can be effectively conjugated with tosylates **4** and **9** to produce UV fluorescent probes maintaining the biological properties of the parent ligand. Due to amide/imide tautomerism, two competing nucleophilic centers are present in the spiperone ketal molecule leading to the formation of mixtures of O- and *N*-alkylated products in reactions with **4** and **9**. If the chosen ligand is unaffected under *Knoevenagel* conditions with malononitrile, or it can be suitably protected, Vis wavelength fluorescent probes can be prepared. The fluorescent properties of these compounds resemble those of *ADMAN* and *DDNP*, supporting the validity of the described approach to the preparation of novel UV and Vis fluorescent dyes for the probing of biological systems.

Experimental

NMR spectra were obtained on Bruker AM 360 WB or DPX 300 spectrometers. ^1H chemical shifts are reported in ppm downfield from *TMS* as internal standard. Deuteriochloroform was used as the solvent unless stated otherwise. Fluorescence spectra were measured on a Perkin Elmer LS 50B luminescence spectrometer. Melting points were determined on a Electrothermal melting point apparatus and are uncorrected. Elemental analyses were performed at the Faculty of Chemistry and

Chemical Technology, University of Ljubljana, or by Galbraith Laboratories Inc., Knoxville, TN. Mass spectra were recorded by Dr. *Bogdan Kralj* at the National Center for Mass Spectrometry of the Republic of Slovenia or by Dr. *Kym Faull* at the mass spectrometry facility at the Department of Chemistry, University of California at Los Angeles. Radial chromatography was performed on a Chromatotron (Harrison Research, 840 Moana Court, Palo Alto, CA 94306). The rotors were prepared as recommended by Harrison Research using EM Silica Gel (Cal. No. 7749–3). Solvents and reagents were from Fisher, Aldrich, or Fluka and were used as received unless noted otherwise. The elemental analysis data compared satisfactorily with the calculated values.

1-(6-Hydroxy-2-naphthyl)-1-ethanone (**1**)

In a 3 l two-neck round bottom flask equipped with a reflux condenser and a dropping funnel, 2 l of HCl ($d = 1.16$) were stirred and heated to boiling. A solution of 6.06 g (30.3 mmol) of 1-(6-methoxy-2-naphthyl)-1-ethanone [9] in a minimum amount of dichloromethane was added, and the mixture was stirred and heated at reflux for 2 h. The hot solution was filtered through a mineral wool plug to remove the oily residue. The solid that separated after cooling was filtered through a glass frit and dissolved in 130 ml of ethyl acetate. The solution was washed with brine, dried over anhydrous magnesium sulfate, and evaporated to give 5 g (89%) **1**. After recrystallization from ethyl acetate the sample melted at 173.5–177°C (Ref. [10]: 170–171°C).

^{1}H NMR: $\delta = 2.71$ (s, 3H, CH_3), 7.18 (dd, 1H, 7-H), 7.19 (d, 1H, 5-H), 7.72 (d, 1H, 4-H), 7.88 (d, 1H, 8-H), 8.00 (dd, 1H, 3-H), 8.40 (d, 1H, 1-H) ppm; $J_{1,3} = 2$ Hz, $J_{3,4} = 8.5$ Hz, $J_{5,7} = 2$ Hz, $J_{7,8} = 8.6$ Hz.

1-6-(Ethyl-(2-hydroxyethyl)-amino)-2-naphthyl-1-ethanone (**2**; $C_{16}H_{19}NO_2$)

A mixture of 1-(6-hydroxy-2-naphthyl)-1-ethanone (**1**, 744 mg, 3.92 mmol), sodium hydrogensulfate (1.66 g, 16 mmol), 2-ethylaminoethanol (2 ml), and water (5 ml) was heated in a steel bomb at 130–140°C for 3 days. After cooling, the mixture was distributed between water and ethyl acetate, and the organic layer was washed with brine, dried, and evaporated. The residue was dissolved in acetone and applied onto a 4 mm dry silica plate for radial chromatography. The plate was eluted with a 1:1 mixture of petroleum ether and ethyl acetate. Appropriate fractions were collected and evaporated to give 125 mg (12%) of **2**. The product melted at 82–85°C.

