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Synthesis and modifications of a small library of 1,4-benzodiazepin-3-ones toward potential inhibitors of the collagen—von Willebrand Factor interaction

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ABSTRACT

A library of 1,4-benzodiazepin-3-one-based turn mimetics were synthesized and tested for their antithrombotic abilities. These mimetics are designed to incorporate amino acid side chains of the previously reported paratope of the inhibitory anti-von Willebrand Factor antibody 82D6A3. Modifications were performed on the scaffolds by alkylation of the N^1-position and acylation of the reduced nitro group. Hereby the number of functional groups incorporated on the core was expanded.

1. Introduction

The process of platelet adhesion, activation and aggregation (primary hemostasis), necessary for the cessation of bleeding at sites of vascular injury, is also responsible for pathological thrombus formation, particularly at sites of high shear. This uncontrolled formation of such aggregates, can lead to vascular occlusion resulting in serious ischemic disorders such as acute myocardial infarction (AMI), unstable angina, or thrombotic stroke. Upon injury subendothelial compounds such as collagen become exposed on cell walls of arteries. The blood plasma protein von Willebrand Factor (VWF) is immobilized by binding to the collagen with its A3-domain. Then blood platelets interact reversibly with the immobilized VWF, which causes them to slow down.[1–5] This allows them to bind irreversibly with collagen. After this adhesion other platelets are activated and aggregate at the place of injury, forming the fatal thrombus. Without VWF the platelets are unable to bind directly to the collagen because of the high blood shear in the arteries. This VWF–collagen interaction, crucial in thrombus formation, is an interesting therapeutical target. Recently, a monoclonal antibody binding to the A3-domain of human VWF called 82D6A3, which blocks the VWF–collagen interaction has been characterized.[6–8] Its antithrombotic effect has already been proven in vivo in a baboon arterial thrombosis model in which no bleeding side effects were detected even at high doses.[9,10] Therefore, the antibody is superior to known antithrombotics such as Aspirin and Reopro, which both interfere with shear-independent platelet aggregation causing increased bleeding risk and resulting in the need for close patient monitoring.[11–13] An orally available drug, with the same antithrombotic characteristics and advantages as the 82D6A3 antibody would be a considerable advancement for cardiovascular treatments.

To this end, only the minimum active sequence and (stereo) structural requirements of the antibody are required. Staelens et al. characterized the paratope of the antibody by X-ray crystallography and mutagenesis studies.[14] The paratope comprises two short sequences of the heavy chain, CDR 1 (Asn 31 and Tyr 32) and CDR3 (Asp 99, Pro 101, Tyr 102, and Tyr 103), which are discontinuous in primary structure, but form one continuous surface patch. Two β-turns of type I and type IV were detected in the paratope conformation.[15–18] β-Turns are one of the secondary elements of peptides, which play next to a structural role, also a functional role as molecular recognition sites in many biological processes. Previously the binding site for collagen within VWF was located on the A3-domain of VWF.[19,20] By comparing binding studies of VWF mutants to collagen, with the Ab 82D6A3-VWF crystal structure, a substantial overlap of the binding site for collagen and the binding site for the Ab on the A3-domain was confirmed. Hereby it was concluded that direct competition for the same interaction site explains the potent action of the Ab. Creating peptidomimetics mimicking the charge distribution and structure of this paratope is the next goal in the development of this new class of potential antithrombotics.

The basic molecular scaffold discussed in this article is the 1,4-benzodiazepin-3-one (Fig. 1). 1,4-Benzodiazepines are privileged scaffolds mimicking peptide secondary structures such as γ-and β-turns making them interesting candidates to mimick the structural features of the paratope.[21–27] Numerous synthetic approaches

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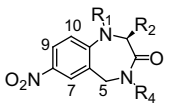


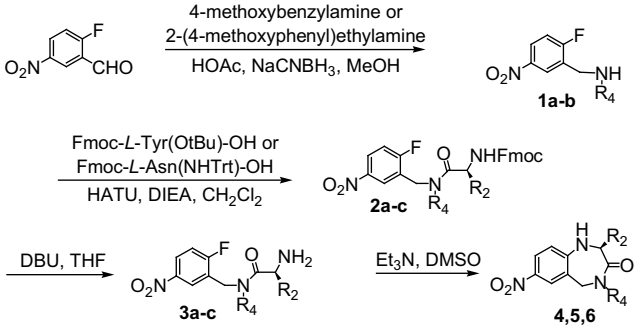
Figure 1. Structure of 1,4-benzodiazepin-3-ones.

have been developed for the preparation of 1,4-benzodiazepin-3-ones.[28–36] Hence these compounds seemed to be an interesting synthetic starting point for this work for which at present no small molecule lead compounds are available.

We applied the strategy of the group of Prof. A. Hallberg because it allows extensive modification of the scaffold.[25,37] The scaffold incorporates the side chains on position R^2 and R^4. With further modifications, the number of side chains can be expanded by R^1-alkylation on nitrogen and by acylation of the latent amine group obtained after reduction of the nitro function (Fig. 1). The basic targets are mimics of the Tyr–Tyr and Asn–Tyr patterns present in a beta turn conformation in the paratope. We also describe a third derivative, which is a homologue of the Tyr–Tyr pattern by incorporating 2-(4-methoxyphenyl)ethylamine instead of 4-methoxybenzylamine as an R^4-group (Fig. 1).

2. Results and discussion

The synthetic pathway toward these bicyclic core structures is outlined in Scheme 1. Reductive amination of 2-fluoro-5-nitrobenzaldehyde with 4-methoxybenzylamine or 2-(4-methoxyfphenyl)ethylamine, was followed by coupling the secondary amine with the fluorenylmethyloxycarbonyl (Fmoc) protected amino acids Fmoc-L-Tyr(O^{t}Bu)-OH or Fmoc-L-Asn(NHTrt)-OH. To ensure a complete reaction, 2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate (HATU) was used as coupling reagent. To prevent nucleophilic aromatic substitution on the activated benzene, the following Fmoc-deprotection was conducted with 8-diazabicyclo[5.4.0]undec-7-ene (DBU). The final key cyclization step, a nucleophilic aromatic substitution of the free amine, proceeded smoothly with triethylamine in DMSO.



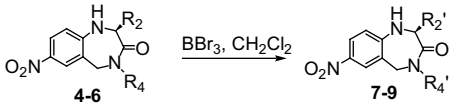
Scheme 1. Synthesis of the basic scaffolds **4**–**6**.

In Table 1 the three different benzodiazepines and their overall yields are given. No significant problems were encountered during their synthesis. The NMR spectra of compounds **2a**–**2c** and **3a**–**3c** showed two rotamers, the signals of which coalesced at elevated temperature.[37]

Table 1
Overall yields of the synthesis of the basic scaffolds

Compound	R^2	R^4	%
4	$CH_2(C_6H_4)O^tBu$	$CH_2(C_6H_4)OMe$	27
5	$CH_2(C_6H_4)O^tBu$	$(CH_2)_2(C_6H_4)OMe$	37
6	$CH_2CONHTrt$	$CH_2(C_6H_4)OMe$	15

The first modification conducted was the deprotection of the groups R^2 and R^4. Because the arylmethylether requires the harshest deprotection conditions, the methyl group was first removed to form the phenol moiety using BBr_3 (Scheme 2). Yields obtained were moderate and unidentified side products were formed. Fortunately extra deprotection steps were avoided since the other two protecting groups, ^{t}Bu and Trt, also were removed in the same step (Table 2).

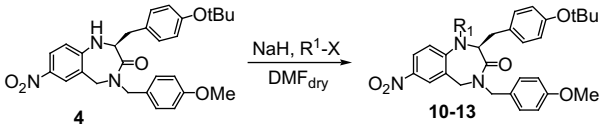


Scheme 2. Deprotection of the R^2 and R^4 groups.

Table 2
Yields of the deprotection of the R^2 and R^4 groups

Compound	$R^{2'}$	$R^{4'}$	%
7	$CH_2(C_6H_4)OH$	$CH_2(C_6H_4)OH$	33
8	$CH_2(C_6H_4)OH$	$(CH_2)_2(C_6H_4)OH$	45
9	CH_2CONH_2	$CH_2(C_6H_4)OH$	52

A second modification is the alkylation of the N^1-position in which the number of functional groups on the scaffold is expanded. Compound **4** was alkylated with four different halides using sodium hydride (NaH) as a base in dry DMF (Scheme 3 and Table 3). Formation of compound **13** was difficult to monitor. After purification, starting material **4** was still recovered.

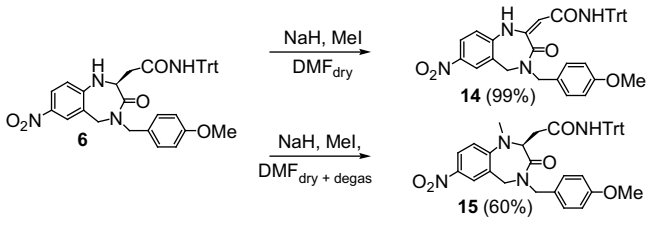


Scheme 3. N^1-Alkylation.

Table 3
Yields of the alkylation of the N^1-position

Compound	R^1-X	%
10	CH_3I	81
11	$BrCH_2(C_6H_5)$	54
12	$BrCH_2CHCH_2$	59
13	$BrCH_2CO_2Me$	32

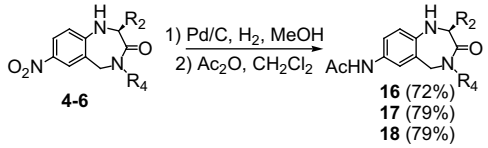
Different attempts for the alkylation of derivative **6** under previously mentioned conditions produced in each case product **14** (Scheme 4). Only when the solvent was degassed, methylated product **15** was obtained in good yield. We believe that compound **14** is formed because of the acidity of the protons next to the amide.



Scheme 4. N^1-Alkylation of compound **6**.

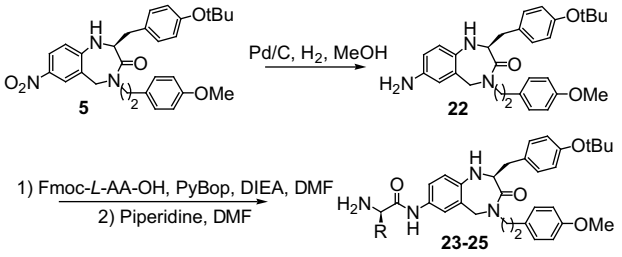
Instead of deprotonation of N^1, one of these protons is deprotonated to form an anion, which reacts with oxygen.

Another way to produce more diversity on the scaffold is to reduce the nitro group and further functionalize the resulting amine. Reduction was effected in a Parr apparatus with palladium/carbon under hydrogen in a quantitative manner. To increase the stability of this electron rich aniline derivate, the amine was immediately capped with acetic anhydride (**16**–**18**) (Scheme 5).



Scheme 5. Reduction of the nitro-group followed by acylation.

Compound **5** was coupled with another Fmoc-protected amino acids of the antibody paratope sequence (**19**–**21**) after reduction of the nitro group **22** (Scheme 6). Because of the low reactivity of the aromatic amine, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (Pybop) was used as the coupling reagent. After coupling overnight the products were obtained in moderate yields. Side products could not be identified or purified. The Fmoc-group was then removed with piperidine in DMF to yield the compounds **23**–**25**, which were difficult to purify (Table 4).



Scheme 6. Coupling of the reduced nitro-group with AA's.