^{1}H NMR: $\delta = 1.27$ (t, 3H, CH_2CH_3), 1.52 (s, 1H, OH), 2.67, (s, 3H, $COCH_3$), 3.56 (t, 2H, NCH_2CH_2O), 3.63 (t, 2H, NCH_2CH_2O), 3.91 (q, 2H, CH_2CH_3), 6.93 (d, 1H, 5-H), 7.19 (dd, 1H, 7-H), 7.62 (d, 1H, 4-H), 7.80 (d, 1H, 8-H), 7.92 (dd, 1H, 3-H), 8.30 (d, 1H, 1-H) ppm; $J_{1,3} = 3$ Hz, $J_{3,4} = 10$ Hz, $J_{5,7} = 3$ Hz, $J_{7,8} = 10$ Hz, $J_{(CH_2CH_3)} = 5.9$ Hz, $J_{(NCH_2CH_2O)} = 5.9$ Hz.

1-6-(4-(Hydroxymethyl)-piperidino)-2-naphthyl-1-ethanone (**3**; $C_{18}H_{21}NO_2$)

A mixture of 1-(6-hydroxy-2-naphthyl)-1-ethanone (**1**, 653 mg, 3.5 mmol), sodium hydrogensulfate (1.6 g, 15.5 mmol), 4-piperidylmethanol [7] (2 g, 17.6 mmol), and water (6 ml) was heated in a steel bomb at 135–142°C for 16 days. After cooling, the reaction mixture was extracted with ethyl acetate. Some product still remained in the residue, so it was further extracted with 5% methanol in dichloromethane. The organic extracts were combined, dried, and evaporated. The residue was chromatographed by radial chromatography (2 mm silica, 2% methanol in dichloromethane) to yield 139 mg (14%) of **3**. After recrystallization from ethyl acetate the compound melted at 180–182°C.

^{1}H NMR: $\delta = 1.44$ (dddd, 2H, 3′a-H, 5′a-H), 1.76 (m, 1H, 4′a-H), 1.91 (bd, 2H, 3′e-H, 5′e-H), 2.68 (s, 3H, $COCH_3$), 2.89 (ddd, 2H, 2′a-H, 6′a-H), 3.58 (d, 2H, OCH_2), 3.94 (bd, 2H, 2′e-H, 6′e-H), 7.10 (d, 1H, 5-H), 7.32 (dd, 1H, 7-H), 7.66 (d, 1H, 4-H), 7.80 (d, 1H, 8-H), 7.94 (dd, 1H, 3-H), 8.32 (d, 1H, 1-H) ppm; $J_{3'a,3'e} = J_{5'a,5'e} = 12.5$ Hz, $J_{2'a,3'a} = J_{6'a,5'a} = 12.5$ Hz, $J_{3'a,4'a} = J_{5'a,4'a} = 12.5$ Hz,

$J_{2'e,3'a} = J_{6'e,5'a} = 4.0$ Hz, $J_{2'a,2'e} = J_{6'a,6'e} = 12.5$ Hz, $J_{2'a,3'e} = J_{6'a,5'e} = 2.6$ Hz, $J_{4a,OCH_2} = 6.4$ Hz, $J_{1,3} = 1.9$ Hz, $J_{3,4} = 8.9$ Hz, $J_{5,7} = 2.3$Hz, $J_{7,8} = 9.0$ Hz.

6-Acetyl-2-(ethyl-2-((4-methylphenyl)-sulfonyloxy)-ethylamino)-naphthalene (**4**; $C_{23}H_{25}NO_4S$)

A solution of **2** (125 mg, 0.486 mmol) in pyridine (3.5 ml) was cooled to −15°C, and *p*-toluenesulfonic anhydride (252 mg, 0.81 mmol) was added with stirring under argon. The reaction mixture was allowed to warm up slowly to room temperature, and stirring was continued for 24 h. Because TLC (silica, 10% ethyl acetate in petroleum ether) revealed that starting material was still present, more *p*-toluensulfonic anhydride (252 mg, 0.81 mmol) was added, and stirring was continued for additional 24 h. The mixture was then cooled in an ice-water bath and distributed between brine and ether. The organic layer was dried and evaporated to leave an oily residue. The product (**4**) was isolated by radial chromatography (1 mm silica, dichloromethane) in 30% yield.