Table 4
Yields of the coupling and the Fmoc-deprotection

Compound	Fmoc-L-AA-OH	%
19	Fmoc-Asn(NHTrt)-OH	35
20	Fmoc-Asp(O^{t}Bu)-OH	68
21	Fmoc-Tyr(O^{t}Bu)-OH	57
23	Fmoc-Asn(NHTrt)-OH	45
24	Fmoc-Asp(O^{t}Bu)-OH	21
25	Fmoc-Tyr(O^{t}Bu)-OH	42

2.1. Determination of inhibition of the VWF–collagen binding

The enzyme-linked immunosorbent assay (ELISA) was performed essentially as described.[38] Briefly, microtiter plates were coated with human collagen type III (Sigma, St. Louis, MO) and blocked with 3% milk powder solution. A 1:80 dilution of human normal plasma (NHP) (prewarmed for 5 min at 37 °C) was pre-incubated (30 min at 37 °C) in presence of 1% DMSO with a serial twofold dilution of either the antibody 82D6A3 or the synthesized compounds, starting at a concentration of 4 μg/ml and 0.100 mM, respectively. Plasma VWF was then allowed to bind collagen for 1.5 h at 37 °C. Rabbit antihuman VWF labeled with horseradish peroxidase was added for 1 h at room temperature. Visualization was obtained with H_2O_2 and *ortho*-phenylenediamine (OPD, Sigma) and the coloring reaction was stopped with 4 M H_2SO_4. The absorbance was determined at 490 nm. After each incubation step, plates were washed with phosphate buffer solution (PBS), 0.1% Tween-20, 3 times after coating and blocking steps and 12 times elsewhere. In Figure 2 the effect of 82D6A3 and some of benzodiazepines on binding of plasma VWF to collagen is shown as determined by ELISA. Absorbance is plotted against the highest concentration compound tested (0.100 mM). As can be derived from the figure 4 μg/ml of the 82D6A3 antibody completely blocks the VWF–collagen interaction. No inhibitory effect on the collagen–VWF binding could be detected after pre-incubation of NHP with the compounds.

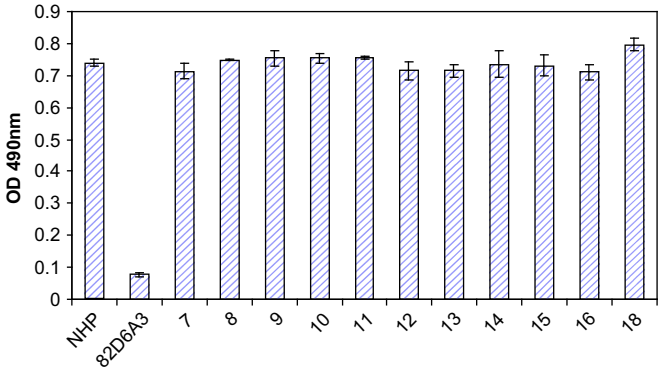


Figure 2. Effect of 82D6A3 and the benzodiazepines on VWF–collagen binding as determined by ELISA. NHP, 82D6A3, 7–16, and 18 are representing the VWF control, the inhibitory antibody and the tested benzodiazepines, respectively. Absorbance (490 nm) is shown in function of the highest concentration tested (0.100 mM). All data point the mean and standard error of single measurements in two independent experiments.

3. Conclusions

In this article we have discussed the synthesis of a small library of 1,4-benzodiazepin-3-one turn mimics based on the synthesis of the group of Prof. A. Hallberg. By this we try to mimic the conformation and the charge distribution of the paratope of the monoclonal antibody 82D6A3 against the A3-domain of VWF. This plasma protein plays a crucial role in the formation of fatal thrombosis in the arteries. Each synthesized molecule was tested for inhibition of the collagen–VWF interaction with Enzyme-Linked Immuno Sorbent Assay (ELISA) in cooperation with the KULAK research group of the Lab of Thrombosis Research. None of these compounds showed the ability to block the interaction.

4. Experimental section

4.1. General methods

All chemical reagents were used without further purification. Solvents for column chromatography and TLC were laboratory grade and distilled before use. TLC was performed on Fluka Chemica Kiezelgel glass plates, column chromatography with silica gel SI 60 (230–400 mesh) and HPLC was performed on an Altech column (25×2.5 cm, 5 μm). NMR spectra were recorded on a Bruker Avance 300 (300 MHz) or a Bruker Avance 400 (400 MHz) spectrometer. NMR samples were run in the indicated solvents and were referenced internally. Chemical shifts (δ) are reported relative to TMS and coupling constants (J) in hertz (Hz). Low-resolution mass spectra were recorded on a HEWLETT–PACKARD instrument (CI-EI) and a Thermo Finnigan LCQ Advantage (ESI). High-resolution mass spectra were recorded on a KRATOS MS50TC instrument. IR spectra were obtained using KBr pellets or NaCl plates. Melting points were determined using a Reichert-Jung Thermovar apparatus and were uncorrected.

4.2. Synthesis of the basic scaffolds

4.2.1. (2-Fluoro-5-nitrophenyl)-N-(4-methoxybenzyl)-methanamine (**1a**)

To a solution of 2-fluoro-5-nitrobenzaldehyde (2 g, 11.83 mmol) in MeOH (20 ml), acetic acid (1.02 ml, 17.74 mmol), and 4-methoxybenzylamine (3.08 ml, 23.66 mmol) was added. After stirring the mixture for 8 h under Ar at room temperature, $NaCNBH_3$ (1.49 g, 23.66 mmol) was added. The solution was stirred another 8 h under the same conditions and afterward quenched by H_2O (100 ml). MeOH was evaporated and the aqueous layer was extracted with EtOAc (3×20 ml). The organic layers were collected, dried ($MgSO_4$), and evaporated. The crude product was purified by column chromatography (1% MeOH in DCM) to afford product **1a** as a yellow oil (2.06 g, 60%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.31 (ddd, J=2.8, 4.2, 8.7 Hz, 1H, 6-H), 8.03 (dd, J=2.8, 6.2 Hz, 1H, 4-H), 7.21 (d, J=8.3 Hz, 2H), 7.08 (t, J=8.9 Hz, 1H, 3-H), 6.80 (d, J=8.3 Hz, 2H), 3.82 (s, 2H, C^1CH_2), 3.70 (s, 5H, OCH_3, $NHCH_2$), 1.30 (s, 1H, $NHCH_2$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 45.78 (d, J_{C-F}=2.3 Hz, C^1CH_2), 53.00 ($NHCH_2$), 55.47 (OCH_3), 114.18 (2CH), 116.45 (d, J_{C-F}=24.7 Hz, CH^3), 124.73 (d, J_{C-F}=10.2 Hz, 4-CH), 126.17 (d, J_{C-F}=6.6 Hz, 6-CH), 129.70 (2CH), 129.96 (d, J_{C-F}=16.2 Hz, 1-C), 132.20 (CCH_2), 144.64 (5-C), 159.16 ($COCH_3$), 164.77 (d, J_{C-F}=257.2 Hz, 2-C) ppm. MS (EI): m/z (%)=289 (95) $[M-H]^+$, 259 (95) $[M-OCH_3]^+$, 169 (25) [-CH_2-ϕ-OMe]$^+$. HRMS (EI) calculated for $C_{15}H_{15}O_3N_2F$: 290.1066; found: 290.1062.

4.2.2. N-(2-Fluoro-5-nitrobenzyl)-2-(4-methoxyphenyl)-ethanamine (**1b**)

Compound **1b** was synthesized by the procedure described for compound **1a**. 2-(4-Methoxyphenyl)ethylamine (3.47 ml, 23.66 mmol) was used in the reaction. The crude product was purified by column chromatography (1% MeOH in DCM) to afford product **1b** as a yellow oil (2.27 g, 64%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.26–8.24 (ddd, J=2.8, 4.5, 8.3 Hz, 1H, 6-H), 8.03–8.00 (dd, J=2.8, 6.3 Hz, 1H, 4-H), 7.09–7.03 (m, 3H, 3-H, 2H), 6.77 (d, J=8.1 Hz, 2H), 3.84 (s, 2H, CH_2NH), 3.68 (s, 3H, OCH_3), 2.86–2.82 (m, 2H, $NHCH_2CH_2$), 2.75–2.73 (m, 2H, $NHCH_2CH_2$), 1.46 (s, 1H, $NHCH_2CH_2$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 35.73 ($NHCH_2CH_2$), 46.40 (d, J_{C-F}=2.5 Hz, C^1CH_2), 51.14 ($NHCH_2CH_2$), 55.34 (OCH_3), 114.23 (2CH), 116.38 (d, J_{C-F}=24.8 Hz, 3-CH), 124.64 (d, J_{C-F}=11.1 Hz, 4-CH), 126.4 (d, J_{C-F}=7.4 Hz, 6-CH), 129.95 (2CH), 130.19 (1-C), 132.11 (CCH_2), 144.68 (5-C), 158.46 ($COCH_3$), 164.68 (d, J_{C-F}=257.6 Hz, 2-C) ppm. MS (EI): m/z (%)=183 (100) $[M-CH_2$-ϕ-OMe$]^+$. HRMS (EI) calculated for $C_{16}H_{17}O_3N_2F$: 304.1223; found: 304.1215.

4.2.3. 9H-Fluoren-9-ylmethyl-1-(4-tert-butoxybenzyl)-2-[(2-fluoro-5-nitro-benzyl)(4-methoxybenzyl)amino]-2-oxoethylcarbamate (**2a**)

To a solution of amine **1a** (2.06 g, 7.10 mmol) in DCM (35 ml), 1 equiv Fmoc-L-Tyr(O^tBu)-OH (3.26 g, 7.10 mmol), 1 equiv HATU (2.69 g, 7.10 mmol), and 2.7 equiv DIPEA (1.95 ml, 21.30 mmol) was added. After stirring the reaction mixture overnight under N_2 at room temperature, H_2O (50 ml) and EtOAc (50 ml) were added. The layers were separated and the aqueous layer was further extracted with EtOAc (3×10 ml). The organic layers were collected, washed with 1 M $KHSO_4$, H_2O, saturated $NaHCO_3$, brine, dried ($MgSO_4$), and evaporated. Purification with column chromatography (1% MeOH in DCM) afforded product **2a** as yellow foam (3.59 g, 67%). MS (ESI, MeOH): 754.5 [MNa^+], 1485.1 [$2MNa^+$].

4.2.4. 9H-Fluoren-9-ylmethyl-1-(4-tert-butoxybenzyl)-2-{(2-fluoro-5-nitro-benzyl)[2-(4-methoxyphenyl)ethyl]amino}-2-oxoethylcarbamate (**2b**)

Amine **1b** (2.27 g, 7.47 mmol) was coupled with Fmoc-L-Tyr(O^tBu)-OH by a procedure similar to that used for compound **2a**. Product **2b** was obtained as a yellow foam (4.69 g, 84%) after column chromatography (1% MeOH in DCM). MS (ESI, MeOH): 746.7 [MH^+], 768.8 [MNa^+], 1508.5 [$2MH^+$], 1513.5 [$2MNa^+$].

4.2.5. 9H-Fluoren-9-ylmethyl-2-[(2-fluoro-5-nitrobenzyl)(4-methoxybenzyl)amino]-2-oxo-1-[(tritylamino)carbonyl]-ethylcarbamate (**2c**)

Amine **1a** (2.27 g, 7.82 mmol) was coupled with Fmoc-L-Asn(NHTrt)-OH by a procedure similar to that used for compound **2a**. Product **2c** was obtained as a yellow foam (3.01 g, 43%) after column chromatography (0.5% MeOH in DCM). MS (ESI, MeOH): 869.5 [MH^+], 891.4 [MNa^+], 1738.2 [$2MH^+$], 1759.3 [$2MNa^+$].