HRMS: calcd. for $C_{23}H_{25}NO_4S$: 411.1504, found: 411.1514; ^{1}H NMR: $\delta = 1.25$ (t, 3H, CH_2CH_3), 2.33 (s, 3H, Ph-CH_3), 2.67 (s, 3H, $COCH_3$), 3.49 (q, 2H, CH_2CH_3), 3.75 (t, 2H, NCH_2CH_2O), 4.25 (t, 2H, NCH_2CH_2O), 6.97 (d, 1H, 5-H), 7.01 (dd, 1H, 7-H), 7.18 and 7.20 (d, 2H, 3′-H, 5′-H), 7.56 (d, 1H, 4-H), 7.69 and 7.72 (d, 2H, 2′-H, 6′-H), 7.75 (d, 1H, 8-H), 7.93 (dd, 1H, 3-H), 8.29 (d, 1H, 1-H) ppm; $J_{1,3} = 1.6$ Hz, $J_{2',6'} = J_{3',5'} = 8.5$ Hz, $J_{7,5} = 2.5$ Hz, $J_{7,8} = 9.2$ Hz, $J_{3,4} = 8.7$ Hz, $J_{(CH_2CH_3)} = 7.1$ Hz, $J_{(NCH_2CH_2O)} = 6.2$ Hz.

1-(6-(Ethyl-2-((8-3-(2-(4-fluorophenyl)-1,3-dioxolan-2-yl)-propyl-4-phenyl-2,4,8-triazaspiro-[4.5]dec-1-en-1-yl)-oxy)-ethylamino)-2-naphthyl)-1-ethanone (**5**; $C_{41}H_{47}FN_4O_4$) *and 1-(6-Ethyl-(2-(8-3-(2-(4-fluorophenyl)-1,3-dioxolan-2-yl)-propyl-1-oxo-4-phenyl-2,4,8-triazaspiro[4.5]dec-2-yl)-ethyl)-amino-2-naphthyl)-1-ethanone* (**6**; $C_{41}H_{47}Fn_4O_4$)

To a solution of sodium hydroxide (1 g) and tetra-*n*-butylammonium hydrogensulfate(VI) (50 mg, 0.15 mmol) in water (2 ml), spiperone ketal (8-3-(2-(4-fluorophenyl)-1,3-dioxolan-2-yl)propyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one [8], 15 mg, 0.034 mmol) was added and the mixture was stirred vigorously. After 10 min, a solution of tosylate **4** (12 mg, 0.03 mmol) in toluene (3 ml) was added, and the reaction mixture was stirred and heated at 90°C for 1 h. After cooling, the reaction mixture was distributed between water and dichloromethane, and the organic layer was washed with brine, dried, and evaporated to leave an oily residue. Radial chromatography (1 mm silica, 2% methanol in dichloromethane) yielded 5 mg (25%) of O-alkyl product **5** and 11 mg (56%) of N-alkyl product **6**.

5: HRMS: calcd. for $C_{41}H_{47}FN_4O_4$: 678.3581, found: 678.3605; ^{1}H NMR: $\delta = 1.45$–2.24 (m, 11H, spiperone CH_2, CH_2CH_3), 2.35–2.84 (m, 6H, spiperone), 2.65 (s, 3H, OCH_3), 3.59 (q, 2H, NCH_2CH_3), 3.76 in 4.05 (m, 4H, OCH_2CH_2O), 3.85 (t, 2H, NCH_2CH_2O), 4.52 (t, 2H, OCH_2CH_2N), 4.99 (s, 2H, NCH_2N), 6.76–6.83 (m, 3H, phenyl, fluorophenyl), 6.93 (d, 1H, 5-H), 6.95–7.04 (m, 2H, phenyl, fluorophenyl), 7.19 (dd, 1H, 7-H), 7.21–7.26 (m, 2H, phenyl, fluorophenyl), 7.39–7.45 (m, 2H, phenyl, fluorophenyl), 7.61 (d, 1H, 4-H), 7.78 (d, 1H, 8-H), 7.93 (dd, 1H, 3-H), 8.30 (d, 1H, 1-H) ppm; $J_{1,3} = 1.5$ Hz, $J_{5,7} = 2.4$ Hz, $J_{3,4} = 9.5$ Hz, $J_{7,8} = 9.2$ Hz, $J_{(CH_2CH_3)} = 7.1$ Hz, $J_{(NCH_2CH_2O)} = 6.3$ Hz.