4.2.6. 2-Amino-3-(4-tert-butoxyphenyl)-N-(2-fluoro-5-nitrobenzyl)-N-(4-methoxybenzyl)propanamide (**3a**)

Fmoc-protected compound **2a** (3.59 g, 4.76 mmol) was dissolved in dry THF (30 ml) and DBU (0.47 ml, 4.76 mmol) was added. After 2 h stirring at room temperature under Ar, THF was evaporated. The crude residue was purified with column chromatography (gradient 0.5–2% MeOH in DCM) and product **3a** was obtained as yellow oil (1.87 g, 74%). Two rotamers were detected. ^{1}H NMR (300 MHz, DMSO): δ 8.18–8.16 (m, 2H, 6-H, 4-H) (major), 7.97–8.03 (m, 2H, 6-H, 4-H) (minor), 7.46–7.37 (m, 1H, 3-H), 7.10–6.92 (m, 4H), 6.81 (d, J=7.7 Hz, 2H), 6.76 (d, J=7.7 Hz, 2H), 4.75 (d, J=14.5 Hz, 1H, C^1CH_2) (minor), 4.61 (d, J=15.3 Hz, 1H, C^1CH_2) (major), 4.40–4.34 (m, 3H, NCH_2, C^1CH_2) (major), 4.15 (d, J=14.5 Hz, 1H, C^1CH_2) (minor), 3.97–3.12 (m, 1H, α-H) (major), 3.84–3.80 (m, 1H, α-H) (minor), 3.71 (s, 3H, OCH_3), 2.95–2.84 (m, 1H, β-H), 2.77–2.68 (m, 1H, β-H), 2.51–1.9 (br s, 2H, NH_2), 1.24 (s, 9H, ^{t}Bu) ppm. ^{13}C NMR (75 MHz, DMSO): δ 29.30 ($C(CH_3)_3$), 42.55 (β-CH_2), 42.82 (CH_2NH) (major), 43.89 (CH_2NH) (minor), 48.46 ($NHCH_2$) (minor), 50.62 ($NHCH_2$) (major), 53.68 (α-CH) (minor), 53.76 (α-CH) (major), 55.67 (OCH_3) (major), 55.77 (OCH_3) (minor), 78.34 ($C(CH_3)_3$), 114.50 (2CH) (minor), 114.77 (2CH) (major), 117.45 (d, J_{C-F}=25.2 Hz, 3-CH) (major), 117.65 (d, J_{C-F}=24.5 Hz, 3-CH) (minor), 124.28 (2CH) (major), 124.38 (2CH) (minor), 125.15 (d, J_{C-F}=6.6 Hz, 6-CH) (minor), 125.83 (d, J_{C-F}=10.7 Hz, 4-CH) (major), 126.06 (d, J_{C-F}=10.7 Hz, 4-CH) (minor), 126.85 (d, J_{C-F}=6.3 Hz, 6-CH) (major), 127.30 (d, J_{C-F}=17.5 Hz, 1-C) (minor), 127.44 (d, J_{C-F}=17.0 Hz, 1-C) (major), 128.89 (2CH) (major), 129.25 (CCH_2) (major), 129.79 (CCH_2) (minor), 130.25 (2CH) (minor), 130.56 (2CH) (minor), 130.62 (2CH) (major), 133.66 (CCH_2) (minor), 133.79 (CCH_2) (major), 144.72 (5-C) (major), 145.00 (5-C) (minor), 154.25 (CO^tBu), 159.34 (COMe), 159.64 (COMe) (major), 164.30 (d, J_{C-F}=255.3 Hz, 2-C) (minor), 164.57 (d, J_{C-F}=255.7 Hz, 2-C) (major), 176.06 (CO) (minor), 176.25 (CO) (major) ppm. MS (EI): m/z (%)=436 (22) $[M-O^tBu]^+$, 346 (15) $[M-CH_2$-ϕ-$O^tBu]^+$. MS (ESI, MeOH): 510.5 [MH^+], 732.1 [MNa^+], 1019.6 [$2MH^+$], 1041.3 [$2MNa^+$].

4.2.7. 2-Amino-3-(4-tert-butoxyphenyl)-N-(2-fluoro-5-nitrobenzyl)-N-[2-(4-methoxyphenyl)ethyl]propanamide (**3b**)

Fmoc-protected compound **2b** (4.69 g, 6.29 mmol) was deprotected by a procedure similar to that used for compound **3a**. The crude residue was purified with column chromatography (gradient 0.5–2% MeOH in DCM) and product **3a** was obtained as yellow oil (2.86 g, 87%). Two conformers were detected. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09–8.06 (m, 1H, 6-H), 7.91–7.87 (m, 1H, 4-H) (major), 7.82–7.80 (m, 1H, 6-H) (minor), 7.00–6.86 (m, 5H), 6.86–6.75 (m, 4H), 4.53 (d, J=15.3 Hz, 1H, C^1CH_2) (major), 4.21 (d, J=15.3 Hz, 1H, C^1CH_2) (major), 4.15 (d, J=17.2 Hz, 1H, C^1CH_2) (minor), 3.97 (d, J=17.2 Hz, 1H, C^1CH_2) (minor), 3.55–3.47 (br s, 4H, OCH_3, α-CH), 3.13–3.02 (m, 2H, $NHCH_2CH_2$), 2.81–2.32 (m, 4H, β-H, $NHCH_2CH_2$), 1.12 (s, 2H, NH_2), 1.12 (s, 9H, ^{t}Bu) (minor), 1.04 (s, 9H, ^{t}Bu) (major) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.59 ($C(CH_3)_3$) (major), 28.66 ($C(CH_3)_3$) (minor), 32.55 (NCH_2CH_2) (minor), 34.14 (NCH_2CH_2) (major), 42.05 (β-CH_2) (major), 42.35 (β-CH_2) (minor), 42.53 (NCH_2CH_2) (major), 44.60 (NCH_2CH_2) (minor), 48.63 (C^1CH_2) (minor), 49.06 (C^1CH_2) (major), 53.24 (α-CH) (major), 53.46 (α-CH)

(minor), 54.95 (OCH_3) (major), 53.61 (OCH_3) (minor), 77.89 ($C(CH_3)_3$), 113.87 (2CH) (minor), 114.09 (2CH) (major), 116.13 (d, J_{C-F}=15.1 Hz, 3-CH) (major), 116.54 (d, J_{C-F}=15.1 Hz, 3-CH) (minor), 123.90 (2CH) (major), 124.02 (2CH) (minor), 124.29 (d, J_{C-F}=5.8 Hz, 6-CH) (minor), 124.71 (d, J_{C-F}=10.8 Hz, 4-CH) (major), 125.17 (d, J_{C-F}=10.8 Hz, 4-CH) (minor), 126.21 (d, J_{C-F}=16.2, 1-C) (minor), 126.44 (d, J_{C-F}=9.2 Hz, 4-CH) (major), 126.49 (d, J_{C-F}=16.2 Hz, 1-C) (major), 129.52 (2CH), 129.59 (2CH) (major), 130.50 (CCH_2), 132.42 (CCH_2) (minor), 132.50 (CCH_2) (major), 144.20 (5-C) (major), 144.45 (5-C) (minor), 154.02 (CO^tBu) (major), 154.21 (CO^tBu) (minor), 158.15 (COMe) (minor), 158.41 (COMe) (major), 163.88 (J_{C-F}=256.6 Hz, 2-C), 164.04 (d, J_{C-F}=256.6 Hz, 2-C), 175.11 (CO) (minor), 175.77 (CO) (major) ppm. MS (EI): *m*/*z* (%)=508 (14) $[M-CH_3]^+$, 450 (30) $[M-O^tBu]^+$. HRMS (EI) calculated for $C_{29}H_{34}O_5N_3F$: 523.2482; found: 523.2469.

4.2.8. 2-Amino-N^1-(2-fluoro-5-nitrobenzyl)-N^1-(4-methoxybenzyl)-N^4-trityl-succinamide (**3c**)

Fmoc-protected compound **2c** (3.01 g, 3.38 mmol) was deprotected by a procedure similar to that used for compound **3a**. The crude residue was purified with column chromatography (gradient 1–10% MeOH in DCM) and product **3c** was obtained as yellow oil (1.31 g, 60%). Two conformers were detected. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.29 (s, 1H, NHTrt) (major), 8.26 (s, 1H, NHTrt) (minor), 8.11–8.03 (m, 2H, 4-H, 6-H) (major), 8.00–7.97 (m, 1H) (minor), 7.22–7.19 (m, 16H, Trt, 3-H), 7.11–6.98 (m, 2H), 6.80 (d, *J*=8.1 Hz, 2H) (major), 6.75 (d, *J*=8.1 Hz, 2H) (minor), 4.69 (d, *J*=14.7 Hz, 1H, C^1CH_2) (minor), 4.63 (d, *J*=15.6 Hz, 1H, NCH_2) (major), 4.57 (d, *J*=16.4 Hz, 1H, C^1CH_2) (major), 4.50 (d, *J*=15.6 Hz, 1H, NCH_2) (major), 4.45 (d, *J*=16.8 Hz, 1H, C^1CH_2) (major), 4.23 (d, *J*=14.8 Hz, 1H, C^1CH_2) (minor), 4.17 (t, *J*=6.4 Hz, 1H, α-H), 3.73 (s, 3H, OCH_3) (major), 3.71 (s, 3H, OCH_3) (minor), 2.56 (d, *J*=6.4 Hz, 2H, β-H), 1.77 (s, 2H, NH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 42.81 (β-CH_2) (major), 42.99 ($NHCH_2$) (major), 43.09 (β-CH_2) (minor), 43.96 ($NHCH_2$) (minor), 48.39 (C^1CH_2) (minor), 49.27 (C^1CH_2) (major), 50.82 (α-CH) (major), 53.61 (α-CH) (minor), 55.35 (OCH_3), 70.56 ($C(\phi)_3$), 114.31 (2CH) (minor), 114.56 (2CH) (major), 116.42 (d, J_{C-F}=24.5 Hz, 3-CH) (major), 116.81 (d, J_{C-F}=25.7 Hz, 3-CH) (minor), 124.81 (d, J_{C-F}=6.5 Hz, 6-CH) (minor), 125.00 (d, J_{C-F}=10.2 Hz, 4-CH) (major), 125.48 (d, J_{C-F}=10.0 Hz, 4-CH) (minor), 125.79 (d, J_{C-F}=15.2 Hz, 1-C) (minor), 126.00 (d, J_{C-F}=6.2 Hz, 6-CH) (major), 126.24 (J_{C-F}=17.2 Hz, 1-C) (major), 126.91 (3CH, Trt), 127.56 (CCH_2), 127.86 (6CH, Trt), 128.28 (2CH) (major), 128.94 (6CH, Trt), 129.47 (2CH) (minor), 144.72 (5-C), 144.89 (3C, Trt), 159.27 (COMe) (minor), 159.47 (COMe) (major), 164.07 (d, J_{C-F}=258.5 Hz, 2-C) (minor), 164.17 (d, J_{C-F}=258.3 Hz, 2-C) (major), 169.51 (CO), 174.92 (CO) (minor), 175.21 (CO) (major) ppm. MS (EI): *m*/*z* (%): 403 (6) $[M-Trt]^+$. HRMS (EI) calculated for $C_{38}H_{35}O_5N_4F$: 646.2591; found: 646.2573.

4.2.9. 2-(4-tert-Butoxybenzyl)-4-(4-methoxybenzyl)-7-nitro-1,2,4,5-tetrahydro-3H-1,4-benzodiazepin-3-one (**4**)

Amine **3a** (1.87 g, 3.67 mmol) was dissolved in DMSO (10 ml), triethylamine (0.19 ml, 11.02 mmol), and H_2O (0.72 ml, 40.41 mmol). After stirring the mixture overnight at room temperature under Ar, EtOAc (50 ml) and saturated $NaHCO_3$ (50 ml) were added. The layers were separated and the organic layer was washed with brine, dried ($MgSO_4$), and evaporated. After column chromatography (1% MeOH in DCM), product **4** was obtained as a yellow solid (1.59 g, 89%). ^{1}H NMR (400 MHz, DMSO): δ 7.78 (dd, *J*=2.6, 9.1 Hz, 1H, 9-H), 7.71 (d, *J*=2.6 Hz, 1H, 7-H), 7.30 (d, *J*=8.3 Hz, 2H), 7.18 (d, *J*=4.1 Hz, 1H, 1-NH), 7.13 (d, *J*=8.6 Hz, 2H), 6.89 (d, *J*=8.3 Hz, 2H), 6.74 (d, *J*=8.6 Hz, 2H), 6.63 (d, *J*=9.1 Hz, 1H, 10-H), 5.45 (d, *J*=17.1 Hz, 1H, 5-H), 5.24–5.19 (m, 1H, 2-H), 4.52 (dd, *J*=15.1 Hz, 2H, NCH_2), 4.03 (d, *J*=17.1 Hz, 1H, 5-H), 3.66 (s, 3H, OCH_3), 3.20 (dd, *J*=6.6, 14.3 Hz, 1H, β-H), 2.84 (dd, *J*=7.1, 14.3 Hz, 1H, β-H), 1.27 (s, 9H, ^{t}Bu) ppm. ^{13}C NMR (100 MHz, DMSO): δ 28.60 ($C(CH_3)_3$), 35.07 (β-CH_2), 48.71 (NCH_2), 49.40 (5-CH_2), 54.53 (2-CH), 55.02 (OCH_3), 77.64 ($C(CH_3)_3$), 113.57 (2CH), 115.05 (10-CH), 118.78 (6-C), 123.27 (2CH), 124.66 (9-CH), 126.26 (7-CH), 129.25 (2CH), 129.82 (CCH_2), 129.84 (2CH), 132.61 (CCH_2), 135.57 (8-C), 152.71 (11-C), 153.47 (CO^tBu), 158.47 (COMe), 168.98 (3-C) ppm. MS (EI): *m*/*z* (%)=326 (64) $[M-CH_2$-ϕ-$O^tBu]^+$. HRMS (EI) calculated for $C_{28}H_{31}O_3N_5$: 489.2263; found: 489.2261. Melting point: 166 °C.

4.2.10. 2-(4-tert-Butoxybenzyl)-4-[2-(4-methoxyphenyl)ethyl]-7-nitro-1,2,4,5-tetrahydro-3H-1,4-benzodiazepin-3-one (**5**)

Amine **3b** (2.86 g, 5.46 mmol) was cyclized by a procedure similar as described for compound **4**. After column chromatography (1% MeOH in DCM), product **5** was obtained as a yellow solid (2.19 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82 (dd, *J*=2.5, 9.0 Hz, 1H, 9-H), 7.53 (d, *J*=2.5 Hz, 1H, 7-H), 7.18 (d, *J*=8.4 Hz, 2H), 6.95 (d, *J*=8.4 Hz, 4H), 6.66 (d, *J*=8.4 Hz, 2H), 6.31 (d, *J*=9.0 Hz, 1H, 10-H), 5.21 (d, *J*=16.9 Hz, 1H, 5-H), 4.90–4.85 (m, 1H, 2-H), 4.51 (d, *J*=2.7 Hz, 1H, 1-NH), 3.90–3.83 (m, 1H, $NHCH_2CH_2$), 3.70 (s, 3H, OCH_3), 3.65–3.50 (m, 2H, 5-H+$NHCH_2CH_2$), 3.36 (dd, *J*=5.5, 14.5 Hz, 1H, β-H), 2.87 (dd, *J*=8.7, 14.5 Hz, 1H, β-H), 2.82–2.72 (m, 2H, NCH_2CH_2), 1.33 (s, 9H, ^{t}Bu) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.87 ($C(CH_3)_3$), 33.64 (NCH_2CH_2), 63.18 (β-CH_2), 51.14 ($NHCH_2CH_2$), 52.56 (5-CH_2), 55.06 (OCH_3), 55.26 (2-CH), 78.48 ($C(CH_3)_3$), 113.81 (2CH), 115.28 (10-CH), 118.31 (6-C), 124.44 (2CH), 125.20 (9-CH), 125.86 (7-CH), 129.60 (2CH), 129.65 (2CH), 130.40 (CCH_2), 131.14 (CCH_2), 137.85 (8-C), 151.16 (11-C), 154.64 (CO^tBu), 158.27 (COMe), 168.81 (3-C) ppm. MS (EI): *m*/*z* (%)=447 (120) $[M-{}^tBu+H]^+$, 369 (19) $[M-CH_2CH_2$-ϕ-OMe+H$]^+$, 340 (85) $[CH_2$-ϕ-$O^tBu]$. HRMS (EI) calculated for $C_{29}H_{33}O_5N_3$: 503.2420; found: 503.2425. Melting point: 147 °C.

4.2.11. 2-[4-(4-Methoxybenzyl)-7-nitro-3-oxo-2,3,4,5-tetrahydro-1H-1,4-benzo-diazepin-2-yl]-N-tritylacetamide (**6**)

Amine **3c** (1.31 g, 2.03 mmol) was cyclized by a procedure similar as described for compound **4**. After column chromatography (1% MeOH in DCM), product **6** was obtained as a yellow solid (1.16 g, 92%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.92 (s, 1H, NHTrt), 7.74 (dd, *J*=2.3, 9.1 Hz, 1H, 9-H), 7.49 (d, *J*=2.3 Hz, 1H, 7-H), 7.23–7.16 (m, 15H, Trt), 7.04 (d, *J*=8.6 Hz, 2H), 6.70 (d, *J*=8.6 Hz, 2H), 6.22 (d, *J*=9.1 Hz, 1H, 10-H), 5.61 (d, *J*=2.8 Hz, 1H, 1-NH), 5.10 (d, *J*=16.4 Hz, 1H, 5-H), 5.12–5.05 (m, 1H, 2-H), 4.57 (dd, *J*=14.8 Hz, 2H, NCH_2), 3.73–3.70 (m, 4H, OCH_3, 5-H), 2.83–2.73 (m, 2H, β-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.00 (β-CH_2), 49.8 (NCH_2), 49.91 (5-CH_2), 52.17 (2-CH), 55.29 (OCH_3), 70.69 ($C(\phi)_3$), 114.15 (2CH), 115.65 (10-CH), 117.82 (6-C), 125.21 (7-CH), 126.00 (9-CH), 127.01 (3CH, Trt), 127.92 (6CH, Trt), 128.44 (CCH_2), 128.72 (6CH, Trt), 129.54 (2CH), 137.47 (8-C), 144.48 (3C, Trt), 151.31 (11-C), 159.35 (COMe), 169.36 (CO), 169.39 (CO) ppm. HRMS (EI) calculated for $C_{38}H_{34}O_5N_4$: 626.2529; found: 626.2516. MS (EI): *m*/*z* (%)=383 (37) $[M-Trt]^+$. Melting point: 117 °C.

4.3. Deprotection of the side chain groups R^2 and R^4

4.3.1. 2-[4-(4-Hydroxybenzyl)-7-nitro-3-oxo-2,3,4,5-tetrahydro-1H-1,4-benzo-diazepin-2-yl]acetamide (**7**)

To a cooled solution of benzodiazepine **4** (50 mg, 0.1 mmol) in dry DCM (2 ml), BBr_3 (0.51 ml of 1 M solution in DCM, 0.51 mmol) was added. After stirring 1 h at 0 °C under Ar, MeOH (5 ml) and H_2O (5 ml) were added. The organic solvents were evaporated and the aqueous layer was extracted with EtOAc (3×2 ml). The organic layer was dried ($MgSO_4$) and evaporated. Product **7** was obtained after purification with column chromatography (EtOAc/heptane 90:10) as a yellow solid (13.83 mg, 33%). ^{1}H NMR (400 MHz, DMSO): δ 9.22 (s, 1H, OH), 9.17 (s, 1H, OH), 7.80 (dd, *J*=2.6, 9.0 Hz, 1H, 9-H), 7.75 (d, *J*=2.6 Hz, 1H, 7-H), 7.19 (d, *J*=8.5 Hz, 2H), 7.13 (d, *J*=4.2 Hz, 1H, 1-NH), 7.03 (d, *J*=8.5 Hz, 2H), 6.68 (d, *J*=8.5 Hz, 2H), 6.63 (d, *J*=9.0 Hz,

1H, 10-H), 6.59 (d, *J*=8.5 Hz, 2H), 5.41 (d, *J*=16.6 Hz, 1H, 5-H), 5.17–5.10 (m, 1H, 2-H), 4.47 (d, *J*=14.6 Hz, 2H, NCH_2), 4.04 (d, *J*=16.6 Hz, 1H, 5-H), 3.15 (dd, *J*=6.3, 14.3 Hz, 1H, β-H), 2.76 (dd, *J*=7.3, 14.3 Hz, 1H, β-H) ppm. ^{13}C NMR (100 MHz, DMSO): δ 35.42 (β-CH_2), 49.20 (NCH_2), 49.81 (5-CH_2), 55.26 (2-CH), 115.32 (2CH), 115.37 (2CH), 115.46 (10-CH), 119.19 (6-C), 125.13 (9-CH), 126.74 (7-CH), 128.41 (CCH_2), 128.53 (CCH_2), 129.70 (2CH), 130.72 (2CH), 135.91 (8-C), 153.20 (11-C), 156.24 (COH), 156.95 (COH), 169.47 (3-C) ppm. MS (EI): *m*/*z* (%): 312 (66) [M−CH_2-ϕ-OH]$^+$, 313 (71) [M−CH_2-ϕ-OH+H], 206 (71) [M−2(CH_2-ϕ-OH)+H]$^+$. HRMS (EI) calculated for $C_{23}H_{21}O_5N_3$: 419.1481; found: 419.1461. Melting point: 115 °C.

*4.3.2. 2-(4-Hydroxybenzyl)-4-[2-(4-hydroxyphenyl)ethyl]-7-nitro-1,2,4,5-tetra-hydro-3H-1,4-benzodiazepin-3-one (**8**)*

Benzodiazepine **5** (50 mg, 0.1 mmol) was deprotected by a procedure similar to that used for product **7**. The crude residue was purified by column chromatography (EtOAc/heptane 80:20) to afford product **8** as a yellow solid (20 mg, 45%). ^{1}H NMR (400 MHz, DMSO): δ 9.17 (s, 1H, OH), 9.11 (s, 1H, OH), 7.96 (d, *J*=1.8 Hz, 1H, 7-H), 7.84 (dd, *J*=1.8, 8.9 Hz, 1H, 9-H), 7.20–7.09 (m, 3H, 2H, 1-NH), 6.90 (d, *J*=8.1 Hz, 2H), 6.72 (d, *J*=8.5 Hz, 2H), 6.71–5.66 (m, 3H, 2H, 10-H), 5.37 (d, *J*=16.6 Hz, 1H, 5-H), 5.09–5.00 (m, 1H, 2-H), 4.14 (d, *J*=16.6 Hz, 1H, 5-H), 3.64–3.43 (m, 2H, NCH_2CH_2), 3.09 (dd, *J*=6.2, 13.9 Hz, 1H, β-H), 2.71 (dd, *J*=6.4, 13.9 Hz, 1H, β-H), 2.56 (t, *J*=7.2 Hz, 2H, NCH_2CH_2) ppm. ^{13}C NMR (100 MHz, DMSO): δ 34.04 (NCH_2CH_2), 35.73 (β-CH_2), 49.47 (NCH_2CH_2), 50.70 (5-CH_2), 55.65 (2-CH), 115.76 (2CH), 115.84 (2CH), 116.91 (10-CH), 119.86 (6-C), 125.69 (9-CH), 126.97 (CCH_2), 127.02 (7-CH), 128.94 (CCH_2), 129.59 (2CH), 130.52 (2CH), 136.42 (8-C), 153.13 (11-C), 156.45 (COH), 156.60 (COH), 169.43 (3-C) ppm. MS (EI): *m*/*z* (%)=383 (37) [M−Trt]$^+$. HRMS (EI) calculated for $C_{24}H_{23}O_5N_3$: 447.1794; found: 447.1796. Melting point: 105 °C.