6: HRMS: calcd. for $C_{41}H_{47}FF_4O_4$: 678.3581, found: 678.3603; ^{1}H NMR: $\delta = 1.20$–1.94 (m, 17H, spiperone CH_2,CH_3CH_2), 2.66 (s, 3H, $COCH_3$), 3.56 (q, 2H, NCH_2CH_3), 3.66 and 4.02 (m, 4H, OCH_2CH_2O), 3.71–3.81 (m, 4H, NCH_2CH_2N), 4.68 (s, 2H, NCH_2N), 6.82–6.90 (m, 2H, phenyl, fluorophenyl), 6.94 (d, 1H, 5-H), 6.98–7.04 (m, 2H, phenyl, fluorophenyl), 7.18 (dd, 1H, 7-H), 7.21–7.26 (m, 3H, phenyl, fluorophenyl), 7.39–7.45 (m, 2H, phenyl, fluorophenyl), 7.60 (d, 1H, 4-H), 7.78 (d, 1H, 8-H), 7.93 (dd, 1H, 3-H), 8.29 (d, 1H, 1-H) ppm; $J_{1,3} = 1.6$ Hz, $J_{3,4} = 9.8$ Hz, $J_{5,7} = 2.4$ Hz, $J_{7,8} = 10.4$ Hz, $J_{(CH_2CH_3)} = 7.1$ Hz.

2-(1-(6-Ethyl-(2-(8-3-(2-(4-fluorophenyl)-1,3-dioxolan-2-yl)-propyl-1-oxo-4-phenyl-2,4,8-triazaspiro[4.5]dec-2-yl)-ethyl)-amino-2-naphthyl)-ethylidene)-malononitrile (**7**; $C_{44}H_{47}FN_6O_3$)

A solution of **6** (13 mg, 0.018 mmol) and malononitrile (6 mg, 0.09 mmol) in pyridine (3 ml) was heated at 85°C under argon for 24 h. After removal of pyridine *in vacuo* at room temperature, the residue was distributed between brine and dichloromethane, and the organic layer was dried and evaporated. The product **7** was isolated by radial chromatography (1 mm silica, 2.5% methanol in dichloromethane; 13.5 mg, 97%).

FAB HRMS: calcd. for $C_{44}H_{48}FN_6O_3$ (M+H): 727.37719, found: 727.3772; ^{1}H NMR: $\delta = 1.25$–1.93 (m, 17H, spiperone CH_2, C*H*$_3$CH$_2$), 2.70 (s, 3H, C=C-CH_3), 3.57 (q, 2H, NC*H*$_2$CH$_3$), 3.64 and 4,03 (m, 4H, OC*H*$_2$C*H*$_2$O), 3,71–3.78 (m, 4H, NC*H*$_2$C*H*$_2$N), 4.68 (s, 2H, NC*H*$_2$N), 6.83–6.88 (m, 2H, phenyl, fluorophenyl), 6.94 (d, 1H, 5-H), 6.96–7.04 (m, 2H, phenyl, fluorophenyl), 7.18 (dd, 1H, 7-H), 7.21–7.25 (m, 3H, phenyl, fluorophenyl), 7.39–7.45 (m, 2H, phenyl, fluorophenyl), 7.56 (dd, 1H, 3-H), 7.63 (d, 1H, 4-H), 7.76 (d, 1H, 9-H), 8.00 (d, 1H, 1-H) ppm; $J_{1,3} = 1.9$ Hz, $J_{3,4} = 8.8$ Hz, $J_{5,7} = 2.4$ Hz, $J_{7,8} = 9.3$ Hz, $J_{(CH_2CH_3)} = 7.1$ Hz.

2-(1-6(Ethyl-(2-8-(4-(4-fluorophenyl)-4-oxobutyl)-1-oxo-4-phenyl-2,4,8-triazaspiro[4.5]dec-2-ylethyl)-amino)-2-naphthylethylidene)-malononitrile (**8**; $C_{42}H_{43}FN_6O_2$)

The ketal protective group was removed by stirring compound **7** (13.5 mg, 0.0186 mmol) in methanol (1 ml) with one drop of concentrated hydrochloric acid for 3 h at room temperature. The reaction mixture was diluted with dichloromethane and washed with a saturated solution of sodium bicarbonate. After evaporation *in vacuo* the residue was purified by radial chromatography (1 mm silica, 2%) methanol in dichloromethane) to give 10 mg (79%) of **8**.