*4.3.3. 2-[4-(4-Hydroxybenzyl)-7-nitro-3-oxo-2,3,4,5-tetrahydro-1H-1,4-benzo-diazepin-2-yl]acetamide (**9**)*

Benzodiazepine **6** (50 mg, 0.08 mmol) was deprotected by a procedure similar to that used for product **7**. The crude residue was purified by column chromatography (8% MeOH in DCM) to afford product **9** as a yellow solid (15.37 mg, 52%). ^{1}H NMR (400 MHz, DMSO): δ 9.22 (s, 1H, OH), 7.81 (dd, *J*=9.0, 2.5 Hz, 1H, 9-H), 7.78 (d, *J*=2.5 Hz, 1H, 7-H), 7.44 (s, 1H, NH_2), 7.25 (d, *J*=3.2 Hz, 1H, 1-NH), 7.04 (d, *J*=8.3 Hz, 2H), 6.87 (s, 1H, NH_2), 6.64 (d, *J*=9.1 Hz, 1H, 10-H), 6.60 (d, *J*=8.3 Hz, 2H), 5.36 (d, *J*=16.6 Hz, 1H, 5-H), 5.26 (m, 1H, 2-H), 4.47 (dd, *J*=14.5 Hz, 2H, NCH_2), 4.02 (d, *J*=16.6 Hz, 1H, 5-H), 2.80 (dd, *J*=15.8, 8.2 Hz, 1H, β-H), 2.43 (dd, *J*=15.8, 2.5 Hz, 1H, β-H) ppm. ^{13}C NMR (100 MHz, DMSO): δ 36.29 (β-CH_2), 49.10 (NCH_2), 49.77 (5-CH_2), 50.96 (2-CH), 115.34 (10-CH), 115.38 (2CH), 119.05 (6-C), 125.23 (9-CH), 126.76 (7-CH),128.28 (CCH_2), 129.60 (2CH), 135.95 (8-C), 153.33 (11-C), 156.96 (C-OH), 169.33 ($CONH_2$), 170.71 (3-C) ppm. MS (EI): *m*/*z* (%): 353 (46) [M−OH]$^+$, 263 (78) [M−CH_2-ϕ-OH]$^+$, 247 (69) [M−NH_2, −CH_2-ϕ-OH]$^+$. HRMS (EI) calculated for $C_{18}H_{18}O_5N_4$: 370.1277; found: 370.1261. Melting point: 142 °C.

4.4. Alkylation of the N^1-position

*4.4.1. 2-(4-tert-Butoxybenzyl)-1-ethyl-4-(4-methoxybenzyl)-7-nitro-1,2,4,5-tetrahydro-3H-1,4-benzodiazepin-3-one (**10**)*

Benzodiazepine **4** (50 mg, 0.1 mmol) was dissolved in dry DMF (5 ml) at 0 °C. NaH (60%) (4 mg, 0.1 mmol) was added and the reaction was stirred for 10 min at 0 °C under Ar. After adding iodoethane (0.01 ml, 0.1 mmol), the solution was further stirred for 4 h at 50 °C. Brine (20 ml) was added and the aqueous layer was extracted with EtOAc (3×5 ml). The organic layers were collected, dried ($MgSO_4$), and evaporated. The crude residue was purified by column chromatography (EtOAc/heptane 50:50) to afford product **10** as a orange solid (42.82 mg, 81%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.93 (dd, *J*=2.5, 9.3 Hz, 1H, 9-H), 7.62 (d, *J*=2.5 Hz, 1H, 7-H), 7.06 (d, *J*=8.2 Hz, 2H), 6.95 (d, *J*=8.5 Hz, 2H), 6.84 (d, *J*=8.2 Hz, 2H), 6.73 (d, *J*=9.3 Hz, 1H, 10-H), 6.69 (d, *J*=8.5 Hz, 2H), 4.67–4.43 (m, 4H, 2-H, N$^4CH_2$, 5-H), 4.16 (d, *J*=16.8 Hz, 1H, 5-H), 3.68 (s, 3H, OCH_3), 3.50–3.26 (m, 3H, β-H, CH_2CH_3), 3.03 (dd, *J*=6.43, 13.6 Hz, 1H, β-H), 1.26 (s, 9H, ^{t}Bu), 1.07 (t, *J*=7.1 Hz, 3H, CH_2CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.29 (CH_2CH_3), 29.49 (C(CH_3)$_3$), 36.27 (β-CH_2), 46.07 (CH_2CH_3), 50.27 (N$^4CH_2$), 51.67 (5-CH_2), 55.67 (2-CH), 64.89 (OCH_3), 78.84 (C(CH$_3$)$_3$), 114.45 (2CH), 117.04 (10-C), 122.49 (6-C), 124.73 (2CH), 125.16 (9-CH), 126.52 (7-CH), 128.95 (CCH_2), 129.81 (2CH), 130.13 (2CH), 132.27 (CCH_2), 138.48 (8-C), 153.90 (11-C), 154.69 (CO^tBu), 159.60 (COMe), 169.82 (CO) ppm. MS (EI): *m*/*z* (%)=354 (76) [M−CH_2-ϕ-O^{t}Bu]$^+$. HRMS (EI) calculated for $C_{30}H_{35}O_5N_3$: 517.2576; found: 517.2570. Melting point: 65 °C.

*4.4.2. 1-Benzyl-2-(4-tert-butoxybenzyl)-4-(4-methoxybenzyl)-7-nitro-1,2,4,5-tetrahydro-3H-1,4-benzodiazepin-3-one (**11**)*

Benzodiazepine **4** (50 mg, 0.1 mmol) was alkylated with benzyl bromide (0.1 mmol, 0.011 ml) by a procedure similar to that used for product **10**. After purification by column chromatography (EtOAc/heptane 30:70), product **11** was obtained as a brown solid (34.93 mg, 59%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89 (dd, *J*=2.6, 9.1 Hz, 1H, 9-H), 7.69 (d, *J*=2.6 Hz, 1H, 7-H), 7.37–7.32 (m, 2H, ϕ), 7.28–7.23 (m, 3H, ϕ), 7.08 (d, *J*=8.4 Hz, 2H), 7.01 (d, *J*=8.5 Hz, 2H), 6.90 (d, *J*=8.4 Hz, 2H), 6.75 (d, *J*=8.5 Hz, 2H), 6.69 (d, *J*=9.1 Hz, 1H, 10-H), 4.94 (dd, *J*=5.2, 8.9 Hz, 1H, 2-H), 4.85 (d, *J*=17.2 Hz, 1H, 5-H), 4.71 (d, *J*=17.5 Hz, 1H, CH_2ϕ), 4.66 (d, *J*=14.5 Hz, 1H, N$^4CH_2$), 4.57 (d, *J*=14.5 Hz, 1H, N$^4CH_2$), 4.43 (d, *J*=17.5 Hz, 1H, CH_2ϕ), 4.15 (d, *J*=17.2 Hz, 1H, 5-H), 3.78 (s, 3H, OCH_3), 3.42 (dd, *J*=8.9, 13.7 Hz, 1H, β-H), 2.95 (dd, *J*=5.2, 13.7 Hz, 1H, β-H), 1.33 (s, 9H, ^{t}Bu) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.87 (C(CH_3)$_3$), 35.37 (β-CH_2), 49.87 (N$^4CH_2$), 51.45 (5-CH_2), 55.24 (CH_2ϕ), 55.26 (OCH_3), 63.72 (2-CH), 78.37 (C(CH$_3$)$_3$), 114.11 (2CH), 117.38 (10-C), 122.39 (6-C), 124.21 (2CH), 124.74 (CH, ϕ), 125.83 (9-C), 126.30 (CH, ϕ), 127.43 (7-C), 128.62 (CCH_2), 128.91 (CH, ϕ), 129.38 (2CH), 129.79 (2CH), 131.89 (CCH_2), 138.03 (CCH_2ϕ), 138.86 (8-C), 154.33 (11-C), 154.44 (CO^tBu), 159.28 (COMe), 168.74 (3-C) ppm. MS (EI): *m*/*z* (%)=416 (100) [M−CH_2-ϕ-O^{t}Bu]$^+$. HRMS (EI) calculated for $C_{35}H_{37}O_5N_3$: 579.2733; found: 579.27460. Melting point: 57 °C.

*4.4.3. 1-Allyl-2-(4-tert-butoxybenzyl)-4-(4-methoxybenzyl)-7-nitro-1,2,4,5-tetrahydro-3H-1,4-benzodiazepin-3-one (**12**)*

Benzodiazepine **4** (50 mg, 0.1 mmol) was alkylated with allyl bromide (0.1 mmol, 0.008 ml) by a procedure similar to that used for product **10**. After purification by column chromatography (EtOAc/heptane 30:70), product **12** was obtained as a brown oil (28.21 mg, 52%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 (dd, *J*=2.7, 9.2 Hz, 1H, 9-H), 7.59 (d, *J*=2.7 Hz, 1H, 7-H), 7.06 (d, *J*=8.4 Hz, 2H), 6.93 (d, *J*=8.6 Hz, 2H), 6.85 (d, *J*=8.4 Hz, 2H), 6.71 (d, *J*=9.2 Hz, 1H, 10-H), 6.67 (d, *J*=8.6 Hz, 2H), 5.82–5.71 (m, 1H, N$^1CH_2CHCH_2$), 5.19–5.12 (m, 2H, N$^1CH_2CHCH_2$), 4.74 (dd, *J*=5.3, 8.7 Hz, 1H, 2-H), 4.68 (d, *J*=16.8 Hz, 1H, 5-H), 4.50 (dd, *J*=14.6 Hz, 2H, N$^4CH_2$), 4.07 (d, *J*=16.8 Hz, 1H, 5-H), 4.05–3.99 (m, 1H, N$^1CH_2CHCH_2$), 3.78–3.72 (m, 1H, N$^1CH_2CHCH_2$), 3.69 (s, 3H, OCH_3), 3.37 (dd, *J*=13.6, 8.7 Hz, 1H, β-H), 3.03 (dd, *J*=13.6, 5.3 Hz, 1H, β-H), 1.26 (s, 9H, ^{t}Bu) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.87 (C(CH_3)$_3$), 35.32 (β-CH_2), 49.81 (N$^4CH_2$), 51.29 (5-CH_2), 53.70 (N$^1CH_2CHCH_2$), 55.25 (OCH_3), 63.50 (2-CH), 78.38 (C(CH$_3$)$_3$), 114.08 (2CH), 117.30 (10-CH), 117.39 (N$^1CH_2CHCH_2$), 122.18 (6-C), 124.22 (2CH), 124.64 (9-CH), 125.76 (7-CH), 128.61 (N$^4CH_2$), 129.39 (2CH), 129.77 (2CH), 131.91 (CCH_2), 134.20 (N$^1CH_2CHCH_2$), 138.61 (8-C), 154.25 (11-C), 154.35 (CO^tBu), 159.27 (COMe), 168.87 (3-C) ppm. MS (EI): *m*/*z* (%)=514 (12) [M−CH_3]$^+$, 487 (10) [M−CH_2CHCH_2-H]$^+$, 367 (100) [M−CH_2CHCH_2, −CH_2-ϕ-OMe]$^+$. HRMS (EI) calculated for $C_{31}H_{35}O_5N_3$: 529.2576; found: 529.2577.