FAB MS: calcd. for $C_{42}H_{44}FN_6O_2$ (M+H): 683.35, found: 683; ^{1}H NMR: $\delta = 1.21$–3.02 (m, 17H, spiperone CH_2, CH_3), 2.71 (s, 3H, C=C-CH_3), 3.56 (q, 2H, NC*H*$_2$CH$_3$), 3.69 (m, 4H, NC*H*$_2$C*H*$_2$N), 4.67 (s, 2H, NC*H*$_2$N), 6.79–7.23 (m, 7H, phenyl, fluorophenyl), 6.95 (d, 1H, 5-H), 7.19 (dd, 1H, 7-H), 7.56 (dd, 1H, 3-H), 7.65 (d, 1H, 4-H), 7.76 (d, 1H, 8-H), 7.97–8.04 (m, 3H, fluorophenyl, 1-H) ppm; $J_{1,3} = 1.9$ Hz, $J_{3,4} = 8.8$ Hz, $J_{5,7} = 2.5$ Hz, $J_{7,8} = 9.1$ Hz, $J_{(CH_2CH_3)} = 7.1$ Hz.

1-(6-Acetyl-2-naphthyl)-4-((4-methylphenyl)-sulfonyloxy)-methylpiperidine (**9**; $C_{25}H_{27}NO_4S$)

A solution of **3** (59 mg, 0.2 mmol) in pyridine (3 ml) was cooled to −15°C, and *p*-toluenesulfonic anhydride (205 mg, 0.6 mmol) was added with stirring under argon. The reaction mixture was allowed to warm up slowly to room temperature during 1 h. It was cooled again and distributed between brine and ether. The organic layer was washed with brine, dried, and evaporated to leave 83 mg (91%) of raw **9**. For elemental analysis it was purified by radial chromatography (1 mm silica, ether). Pure **9** melted at 135–137°C.

^{1}H NMR: $\delta = 1.39$ (dddd, 2H, 3a-H, 5a-H), 1.84 (bd, 2H, 3e-H, 5e-H), 1.93 (m, 1H, 4a-H), 2.46 (s, 3H, PhC*H*$_3$), 2.67 (s, 3H, COC*H*$_3$), 2.83 (ddd, 2H, 2a-H, 6a-H), 3.87 (bd, 2H, 2e-H, 6e-H), 3.92 (d, 2H, OC*H*$_2$), 7.06 (d, 1H, 5′-H), 7.27 (dd, 1H, 7′-H), 7.36 (d, 2H, 3″-H, 5″-H (Ts)), 7.65 (d, 1H, 4′-H), 7.79 (d, 1H, 8′-H), 7.80 (d, 2H, 2″-H, 6″-H (Ts)), 7.94 (dd, 1H, 3′-H), 8.32 (d, 1H, 1′-H) ppm; $J_{3a,3e} = J_{5a,5e} = 12.4$ Hz, $J_{2a,3a} = J_{6a,5a} = 12.4$ Hz, $J_{3a,4a} = J_{5a,4a} = 12.4$ Hz, $J_{2e,3a} = J_{6e,5a} = 4.0$ Hz, $J_{2a,2e}$, $J_{6a,6e} = 12.4$ Hz, $J_{2a,3e} = J_{6a,5e} = 2.4$ Hz, $J_{4a,OCH_2} = 6.5$ Hz, $J_{1',3'} = 1.6$ Hz, $J_{3',4'} = 8.7$ Hz, $J_{5',7'} = 2.4$ Hz, $J_{7',8'} = 9.5$ Hz, $J_{2'',3''} = J_{5'',6''} = 8.1$ Hz.

*1-(6-(4-((8-3-(2-(4-Fluorophenyl)-1,3-dioxolan-2-yl)-propyl-4-phenyl-2,4,8-triazaspiro[4.5]dec-1-en-1-yl)-oxy)-methylpiperidino)-2-naphthyl)-1-ethanone (***10**; $C_{43}H_{49}FN_4O_4$) *and 1-(6-4-((8-3-(2-(4-fluorophenyl)-1,3-dioxolan-2-yl)-propyl-1-oxo-4-phenyl-2,4,8-triazaspiro[4.5]dec-2-yl)-methyl)-piperidino-2-naphthyl)-1-ethanone* (**11**; $C_{43}H_{49}FN_4O_4$)

To a solution of sodium hydroxide (1 g) and tetra-*n*-butylammonium hydrogensulfate(VI) (50 mg, 0.15 mmol) in water (2 ml), spiperone ketal (100 mg, 0.2 mmol) was added, and the mixture was

stirred vigorously. After 10 minutes, a solution of tosylate **9** (98 mg, 0.2 mmol) in toluene (10 ml) was added, and the reaction mixture was stirred at room temperature for 11 days. It was then distributed between brine and dichloromethane, and the organic layer was dried and evaporated to leave 190 mg of an oily residue. Radial chromatography (1 mm silica, dichloromethane followed by 2% methanol in dichloromethane) yielded 27 mg (17%) of O-alkayl **10** and 92 mg (58%) of N-alkyl product **11**.

10: HRMS: calcd. for $C_{43}H_{49}FN_4O_4$: 704.3738, found: 704.3760; ^{1}H NMR: $\delta = 1.46$–1.90 (m, 10H, 3′a-H, 5′a-H, 3′e-H, 5′e-H, spiperone), 1.88 (m, 1H, 4′a-H), 2.15 and 2.38 (b, 4H, spiperone), 2.67 (s, 3H, $COCH_3$), 2.80 (m, 4H, spiperone), 2.95 (m, 2H, 2′a-H, 6′a-H), 3.75 (m, 2H, OCH_2CH_2O), 3.87 (m, 2H, 2′e-H, 6′e-H), 3.92 (m, 2H, OCH_2CH_2O), 4.19 (d, 2H, OCH_2), 4.97 (s, 2H, NCH_2N), 6.7–6.9 (m, 3H, Ph), 7.01 (m, 2H, Ph), 7.11 (d, 1H, 5-H), 7.23 (m, 2H, Ph), 7.32 (dd, 1H, 7-H), 7.41 (m, 2H, Ph), 7.66 (d, 1H, 4-H), 7.81 (d, 1H, 8-H), 7.95 (dd, 1H, 3-H), 8.32 (d, 1H, 1-H) ppm; $J_{2'a,2'e} = J_{6'a,6'e} = 12.4$ Hz, $J_{2'a,3'e} = J_{6'a,5'e} = 2.6$ Hz, $J_{4'a,OCH_2} = 6.1$ Hz, $J_{1,3} = 2.1$ Hz, $J_{3,4} = 8.8$ Hz, $J_{5,7} = 2.1$ Hz, $J_{7,8} = 9.1$ Hz.

11: HRMS: calcd. for $C_{43}H_{49}FN_4O_4$: 704.3738, found: 704.3710; ^{1}H NMR: $\delta = 1.50$ (dddd, 2H, 3′a-H, 5′a-H), 1.55–1.70 (m, 4H, spiperone), 1.84 (bd, 2H, 3′e-H, 5′e-H), 1.92 (m, 2H, spiperone), 1.98 (m, 1H, 4′a-H), 2.42 (m, 2H, spiperone), 2.67 (s, 3H, $COCH_3$), 2.69 (m, 2H, spiperone), 2.83 (m, 4H, spiperone), 2.88 (m, 2H, 2′a-H, 6′a-H), 3.35 (d, 2H, 4′-CH_2N), 3.75 (m, 2H, OCH_2CH_2O), 3.92 (bd, 2H, 2′e-H, 6′e-H), 4.02 (m, 2H, OCH_2CH_2O), 4.71 (s, 2H, NCH_2N), 6.88 (m, 1H, Ph), 6.91 (m, 2H, Ph), 7.01 (m, 2H, Ph), 7.08 (bs, 1H, 5-H), 7.27 (m, 3H, 7-H, Ph), 7.43 (m, 2H, Ph), 7.65 (d, 1H, 4-H), 7.79 (d, 1H, 8-H), 7.94 (dd, 1H, 3-H), 8.32 (bs, 1H, 1-H) ppm; $J_{3'a,3'e}) = J_{5'a,5'e} = 12.4$ Hz, $J_{2'a,3'a} =$ Hz, $J_{2'a,2e} = J_{6'a,6'e} = 12.8$ Hz, $J_{2'a,3'e} = J_{6'a,5'e} = 2.4$ Hz, $J_{4'a,4'\text{-}CH_2N} = 7.3$ Hz, $J_{1,3} = 1.9$ Hz, $J_{3,4} = 8.8$ Hz, $J_{5,7} = 2.1$ Hz, $J_{7,8} = 9.2$ Hz.