4.4.4. 1-Phenyl-2-(4-tert-butoxybenzyl)-4-(4-methoxybenzyl)-7-nitro-1,2,4,5-tetrahydro-3H-1,4-benzodiazepin-3-one (**13**)

Benzodiazepine **4** (50 mg, 0.1 mmol) was alkylated with methylbromoacetate (0.1 mmol, 0.010 ml) by a procedure similar to that used for product **10**. After purification by HPLC (1% MeOH in DCM), product **13** was obtained as a yellow-brown oil (17.95 mg, 32%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99 (dd, J=2.3, 9.1 Hz, 1H, 9-H), 7.70 (d, J=2.3 Hz, 1H, 7-H), 7.19 (d, J=8.3 Hz, 2H), 7.00 (d, J=8.4 Hz, 2H), 6.92 (d, J=8.3 Hz, 2H), 6.73 (d, J=8.4 Hz, 2H), 6.69 (d, J=9.1 Hz, 1H, 10-H), 4.94 (d, J=17.2 Hz, 1H, 5-H), 4.88 (dd, J=4.4, 9.4 Hz, 1H, 2-H), 4.73 (d, J=14.5 Hz, 1H, N^4CH_2), 4.43 (d, J=14.5 Hz, 2H, N^4CH_2), 4.33 (d, J=18.6 Hz, 1H, N^1CH_2), 4.00 (d, J=17.2 Hz, 1H, 5-H), 3.90 (d, J=18.6 Hz, 1H, N^1CH_2), 3.78 (s, 3H, OCH_3), 3.73 (3H, OCH_3), 3.44 (dd, J=9.4, 12.9 Hz, 1H, β-H), 3.02 (dd, J=4.4, 13.2 Hz, 1H, β-H), 1.31 (s, 9H, ^{t}Bu) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.86 (C(CH_3)$_3$), 34.14 (β-CH_2), 49.61 (N^4CH_2), 51.46 (5-CH_2), 52.65 (N^1CH_2), 52.60 (OCH_3), 55.25 (OCH_3), 61.64 (2-CH), 78.42 (C(CH_3)$_3$), 114.06 (2CH), 117.32 (10-CH), 122.61 (6-C), 124.28 (2CH), 124.74 (9-CH), 125.90 (7-CH), 128.55 (CCH_2N), 129.34 (2CH), 129.91 (2CH), 131.42 (CCH_2), 139.3 (8-C), 154.16 (11-C), 154.34 (CO^tBu), 159.22 (COMe), 168.54 (3-C), 170.86 (CO_2CH_3) ppm. MS (EI): m/z (%)=398 (94) [M−CH_2-ϕ-O^{t}Bu]$^+$. HRMS (EI) calculated for $C_{31}H_{35}O_7N_3$: 561.2475; found: 561.2491.

4.4.5. 2-[4-(4-Methoxybenzyl)-7-nitro-3-oxo-1,3,4,5-tetrahydro-2H-1,4-benzodiazepin-2-ylidene]-N-tritylethanamide (**14**)

Product **14** was obtained by attempts of alkylation of benzodiazepine **6** (50 mg, 0.08 mmol) with iodomethane (0.015 ml, 0.08 mmol) by a procedure similar to that used for product **10**. After purification by column chromatography (0.5% MeOH in DCM), product **14** was obtained as a yellow solid (32.40 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.54 (s, 1H, NHTrt), 7.99 (dd, J=2.3, 8.7 Hz, 1H, 9-H), 7.52 (d, J=2.3 Hz, 1H, 7-H), 7.35–7.10 (m, 15H, Trt), 7.15 (d, J=8.5 Hz, 2H), 6.90 (s, 1H, NH), 6.85 (d, J=8.7 Hz, 1H, 10-H), 6.83 (d, J=8.5 Hz, 2H), 5.85 (s, 1H, C^2CH), 4.59 (s, 2H, 5-H), 4.19 (s, 2H, N^4CH_2), 3.79 (s, 3H, OCH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 47.22 (N^4CH_2), 50.05 (5-CH_2), 55.33 (OCH_3), 70.78 (C(ϕ)$_3$), 98.06 (C^2CH), 114.32 (2CH), 120.02 (10-CH), 123.39 (7-CH), 123.74 (6-C), 125.01 (9-CH), 127.11 (3CH, Trt), 127.47 (CCH_2), 128.04 (6CH, Trt), 128.25 (6CH, Trt), 129.97 (2CH), 141.76 (2-C), 144.48 (8-C), 145.80 (3C, Trt), 149.11 (11-C), 159.77 (COMe), 162.93 (CONHTrt), 168.52 (3-C) ppm. MS (EI): m/z (%)=381 (15) [M−Trt]$^+$. HRMS (EI) calculated for $C_{38}H_{32}O_5N_4$: 624.2372; found: 624.2362. Melting point: 133 °C.

4.4.6. 2-[4-(4-Methoxybenzyl)-1-methyl-7-nitro-3-oxo-tetrahydro-1H-1,4-benzodiazepin-2-yl]-N-tritylacetamide (**15**)

Benzodiazepine **6** (50 mg, 0.08 mmol) was dissolved in degassed and dry DMF (5 ml) at 0 °C. NaH (60%) (8 mg, 0.16 mmol) was added and the reaction was stirred for 10 min at 0 °C under Ar. After adding iodomethane (0.015 ml, 0.08 mmol), the solution was further stirred for 4 h at 50 °C. Brine (20 ml) was added and the aqueous layer was extracted with EtOAc (3×5 ml). The organic layers were collected, dried ($MgSO_4$), and evaporated. The crude residue was purified by column chromatography (0.5% MeOH in DCM) to afford product **15** as a yellow solid (30.67 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98 (dd, J=2.7, 9.2 Hz, 1H, 9-H), 7.70 (d, J=2.7 Hz, 1H, 7-H), 7.37–7.18 (m, 15H, Trt), 7.10 (s, 1H, NH), 7.02 (d, J=8.5 Hz, 2H), 6.79 (d, J=9.2 Hz, 1H, 10-H), 6.73 (d, J=8.6 Hz, 2H), 5.10 (d, J=17.0 Hz, 1H, 5-H), 5.11–5.06 (m, 1H, 2-H), 4.86 (d, J=14.6 Hz, 1H, N^4CH_2), 4.37 (d, J=14.7 Hz, 1H, N^4CH_2), 3.89 (d, J=17.0 Hz, 1H, 5-H), 3.78 (s, 3H, OCH_3), 3.31 (dd, J=13.8, 10.0 Hz, 1H, β-H), 2.99 (s, 3H, N^1CH_3), 2.64 (dd, J=13.8, 3.5 Hz, 1H, β-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 36.90 (β-CH_2), 37.21 (N^1CH_3), 49.58 (NCH_2), 51.68 (5-CH_2), 55.29 (2-CH), 55.74 (OCH_3), 70.68 (C(ϕ)$_3$), 114.21 (2 CH), 118.98 (10-C), 122.77 (6-C), 124.42 (7-CH), 125.79 (9-CH), 127.09 (3CH, Trt), 128.02 (6CH, Trt), 128.35 (CCH_2), 128.62 (6CH, Trt), 129.32 (2CH), 139.07 (8-C), 144.44 (3C, Trt), 155.70 (11-C), 159.20 (COMe), 168.29 (CO), 169.65 (CO) ppm. MS (EI): m/z (%)=397 (33) [M−Trt]$^+$, 354 (17) [M−CONHTrt]$^+$, 243 (100) [Trt]$^+$. HRMS (EI) calculated for $C_{39}H_{36}O_5N_4$: 640.2685; found: 640.2656. Melting point: 93 °C.

4.5. Acylation of the reduced nitro group

4.5.1. N-[2-(4-tert-Butoxybenzyl)-4-(4-methoxybenzyl)-3-oxo-2,3,4,5-tetra-hydro-1H-1,4-benzodiazepin-7-yl]acetamide (**16**)

To a solution of benzodiazepinone **4** (30 mg, 0.06 mmol) in MeOH (3 ml), 3 mg Pd (10% on carbon) catalyst was added. After 3 h shaking under hydrogen (2.7 atm) in the Parr apparatus, the catalyst was filtrated off and washed. MeOH was evaporated and a white oil was obtained. The aniline derivate was dissolved without further purification in DCM (3 ml) and acetic anhydride (0.05 ml, 0.06 mmol) was added. After stirring the reaction mixture for 2 h, DCM was evaporated and the residue was redissolved in EtOAc. The solution was washed with saturated $NaHCO_3$, brine, dried ($MgSO_4$), and evaporated. Purification with column chromatography (EtOAc/heptane 95:5) afforded **16** as a light yellow solid (22.14 mg, 72%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.20 (d, J=8.3 Hz, 2H), 7.19–7.17 (m, 1H, 7-H), 7.13 (d, J=8.3 Hz, 2H), 7.01 (d, J=0.8 Hz, 1H, NH), 6.94 (d, J=8.3 Hz, 2H), 6.95–6.93 (m, 1H, 9-H), 6.79 (d, J=8.3 Hz, 2H), 6.33 (d, J=8.5 Hz, 1H, 10-H), 5.07 (d, J=16.4 Hz, 1H, 5-H), 4.90 (d, J=14.6 Hz, 1H, NCH_2), 4.79–4.76 (m, 1H, 2-H), 4.30 (d, J=14.6 Hz, 1H, NCH_2), 3.77 (s, 3H, OCH_3), 3.74 (d, J=16.4 Hz, 1H, 5-H), 3.64 (s, 1H, NH), 3.36 (dd, J=14.4, 4.9 Hz, 1H, β-H), 2.89 (dd, J=14.4, 8.6 Hz, 1H, β-H), 2.08 (s, 3H, CH_3CO), 1.34 (s, 9H, ^{t}Bu) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.18 (CH_3CO), 28.88 (C(CH_3)$_3$), 36.92 (β-H), 49.73 (NCH_2), 50.34 (5-CH_2), 55.27 (OCH_3), 56.30 (2-CH), 78.41 (C(CH_3)$_3$), 114.03 (2CH), 117.19 (10-CH), 120.40 (6-C), 121.43 (9-CH), 122.00 (7-CH), 124.32 (2CH), 128.80 (CCH_2), 129.24 (CCH_2), 129.50 (2CH), 129.69 (2CH), 132.21 (8-C), 142.58 (11-C), 154.30 (CO^tBu), 159.07 (COMe), 168.07 (CO), 170.56 (CO) ppm. MS (EI): m/z (%)=338 (68) [M−CH_2-ϕ-O^{t}Bu]$^+$. HRMS (EI) calculated for $C_{30}H_{35}O_4N_3$: 501.2627; found: 501.2634. Melting point: 183 °C.

4.5.2. N-{2-(4-tert-Butoxybenzyl)-4-[2-(4-methoxyphenyl)-ethyl]-3-oxo-2,3,4,5-tetrahydro-1H-1,4-benzodiazepin-7-yl}-acetamide (**17**)

Benzodiazepinone **5** (30 mg, 0.06 mmol) was reduced and acylated by the same procedure as described for product **16**. After column chromatography (EtOAc/heptane 95:5), product **17** was obtained as a light yellow solid (24.27 mg, 79%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.24–7.23 (m, 1H, 7-H), 7.17 (d, J=8.4 Hz, 2H), 7.03 (d, J=1.5 Hz, 1H, NH), 7.00 (d, J=8.4 Hz, 2H), 6.93–6.90 (m, 1H, 9-H), 6.94 (d, J=8.4 Hz, 2H), 6.73 (d, J=8.4 Hz, 2H), 6.32 (d, J=8.5 Hz, 1H, 10-H), 5.14 (d, J=16.4 Hz, 1H, 5-H), 4.72–4.66 (m, 1H, 2-H), 3.78–3.70 (m, 1H, NCH_2CH_2), 3.76 (s, 3H, OCH_3), 3.66–3.59 (m, 3H, NH, NCH_2CH_2, 5-H), 3.30 (dd, J=5.1, 14.3 Hz, 1H, β-H), 2.82 (dd, J=8.4, 14.3 Hz, 1H, β-H), 2.74 (t, J=6.9 Hz, 2H, NCH_2CH_2), 2.09 (s, 3H, CH_3CO), 1.33 (s, 9H, ^{t}Bu) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.18 (CH_3CO), 28.87 (C(CH_3)$_3$), 33.78 (NCH_2CH_2), 36.78 (β-CH_2), 50.78 (5-CH_2), 52.73 (NCH_2CH_2), 55.23 (OCH_3), 56.15 (2-CH), 78.39 (C(CH_3)$_3$), 113.92 (2CH), 117.16 (10-CH), 120.70 (6-C), 121.40 (9-CH), 121.90 (7-CH), 124.32 (2CH), 129.68 (2CH), 129.74 (2CH), 130.99 (CCH_2), 132.24 (8-C), 142.50 (11-C), 150.73 (CH_2C), 154.27 (CO^tBu), 158.12 (COMe), 168.13 (3-C), 170.23 (CH_3CO) ppm. MS (EI); m/z (%): 352 (100) [M−CH_2-ϕ-O^{t}Bu]$^+$. HRMS (EI) calculated for $C_{31}H_{37}O_4N_3$: 515.2784; found: 515.2788. Melting point: 174 °C.