2-(1-(6-4-((8-3-(2-(4-Fluorophenyl)-1,3-dioxolan-2-yl)-propyl-1-oxo-4-phenyl-2,4,8-triazaspiro[4.5]dec-2-yl)-methyl)-piperidino-2-naphthyl)-ethylidene)malononitrile (**12**; $C_{46}H_{49}FN_6O_3$)

Using the procedure described for the synthesis of **7**, compound **11** was transformed into the malononitrile derivative **12**. It was purified by radial chromatography on a 1 mm silica plate using 2% MeOH in CH_2Cl_2 as the solvent.

FAB HRMS: calcd. for $C_{46}H_{50}FN_6O_3$ (M+H): 753.3928, found: 753.3940; ^{1}H NMR: $\delta = 1.60$–2.1 (m, 11H, spiperone, 3′a-H, 3′e-H, 4′a-H, 5′a-H, 5′e-H), 2.40 (m, 2H, spiperone), 2.71 (s, 3H, C=CCH_3), 2.60–2.80 (m, 6H, spiperone), 2.91 (m, 2H, 2′a-H, 6′a-H), 3.37 (d, 2H, 4′-CH_2N), 3.75 (m, 2H, OCH_2CH_2O), 3.94 (bd, 2H, 2′e-H, 6′e-H), 4.02 (m, 2H, OCH_2CH_2O), 4.72 (s, 2H, NCH_2N), 6.85–6.95 (m, 3H, Ph), 7.01 (m, 2H, fluorophenyl), 7.07 (d, 1H, 5-H), 7.31 (m, 3H, 7-H, Ph), 7.41 (m, 2H, fluorophenyl), 7.56 (dd, 1H, 3-H), 7.69 (d, 1H, 4-H), 7.77 (d, 1H, 8-H), 8.01 (d, 1H, 1-H) ppm; $J_{2'a,3'a} = J_{5'a,6a} = 12.8$ Hz, $J_{2'a,2'e} = J_{6'a,6'e} = 12.8$ Hz, $J_{4'a,4'\text{-}CH_2N} = 7.6$ Hz, $J_{1,3} = 1.8$ Hz, $J_{3,4} = 8.6$ Hz, $J_{5,7} = 2.2$ Hz, $J_{7,8} = 9.4$ Hz, $J_{2'a,3'e} = J_{5'e,6'a} = 1.8$ Hz, $J_{H,F} = 8.7$ and 6.2 Hz.

2-(1-6-(4-(8-(4(4-Fluorophenyl)-4-oxobutyl)-1-oxo-4-phenyl-2,4,8-triazaspiro[4,5]dec-2-ylmethyl)-piperidino)-2-naphthylethylidene)-malononitrile (**13**; $C_{44}H_{45}FN_6O_2$)

The ketal protective group was removed from **12** as described for **8** to give **13** in quantitative yield.

FAB HRMS: calcd. for $C_{44}H_{46}FN_6O_2$ (M+H): 709.3666, found: 709.3689; ^{1}H NMR: $\delta = 1.60$–2.1 (m, 11H, spiperone, 3′a-H, 3′e-H, 4′a-H, 5′a-H, 5′e-H), 2.5–2.71 (m, 4H, spiperone), 2.73 (s, 3H, C=C-CH_3), 2.8–3.1 (m, 6H, spiperone, 2′a-H, 6′a-H), 3.38 (d, 2H, 4′-CH_2N), 3.96 (bd, 2H, 2′e-H, 6′e-H), 4.74 (s, 2H, NCH_2N), 6.91 (m, 3H, phenyl), 7.1 (d, 1H, 5-H), 7.15 (m, 2H, fluorophenyl),

7.24–7.30 (m, 2H, Ph), 7.34 (dd, 1H, 7-H), 7.58 (dd, 1H, 3-H), 7.72 (d, 1H, 4-H), 7.79 (d, 1H, 8-H), 8.01–8.08 (m, 3H, fluorophenyl, 1-H) ppm; $J_{1,3} = 2.0$ Hz, $J_{3,4} = 8.6$ Hz, $J_{5,7} = 2.4$ Hz, $J_{7,8} = 9.2$ Hz, $J_{4'a,4'\text{-}CH_2N} = 7.4$ Hz, $J_{2'a,2'e} = J_{6'a,6'e} = 13.0$ Hz, $J_{2'a,3'a} = J_{5'a,6'a} = 12.5$ Hz, $J_{2'a,3'e} = J_{5'e,6'a} = 1.9$ Hz, $J_{H,F} = 8.7$ and 6.2 Hz.

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