4.5.3. 2-[7-(Acetylamino)-4-(4-methoxybenzyl)-3-oxo-2,3,4,5-tetrahydro-1H-1,4-benzodiazepin-2-yl]-N-tritylacetamide (**18**)

Benzodiazepinone **6** (30 mg, 0.05 mmol) was reduced and acylated by the same procedure as described for product **16**. After column chromatography (EtOAc/heptane 95:5), product **18** was

obtained as a light yellow solid (24.15 mg, 79%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H, NHTrt), 7.58 (s, 1H, 7-H), 7.24–7.18 (m, 15H, Trt), 7.02 (d, J=8.1 Hz, 2H), 6.99 (s, 1H, NH), 6.73 (d, J=8.1 Hz, 2H), 6.67 (d, J=8.1 Hz, 1H, 9-H), 6.07 (d, J=8.2 Hz, 1H, 10-H), 4.96 (d, J=16.6 Hz, 1H, 5-H), 4.89–4.86 (m, 1H, 2-H), 4.83 (d, J=14.6 Hz, 1H, NCH_2), 4.14 (d, J=14.6 Hz, 1H, NCH_2), 3.6 (s, 1H, NH), 3.74 (s, 3H, OCH_3), 3.59 (d, J=16.6 Hz, 1H, 5-H), 2.86–2.74 (m, 2H, β-H), 1.93 (s, 3H, CH_3CO) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.01 (CH_3CO), 39.24 (β-CH_2), 49.74 (NCH_2), 50.62 (5-CH_2), 52.64 (OCH_3), 55.27 (2-CH), 70.63 ($C(\phi)_3$), 114.10 (2CH), 117.34 (10-CH), 119.42 (6-C), 121.75 (9-CH), 122.11 (7-CH), 126.90 (3CH, Trt), 127.85 (6CH, Trt), 128.73 (6CH, Trt), 128.88 (CCH_2), 129.34 (2CH), 134.82 (8-C), 141.18 (11-C), 144.59 (3C, Trt), 159.07 (COMe), 168.43 (CO), 169.74 (CO), 170.71 (CO) ppm. MS (EI): m/z (%): 395 (100) [M−Trt]$^+$. HRMS (EI) calculated for $C_{40}H_{38}O_4N_4$: 638.2893; found: 638.2905. Melting point: 132 °C.

4.5.4. 7-Amino-2-(4-tert-butoxybenzyl)-4-[2-(4-methoxyphenyl)-ethyl]-1,2,4,5-tetrahydro-3H-1,4-benzodiazepin-3-one (**22**)

Benzodiazepinone **5** (100 mg, 0.19 mmol) was dissolved in MeOH (5 ml) and 5 mg Pd (10% on carbon) was added. After 3 h shaken under hydrogen (40 atm) in the Parr apparatus, the catalyst was filtrated off and washed. MeOH was evaporated and product **22** was obtained as a white oil (88.97 mg, 99%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.18 (d, J=8.3 Hz, 2H), 7.03 (d, J=8.4 Hz, 2H), 6.93 (d, J=8.4 Hz, 2H), 6.77 (d, J=8.4 Hz, 2H), 6.42 (d, J=8.1 Hz, 1H, 7-H), 6.31 (d, J=8.1 Hz, 1H, 9-H), 6.17 (s, 1H, 10-H), 4.93 (d, J=16.3 Hz, 1H, 5-H), 4.57–4.46 (m, 1H, 2-H), 3.75 (s, 3H, OCH_3), 3.74–3.54 (m, 3H, 5-H, NCH_2CH_2), 3.30 (dd, J=5.0, 14.2 Hz, 1H, CH_2), 3.16–2.76 (br s, 2H, NH_2), 2.82 (dd, J=8.4, 14.2 Hz, 1H, CH_2^{β}), 2.75 (t, J=6.8 Hz, 2H, NCH_2CH_2), 1.28 (s, 9H, ^{t}Bu) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.87 (C(CH_3)$_3$), 33.75 (NCH_2CH_2), 37.29 (β-CH_2), 50.91 (5-CH_2), 52.56 (NCH_2CH_2), 55.22 (OCH_3), 57.66 (2-CH), 78.38 ($C(CH_3)_3$), 113.87 (2CH), 116.14 (9-CH), 116.41 (10-CH), 119.20 (7-CH), 123.34 (6-C), 124.26 (2CH), 129.79 (4CH), 131.07 (CCH_2), 132.79 (CCH_2), 138.12 (8-C), 138.71 (11-C), 154.07 (CO^tBu), 158.11 (COMe), 170.81 (CO) ppm. HRMS (EI) calculated for $C_{29}H_{35}O_3N_3$: 473.2678; found: 473.2672.

4.5.5. Compound (**19**)

Aniline derivate **22** (88.97 mg, 0.19 mmol) was dissolved without further purification in dry DMF (10 ml). Pybop (97.87 mg, 0.19 mmol), Fmoc-L-Asn(NHTrt)-OH (0.113 mg, 0.19 mmol), and DIPEA (0.017 ml, 0.19 mmol) were added. After stirring the solution overnight under Ar at room temperature, DMF was evaporated and the crude mixture was purified by column chromatography (EtOAc/heptane 50:50). Product **23** was obtained as a pale white solid (69.89 mg, 35%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.57 (s, 1H), 7.71 (d, J=7.2 Hz, 2H), 7.53 (d, J=7.2 Hz, 2H), 7.35 (t, J=7.2 Hz, 2H), 7.28–7.04 (m, 21H), 6.98 (d, J=8.1 Hz, 2H), 6.97–6.91 (m, 4H), 6.72 (d, J=8.4 Hz, 2H), 6.48 (d, J=5.3 Hz, 1H), 6.31 (d, J=9.1 Hz, 1H), 5.12 (d, J=16.2 Hz, 1H, 5-H), 4.74–4.66 (m, 1H, α-H), 4.66–4.56 (m, 1H, α-H), 4.38 (d, J=6.8 Hz, 2H, Fmoc), 4.15 (t, J=6.8 Hz, 1H, Fmoc), 3.70 (s, 3H, OCH_3), 3.67–3.61 (m, 2H, NCH_2CH_2), 3.56 (d, J=16.2 Hz, 1H, 5-H), 3.32 (dd, J=5.3, 14.4 Hz, 1H, β-H), 3.14–3.04 (m, 1H, β-H), 2.82 (dd, J=8.4, 14.4 Hz, 1H, β-H), 2.78–2.68 (m, 2H, NCH_2CH_2), 2.63 (dd, J=6.6, 15.5 Hz, 1H, β-H), 2.33 (s, 9H, ^{t}Bu) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.92 (C(CH_3)$_3$), 33.78 (NCH_2CH_2), 36.76 (β-CH_2), 38.97 (β-CH_2), 47.15 (CH-Fmoc), 50.62 (NCH_2CH_2), 51.93 (α-CH), 52.62 (5-CH_2), 55.23 (α-CH), 56.06 (OCH_3), 67.25 (CH_2-Fmoc), 71.00 ($C(\phi)_3$), 78.43 ($C(CH_3)_3$), 113.92 (2CH), 117.14 (7-CH), 120.08 (2CH, Fmoc), 120.36 (6-C), 121.46 (2CH, 9-CH, 7-CH), 124.37 (2CH, Fmoc), 125.09 (2CH), 127.13 (2CH, Fmoc), 127.19 (2CH, Fmoc), 127.80 (3CH, Trt), 128.06 (3CH, Trt), 128.37 (CCH), 128.64 (6CH, Trt), 129.79 (4CH), 130.86 (CCH_2), 132.36 (8-C), 141.34 (2C, Fmoc), 142.58 (11C), 143.71 (2C, Fmoc), 144.16 (CO), 145.54 (3C, Trt), 154.26 (CO), 156.30 (CO^tBu), 158.15 (COMe), 168.49 (CO), 170.17 (CO) ppm. MS (ESI, MeOH): 1074.9 [MNa^+]. Melting point: 131 °C.

4.5.6. Compound (**20**)

Aniline derivate **22** (88.97 mg, 0.19 mmol) was coupled to Fmoc-L-Asp(O^{t}Bu)-OH (0.078 mg, 0.19 mmol) by a procedure similar to that used for product **23**. After purification by column chromatography (EtOAc/DCM 10:90), product **23** was obtained as a pale white solid (111 mg, 68%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (br s, 1H), 7.75 (d, J=7.4 Hz, 2H), 7.57 (d, J=7.4 Hz, 2H), 7.37 (t, J=7.4 Hz, 2H), 7.32–7.24 (m, 3H), 7.18 (d, J=8.5 Hz, 2H), 7.02–6.92 (m, 6H), 6.73 (d, J=8.5 Hz, 2H), 6.33 (d, J=8.8 Hz, 1H), 6.14–6.03 (br s, 1H), 5.13 (d, J=16.6 Hz, 1H, 5-H), 4.72–4.66 (m, 1H, α-H), 4.66–4.57 (m, 1H, α-H), 4.45 (d, J=6.8 Hz, 2H, Fmoc), 4.21 (t, J=6.8 Hz, 1H, Fmoc), 3.74 (s, 3H, OCH_3), 3.68 (dd, J=7.1, 14.5 Hz, 2H, NCH_2CH_2), 3.60 (d, J=16.6 Hz, 1H, 5-H), 3.31 (dd, J=5.4, 14.2 Hz, 1H, β-H), 2.92 (dd, J=3.3, 16.9 Hz, 1H, β-H), 2.83 (dd, J=8.3, 14.2 Hz, 1H, β-H), 2.75 (t, J=7.1 Hz, 2H, NCH_2CH_2), 2.67 (dd, J=7.1, 16.9 Hz, 1H, β-H), 1.44 (s, 9H, ^{t}Bu), 1.33 (s, 9H, ^{t}Bu) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.05 (C(CH_3)$_3$), 28.87 (C(CH_3)$_3$), 33.75 (NCH_2CH_2), 36.71 (β-CH_2), 37.53 (β-CH_2), 47.15 (CH, Fmoc), 50.74 (NCH_2CH_2), 51.75 (α-CH), 52.68 (5-CH_2), 55.22 (OCH_3), 56.01 (α-CH), 67.35 (CH_2, Fmoc), 78.41 ($C(CH_3)_3$), 82.11 ($C(CH_3)_3$), 113.89 (2CH), 117.16 (10CH), 120.06 (2CH, Fmoc), 120.52 (6-C), 121.23 (9-CH), 121.50 (7-CH), 124.34 (2CH), 124.97 (2CH, Fmoc), 127.11 (2CH, Fmoc), 127.81 (2CH, Fmoc), 128.30 (CCH_2), 129.71 (2CH), 129.75 (2CH), 130.09 (CCH_2), 132.27 (8-C), 141.34 (2C, Fmoc), 142.65 (11-C), 143.63 (2C, Fmoc), 154.23 (COMe), 158.12 (CO^tBu), 163.38 (CO), 171.3 (CO), 170.18 (CO), 168.19 (CO) ppm. MS (ESI, MeOH): 867.7 [MH^+], 889.7 [MNa^+], 1733.5 [$2MH^+$]. Melting point: 83 °C.

4.5.7. Compound (**21**)

Aniline derivate **22** (87.32 mg, 0.19 mmol) was coupled to Fmoc-L-Tyr(O^{t}Bu)-OH (0.078 mg, 0.19 mmol) by a procedure similar to that used for product **23**. After purification by column chromatography (EtOAc/heptane 50:50), product **23** was obtained as a pale white solid (79.88 mg, 46%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (d, J=7.3 Hz, 2H), 7.52 (d, J=7.3 Hz, 2H), 7.47–7.42 (br s, 1H), 7.36 (t, J=7.4 Hz, 2H), 7.29–7.25 (m, 4H), 7.17 (d, J=8.4 Hz, 2H), 7.12–7.04 (m, 2H), 7.01 (d, J=8.2 Hz, 2H), 6.97 (d, J=8.4 Hz, 2H), 6.94 (d, J=8.4 Hz, 2H), 6.80 (s, 2H), 6.70 (d, J=8.4 Hz, 2H), 6.24 (d, J=8.8 Hz, 1H), 5.17 (d, J=16.5 Hz, 1H, 5-H), 4.70–4.62 (m, 1H, α-H), 4.51–4.26 (m, 3H, α-H, Fmoc), 4.16 (t, J=6.7 Hz, 1H, Fmoc), 3.71 (s, 3H, OCH_3), 3.69–3.58 (m, 2H, NCH_2CH_2), 3.52 (d, J=16.5 Hz, 1H, 5-H), 3.30 (dd, J=5.3, 14.4 Hz, 1H, β-H), 3.16–2.96 (m, 2H, β-H), 2.81 (dd, J=8.4, 14.4 Hz, 1H, β-H), 2.72 (t, J=7.2 Hz, 2H, NCH_2CH_2), 1.33 (s, 9H, ^{t}Bu), 1.28 (s, 9H, ^{t}Bu) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.83 (C(CH_3)$_3$), 28.87 (C(CH_3)$_3$), 33.74 (NCH_2CH_2), 36.70 (β-CH_2), 38.18 (β-CH_2), 47.12 (CH, Fmoc), 50.72 (NCH_2CH_2), 52.60 (5-CH_2), 55.24 (OCH_3), 55.96 (α-CH), 57.03 (α-CH), 67.19 (CH_2, Fmoc), 78.41 ($C(CH_3)_3$), 78.47 ($C(CH_3)_3$), 113.91 (2CH), 117.05 (10-CH), 120.02 (2CH, Fmoc), 120.40 (9-CH), 121.56 (7-CH), 121.81 (6-C), 124.34 (2CH), 124.37 (2CH), 124.98 (2CH), 127.12 (2CH, Fmoc), 127.79 (2CH, Fmoc), 128.23 (CCH_2), 128.45 (CCH_2), 129.70 (2CH), 129.75 (2CH), 129.84 (2CH), 130.91 (CCH_2), 132.25 (8-C), 141.31 (2C, Fmoc), 142.77 (11-C), 143.66 (2C, Fmoc), 154.25 (CO^tBu), 154.57 (CO^tBu), 158.07 (COMe), 168.89 (CO), 170.13 (CO) ppm. MS (ESI, MeOH): 937.9 [MNa^+], 1851.4 [$2MNa^+$]. Melting point: 110 °C.

4.5.8. N^1-{2-(4-tert-Butoxybenzyl)-4-[2-(4-methoxyphenyl)ethyl]-3-oxo-2,3,4,5-tetrahydro-1H-1,4-benzodiazepin-7-yl}-N^4-tritylaspartamide (**23**)

Fmoc-protected compound **19** (69.89 mg, 0.06 mmol) was dissolved in a DMF/piperidine (4:1) mixture and stirred for 1 h at room temperature. After evaporation of the solvent, purification by column chromatography (2% MeOH in DCM) and preparative TLC (1% MeOH in DCM), compound **23** was obtained as an oil (24.80 mg,

45%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.32 (s, 1H), 7.43–7.08 (m, 18H), 7.04–6.92 (m, 5H), 6.74 (d, J=8.6 Hz, 2H), 6.31 (d, J=8.6 Hz, 1H), 5.13 (d, J=16.1 Hz, 1H, 5-H), 4.74–4.65 (m, 1H, α-H), 3.74 (s, 3H, OCH_3), 3.67 (t, J=7.2 Hz, 2H, NCH_2CH_2), 3.70–3.63 (m, 1H, α-H), 3.60 (d, J=16.1 Hz, 1H, 5-H), 3.32 (dd, J=5.1, 14.4 Hz, 1H, β-H), 2.88–2.63 (m, 5H, β-H, β-H, NCH_2CH_2), 2.32–2.07 (br s, 2H, NH_2), 1.34 (s, 9H, ^{t}Bu) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 29.00 ($C(CH_3)_3$), 33.73 (NCH_2CH_2), 36.78 (β-CH_2), 41.05 (β-CH_2), 50.10 (NCH_2CH_2), 52.62 (5-CH_2), 52.73 (α-CH), 55.22 (OCH_3), 56.14 (α-CH), 70.62 ($C(\phi)_3$), 78.38 ($C(CH_3)_3$), 113.87 (2CH), 117.26 (10CH), 120.59 (6-C), 120.68 (10-CH), 120.73 (9-CH), 124.31 (2CH), 127.01 (3CH, Trt), 127.94 (6CH, Trt), 128.54 (6CH, Trt), 128.76 (CCH_2), 129.81 (2CH), 129.73 (2CH), 130.90 (CCH_2), 132.34 (8-C), 142.27 (11-C), 144.41 (NHCO), 144.45 (3C, Trt), 154.23 (CO^tBu), 158.11 ($COMe$), 170.16 (CO), 170.24 (CO) ppm. MS (EI); m/z (%): 529 (100) [M−CH_2-ϕ-O^{t}Bu]$^+$. MS (ESI, MeOH): 852.6 [MNa^+], 1762.3 [$2MNa^+$].

4.5.9. N^1-{2-(4-tert-Butoxybenzyl)-4-[2-(4-methoxyphenyl)ethyl]-3-oxo-2,3,4,5-tetrahydro-1H-1,4-benzodiazepin-7-yl}-O-(tert-butyl)asparaginate (**24**)

Fmoc-protected compound **20** (111 mg, 0.1 mmol) was dissolved in a DMF/piperidine (4:1) mixture and stirred for 1 hour at room temperature. After evaporation of the solvent, purification by column chromatography (10% EtOAc in DCM) and preparative TLC (1% MeOH in DCM), compound **24** was obtained as an oil (17.33 mg, 21%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.24 (s, 1H), 7.22 (d, J=1.7 Hz, 1H), 7.19 (d, J=8.2 Hz, 2H), 7.06–7.00 (m, 3H), 6.94 (d, J=8.3 Hz, 2H), 6.75 (d, J=8.3 Hz, 2H), 6.35 (d, J=8.5 Hz, 1H), 5.16 (d, J=16.5 Hz, 1H, 5-H), 4.74–4.65 (m, 1H, α-H), 3.76 (s, 3H, OCH_3), 3.76–3.70 (m, 2H, NCH_2CH_2), 3.66 (d, J=16.5 Hz, 1H, 5H), 3.57–3.53 (m, 1H, α-H), 3.32 (dd, J=5.1, 14.3 Hz, 1H, β-H), 2.89 (dd, J=3.6, 16.5 Hz, 1H, β-H), 2.83 (dd, J=8.7, 14.3 Hz, 1H, β-H), 2.76 (t, J=7.0 Hz, 2H, NCH_2CH_2), 2.60 (dd, J=8.1, 16.6 Hz, 1H, β-H), 1.93–1.70 (br s, 2H, NH_2), 1.45 (s, 9H, ^{t}Bu), 1.34 (s, 9H, ^{t}Bu) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.11 ($C(CH_3)_3$), 28.86 ($C(CH_3)_3$), 33.76 (NCH_2CH_2), 36.77 (β-CH_2), 40.36 (β-CH_2), 50.71 (NCH_2CH_2), 52.40 (α-CH), 52.70 (5-CH_2), 55.22 (OCH_3), 56.12 (α-CH), 78.38 ($C(CH_3)_3$), 81.33 ($C(CH_3)_3$), 113.85 (2CH), 117.25 (10-CH), 120.42 (9-CH), 120.67 (7-CH), 120.71 (6-C), 124.31 (2CH), 129.71 (2CH), 129.75 (2CH), 128.89 (CCH_2), 130.92 (CCH_2), 132.33 (8-C), 142.20 (11-C), 154.21 (CO^tBu), 158.10 ($COMe$), 170.02 (CO), 171.04 (CO), 171.16 (CO) ppm. MS (EI); m/z (%): 481 (26) [M−CH_2-ϕ-O^{t}Bu]$^+$. HRMS (EI) calculated for $C_{37}H_{48}O_6N_4$: 644.3574; found: 644.3583.

4.5.10. N-{2-(4-tert-butoxybenzyl)-4-[2-(4-methoxyphenyl)ethyl]-3-oxo-2,3,4,5-tetrahydro-1H-1,4-benzodiazepin-7-yl}-O-(tert-butyl)tyrosinamide (**25**)

Fmoc-protected compound **21** (79.88 mg, 0.08 mmol) was dissolved in a DMF/piperidine (4:1) mixture and stirred for 1 h at room temperature. After evaporation of the solvent, purification by column chromatography (EtOAc/heptane 80:20) and HPLC (100% EtOAC), compound **23** was obtained as an oil (25.40 mg, 42%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.06 (s, 1H), 7.23 (d, J=1.0 Hz, 1H), 7.18 (d, J=8.4 Hz, 2H), 7.11 (d, J=8.4 Hz, 2H), 7.05–6.98 (m, 3H), 6.97–6.91 (m, 4H), 6.75 (d, J=8.4 Hz, 2H), 6.35 (d, J=8.5 Hz, 1H), 5.15 (d, J=16.4 Hz, 1H, 5-H), 4.75–4.65 (m, 1H, α$^-$H), 3.75 (s, 3H, OCH_3), 3.74–3.64 (m, 4H, 5-H, α$^-$H, NCH_2CH_2), 3.32 (dd, J=5.3, 14.4 Hz, 1H, β-H), 3.27 (dd, J=3.8, 14.1 Hz, 1H, β-H), 2.83 (dd, J=8.3, 14.4 Hz, 1H, β-H), 2.76 (t, J=7.0 Hz, 2H, NCH_2CH_2), 2.73 (dd, J=9.3, 14.1 Hz, 1H, β-H), 1.98–1.81 (br s, 2H, NH_2), 1.45 (s, 9H, ^{t}Bu), 1.34 (s, 9H, ^{t}Bu) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.11 ($C(CH_3)_3$), 28.86 ($C(CH_3)_3$), 33.78 (NCH_2CH_2), 36.80 (β-CH_2), 40.06 (β-CH_2), 50.71 (NCH_2CH_2), 52.71 (5-CH_2), 55.20 (OCH_3), 56.21 (α-CH), 56.72 (α-CH), 78.35 ($C(CH_3)_3$), 78.39 ($C(CH_3)_3$), 113.89 (2CH), 117.27 (10-CH), 120.53 (6-C), 120.79 (7-CH), 120.81 (9-CH), 124.26 (2CH), 124.33 (2CH), 129.67 (2CH), 129.70 (2CH), 129.75 (2CH), 128.89 (CCH_2), 130.95 (CCH_2), 132.31 (CCH_2), 132.33 (8-C), 142.26 (11-C), 154.27 ($COMe$), 154.44 (CO^tBu), 158.14 ($COMe$), 170.22 (2CO) ppm. MS (EI); m/z (%): 529 (100) [M−CH_2-ϕ-O^{t}Bu]$^+$. HRMS (EI) calculated for $C_{42}H_{52}O_5N_3$: 693.4016; found: 693.3996.

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